



ORIGINAL ARTICLE

Long-term outcome and prognostic factors of combined hepatocellular carcinoma and cholangiocarcinoma after curative resection

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Abstract

Background: Combined hepatocellular carcinoma and cholangiocarcinoma (cHCC-CC) is a rare subtype of primary liver cancers. Its prognostic factors remain unclear. The study aimed to evaluate its long-term outcome and prognostic factors by retrospectively reviewing the series of cHCC-CC after curative resection from our institute.

Methods: A total of 55 pathologically confirmed cHCC-CC patients undergoing curative resections between January 2003 and January 2018 at the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China) were included. The clinicopathological and follow-up data were retrieved. Overall survival (OS) and recurrence-free survivals (RFS) were analysed by Kaplan–Meier curve. The independent prognostic factors were determined by using univariate and multivariate Cox analyses.

Results: There were 41 males and 14 females, with a median age of 51.0 (interquartile range, 44.0–60.0) years. The 1-, 3-, and 5-year OS and RFS rates in cHCC-CC were 80.0%, 25.5%, and 16.4%, respectively, and 52.7%, 21.8%, and 10.9%, respectively. The median OS and RFS were 24.9 and 14.5 months, respectively. Univariate and multivariate analyses revealed that elevated alpha-fetal protein (AFP) and/or CA19-9, vascular invasion, local extra-hepatic invasion, and lymph-node metastasis (LNM) were independent unfavorable prognostic factors for OS and RFS (all $P < 0.005$). Furthermore, elevated AFP and/or CA19-9 were independent unfavorable prognostic factors in various subgroups of cHCC-CC, including patients aged < 60 years, positive hepatitis B surface antigen, cirrhosis, single tumor, tumor size ≥ 5 cm, no vascular invasion, no LNM, and no local extra-hepatic invasion (all $P < 0.05$).

Conclusions: Elevated AFP and/or CA19-9, vascular invasion, local extra-hepatic invasion, and LNM were independent unfavorable prognostic factors for long-term survival of cHCC-CC undergoing curative resections. Patients with normal levels of AFP and CA19-9 had better prognosis.

Key words: hepatocellular carcinoma; cholangiocarcinoma; AFP; CA19-9; prognosis

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Introduction

Combined hepatocellular carcinoma and cholangiocarcinoma (cHCC-CC) is one of the uncommon types of primary liver cancers, accounting for 0.4%–14.2% of all primary liver carcinomas [1, 2]. It contains elements of both hepatocellular carcinoma (HCC) and intra-hepatic cholangiocarcinoma (ICC). Because of a lack of specific clinical manifestations, its diagnosis is dependent on pathological examination. Regarding the treatment of cHCC-CC, curative resection offers the greatest hope of a cure [3]. However, the prognosis of cHCC-CC is worse than either HCC or ICC after surgery [4]. The prognostic factors for cHCC-CC patients are still unclear due to its rarity.

α -Fetoprotein (AFP) is a widely accepted biomarker for HCC [5]. It has been proposed that AFP can be used as a prognostic indicator for HCC patients following liver resections or other therapies [6–8]. On the other hand, carbohydrate antigen 19–9 (CA19–9) has been proposed as an independent prognostic factor for ICC patients undergoing curative resections [9, 10]. The clinical significance of either AFP or CA 19-9 in cHCC-CC remains unknown.

The aim of this study was to evaluate the clinical significance of AFP and CA19-9 in cHCC-CC patients after curative resections, including its association with other clinicopathological features and prognostic values.

Materials and methods

Patients

A total of 55 patients with pathologically proven cHCC-CC, who underwent curative liver resections between January 2003 and January 2018 at the First Affiliated Hospital of Sun Yat-sen University (Guangzhou, China), were included in the present study. The following patients were excluded from the study, including non-curative resection or unresectable tumors ($n=8$), presence of other malignancies ($n=2$), and hospital mortality ($n=1$).

Routine blood tests, biochemistry tests, serum AFP level, and serum CA19-9 level within 7 days before surgery were assessed. The cut-off values for AFP and CA19-9 were 20 μ g/L and 40 U/mL, respectively as previously reported [11, 12]. Any two of the radiological assessments, including ultrasound, contrast-enhanced computed tomography (CT)/magnetic resonance imaging (MRI), and positron emission tomography/computed tomography (PET/CT), were performed routinely.

Informed consent was obtained from each patient and this study was approved by the Ethics Committee of the First Affiliated Hospital of Sun Yat-sen University.

Treatment and follow-up

All patients received curative liver resections. The indications for surgical resection were as follows: (i) absence of distant metastasis, (ii) possibility of surgical resection with curative intention, and (iii) presence of adequate liver function required for liver resection. Patients were regularly followed by ultrasound, or contrast-enhanced CT/MRI, and titration of serum AFP and CA19-9 after discharge from the hospital. Recurrence was defined as the intra-hepatic and/or extra-hepatic lesions confirmed by contrast-enhanced CT/MRI and/or PET-CT or pathological confirmation (percutaneous fine-needle biopsy).

Overall survival (OS) was the interval from the date of surgery to the date of death or the last follow-up. Recurrence-free survival (RFS) was the interval from the date of surgery to the date of recurrence.

Statistical analysis

Statistical analyses were performed by using SPSS version 24.0 (IBM, New York, USA). Data are presented as mean \pm standard deviation or median with interquartile range (IQR). The differences between categorical variables were detected by using chi-square test or Fisher's exact test. Kaplan–Meier curve and log-rank test was used to compare the differences in survival between two groups. Multivariate Cox analysis was performed to determine the independent prognostic factors based on the statistically significant factors selected by univariate analyses and the results were presented as hazard ratios (HRs) with 95% confident intervals (CIs). A two-tailed P -value of <0.05 was considered significantly different.

Results

Patients' clinicopathological features

The clinicopathological features of cHCC-CC patients are summarized in Table 1. All patients were followed after discharged until December 2018. The median follow-up period was 20.0 months (IQR: 12.0–36.0 months). Forty-one males (74.5%) and 14 females (25.5%) were included in this study, with a median age of 51.0 (IQR: 44.0–60.0) years. Serum AFP and CA19-9 levels were elevated in 30 (54.5%) and 25 (45.5%) patients, respectively. Both AFP and CA19-9 were elevated in 13 patients (23.6%). It was shown that elevated AFP and/or CA19-9 was significantly associated with tumor size ≥ 5 cm ($P=0.002$), presence of vascular invasion ($P=0.025$), and incomplete/absent tumor capsule ($P=0.014$).

Long-term outcome and prognostic factors in cHCC-CC patients

The 1-, 3-, and 5-year OS rates of the cHCC-CC patients were 80.0%, 25.5%, and 16.4%, respectively. The 1-, 3-, and 5-year RFS rates of the cHCC-CC patients were 52.7%, 21.8%, and 10.9%, respectively. The median OS and RFS of cHCC-CC patients were 24.9 and 14.5 months, respectively.

With respect to the OS of cHCC-CC patients, the survival analyses showed that elevated AFP and/or CA19-9, vascular invasion, local extra-hepatic invasion, and lymph-node metastasis (LNM) were unfavorable prognostic factors (all $P < 0.001$; Figure 1). Furthermore, the multivariate analysis revealed that elevated AFP and/or CA19-9, vascular invasion, local extra-hepatic invasion, and LNM were independent unfavorable prognostic factors (all $P < 0.01$; Table 2).

In terms of the RFS of cHCC-CC patients, the survival analyses showed that elevated AFP and/or CA19-9, vascular invasion, local extra-hepatic invasion, and LNM (all $P < 0.001$; Figure 2) were unfavorable prognostic factors. Furthermore, the multivariate analysis revealed that elevated AFP and/or CA19-9, vascular invasion, local extra-hepatic invasion, and LNM were independent unfavorable factors (all $P < 0.05$; Table 3).

Table 1. Clinicopathological features of patients with cHCC-CC

Variable	Normal AFP and CA19-9 (n = 13)	Elevated AFP and/or CA19-9 (n = 42)	P-value ^b
Sex			0.050
Male	7 (53.8)	34 (81.0)	
Female	6 (46.2)	8 (19.0)	
Age (years)			0.111 ^c
<60	12 (92.3)	27 (64.3)	
≥60	1 (7.7)	15 (35.7)	
Symptoms			0.232 ^c
No	9 (69.2)	19 (45.2)	
Yes	4 (30.8)	23 (54.8)	
Alcohol			1.000 ^c
No	11 (84.6)	37 (88.1)	
Yes	2 (15.4)	5 (11.9)	
Family history of liver cancer			1.000 ^c
No	12 (92.3)	39 (92.9)	
Yes	1 (7.7)	3 (7.1)	
HBsAg			0.614
Negative	5 (38.5)	13 (31.0)	
Positive	8 (61.5)	29 (69.0)	
AFP (μg/L)			<0.001 ^d
<20	13 (100)	12 (28.6)	
≥20	0 (0)	30 (71.4)	
CA19-9 (U/mL)			<0.001 ^d
<40	13 (100)	17 (40.5)	
≥40	0 (0)	25 (59.5)	
ASA classification ^a			0.271 ^c
I + II	10 (76.9)	39 (92.9)	
III + IV	3 (23.1)	3 (7.1)	
Tumor size (cm)			0.002 ^c
<5	9 (69.2)	8 (19.0)	
≥5	4 (30.8)	34 (81.0)	
Tumor number			0.562 ^d
Single	13 (100)	38 (90.5)	
Multiple	0 (0)	4 (9.5)	
Cirrhosis			0.614
No	5 (38.5)	13 (31.0)	
Yes	8 (61.5)	29 (69.0)	
Blood transfusion			0.304 ^c
No	12 (92.3)	31 (73.8)	
Yes	1 (7.7)	11 (26.2)	
Vascular invasion			0.025 ^d
No	13 (100)	29 (69.0)	
Yes	0 (0)	13 (31.0)	
Local extra-hepatic invasion			0.179 ^d
No	13 (100)	35 (83.3)	
Yes	0 (0)	7 (16.7)	
Lymph-node metastasis			0.050 ^d
No	13 (100)	31 (73.8)	
Yes	0 (0)	11 (26.2)	
Tumor capsule			0.014 ^c
Incomplete/absence	3 (23.4)	28 (66.7)	
Complete	10 (76.9)	14 (33.3)	

All values are presented as number of patients followed by percentage in parentheses.

^aAmerican Society of Anesthesiologists classification.

^bChi-square test.

^cChi-square test (correction for continuity).

^dFisher's exact test.

However, OS ($P = 0.320$) and RFS ($P = 0.899$) were not significantly different between patients with both elevated AFP and CA19-9 and those with elevated AFP or CA19-9.

Prognostic values of elevated AFP and/or CA19-9 in different subgroups of cHCC-CC patients

The predictive values of combined AFP and CA19-9 for OS and RFS were further investigated in various subgroups of cHCC-CC patients (Figures 3 and 4). It was shown that, among patients with normal AFP and CA19-9, the OS and RFS were significantly longer in the patients aged <60 years, with positive hepatitis B surface antigen (HBsAg), cirrhosis, single tumor, tumor size ≥5 cm, no vascular invasion, no LNM, and no local extra-hepatic invasion than in their controls (all $P < 0.05$).

Discussion

cHCC-CC is a rare subtype of primary liver cancers consisting of elements of both hepatocellular carcinoma and intra-hepatic cholangiocarcinoma. Its proportion in liver cancers varies from 1.0% to 6.3% in Asian areas to 2.4%–14.2% in Western countries [13, 14]. Previous studies have demonstrated that the prognosis for cHCC-CC patients is poorer than that for patients with either HCC or ICC [1, 14, 15]. However, the prognostic factors of cHCC-CC have not been well documented. Clinically, the detection of AFP and CA19-9 is widely used for post-operative surveillance of HCC and ICC, respectively [16, 17]. It has been revealed that the combination of AFP and CA19-9 could contribute to the pre-operative diagnosis of cHCC-CC [18]. However, it has not been reported whether the combination of AFP and CA19-9 could predict the prognosis of cHCC-CC patients.

Among the patients enrolled in the study, patients with elevated AFP and elevated CA19-9 accounted for 54.5% and 45.5% of the total, respectively, which is comparable to the previous literature [19]. In the present study, we found that elevated AFP and/or CA19-9 was associated with larger tumor size, presence of vascular invasion, and incomplete/absence of tumor capsule. According to the proposed research, these three characteristics are significantly associated with poor prognosis in patients with cHCC-CC [19, 20]. Furthermore, survival analyses showed that cHCC-CC patients with preoperative elevated AFP and/or CA19-9 had worse prognosis after surgery compared with patients with normal AFP and CA19-9, even in various subgroups of patients. However, as a single indicator, AFP or CA19-9 alone could not independently predict the long-term prognosis of patients with cHCC-CC, which is the same as most studies reported [20–22]. This may be related to the biological properties of cHCC-CC itself, as it is not identical to either HCC or ICC. Moreover, there existed no significant differences in prognosis between patients with simultaneous elevation of AFP and CA19-9 and those with single-marker elevation. It may be due to the difference in the proportion of hepatocellular carcinoma and cholangiocarcinoma components in cHCC-CC tumors. Specifically, in patients with cHCC-CC whose main component is liver cancer, the increase in AFP is associated with a poor prognosis. Similarly, an increase in CA19-9 is associated with a poor prognosis in patients with cholangiocarcinoma as a major component. Therefore, for patients with cHCC-CC, a single AFP or CA19-9 does not accurately reflect the long-term prognosis of the patient. Considering the complexity of the tumor, combining the two indicators may be more clinically meaningful.

The predictive values of combining AFP and CA19-9 could be useful in clinical settings. First, it could be used to preoperatively predict the long-term outcome of cHCC-CC patients undergoing surgical resection as an independent prognostic factor. Second, in order to detect recurrence at an early stage, for patients with elevated AFP and/or CA19-9, the interval of

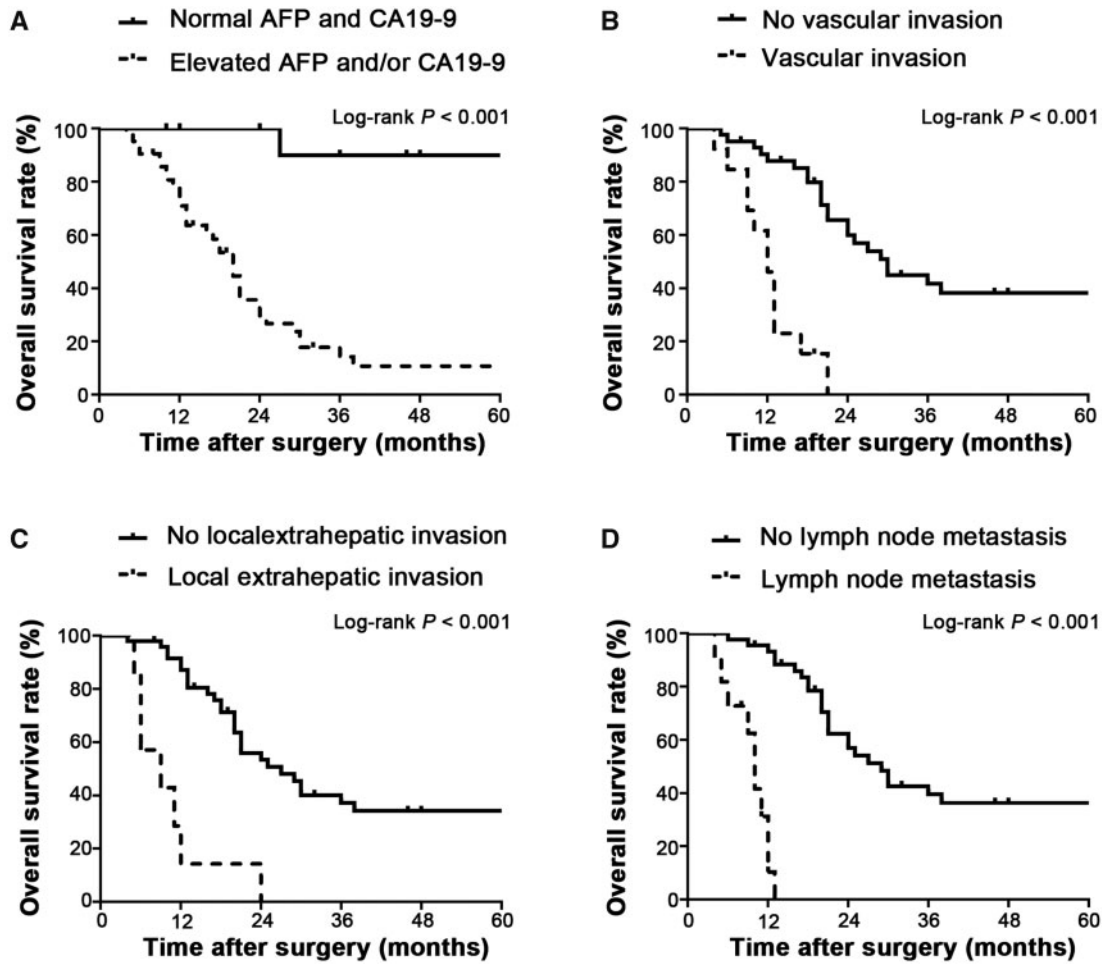


Figure 1. Kaplan–Meier curves showing the prognostic value of elevated AFP and/or CA19-9 (A), vascular invasion (B), local extrahepatic invasion (C), and lymph-node metastasis (D) for the overall survival of cHCC-CC patients after curative resections. cHCC-CC, combined hepatocellular carcinoma and cholangiocarcinoma; AFP, alpha-fetal protein; CA19-9, carbohydrate antigen 19-9.

Table 2. Univariate and multivariate analysis for OS of patients with cHCC-CC

Variable	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Combined AFP and CA19-9 (normal vs elevated)	0.11 (0.02–0.56)	0.008	0.20 (0.07–0.59)	0.004
Vascular invasion (no vs yes)	2.24 (1.01–4.97)	0.048	4.90 (1.86–12.90)	0.001
Local extra-hepatic invasion (no vs yes)	5.59 (1.06–29.58)	0.043	4.91 (1.77–13.63)	0.002
Lymph-node metastasis (no vs yes)	9.45 (1.23–72.40)	0.031	6.95 (2.15–22.44)	0.001
Age (years) (<60 vs ≥60)	1.59 (0.81–3.10)	0.174		
Alcohol (no vs yes)	0.69 (0.15–3.20)	0.633		
Family history of liver cancer (no vs yes)	0.98 (0.27–3.63)	0.979		
HBsAg (negative vs positive)	1.55 (0.49–4.92)	0.457		
ASA classification (I+II vs III+IV)	0.73 (0.22–2.47)	0.615		
Blood transfusion (no vs yes)	0.58 (0.27–1.26)	0.172		
AFP (µg/L) (<20 vs ≥20)	0.39 (0.14–1.10)	0.074		
CA19-9 (U/mL) (<40 vs ≥40)	0.39 (0.13–1.18)	0.097		
Cirrhosis (no vs yes)	1.11 (0.40–3.08)	0.847		
Tumor size (cm) (<5 vs ≥5)	1.86 (0.63–5.54)	0.263		
Tumor number (single vs multiple)	0.87 (0.14–5.45)	0.883		
Tumor capsule (incomplete/absence vs complete)	1.63 (0.85–3.14)	0.145		

AFP, α-Fetoprotein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen; ASA, American Society of Anesthesiologists.

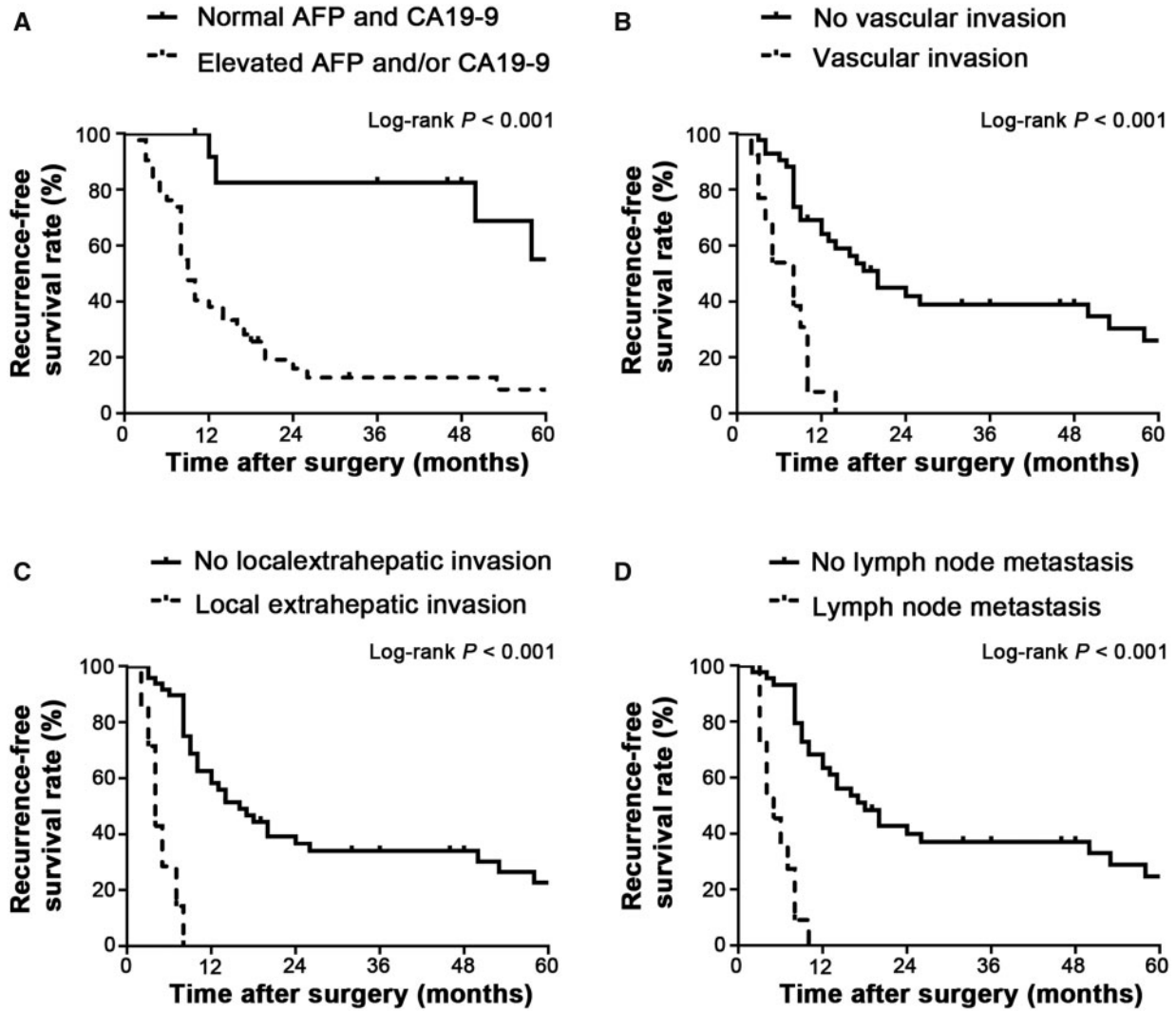


Figure 2. Kaplan–Meier curves showing the prognostic value of elevated AFP and/or CA19–9 (A), vascular invasion (B), local extra-hepatic invasion (C), and lymph-node metastasis (D) for the recurrence-free survival of cHCC-CC patients after curative resections. cHCC-CC, combined hepatocellular carcinoma and cholangiocarcinoma; AFP, alpha-fetal protein; CA19–9, carbohydrate antigen 19–9.

Table 3. Univariate and multivariate analysis for RFS of patients with cHCC-CC

Variable	Univariate		Multivariate	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Combined AFP and CA19-9 (normal vs elevated)	0.25 (0.07–0.96)	0.043	0.24 (0.09–0.64)	0.005
Vascular invasion (no vs yes)	7.74 (2.40–24.99)	0.001	2.89 (1.23–6.76)	0.014
Local extra-hepatic invasion (no vs yes)	7.47 (1.41–39.67)	0.018	7.33 (2.36–22.81)	0.001
Lymph-node metastasis (no vs yes)	6.61 (1.73–25.20)	0.006	2.88 (1.07–7.74)	0.036
Age (years) (<60 vs ≥60)	0.52 (0.23–1.14)	0.103		
Alcohol (no vs yes)	1.24 (0.31–4.88)	0.760		
Family history of liver cancer (no vs yes)	0.72 (0.19–2.77)	0.633		
HBsAg (negative vs positive)	1.31 (0.42–4.15)	0.644		
ASA classification (I + II vs III + IV)	0.58 (0.18–1.92)	0.371		
Blood transfusion (no vs yes)	0.85 (0.30–2.44)	0.762		
AFP (μg/L) (<20 vs ≥20)	1.05 (0.42–2.63)	0.919		
CA19-9 (U/mL) (<40 vs ≥40)	1.08 (0.37–3.17)	0.887		
Cirrhosis (no vs yes)	0.82 (0.28–2.41)	0.712		
Tumor size (cm) (<5 vs ≥5)	1.01 (0.38–2.71)	0.981		
Tumor number (single vs multiple)	0.28 (0.05–1.68)	0.164		
Tumor capsule (incomplete/absence vs complete)	1.48 (0.78–2.82)	0.232		

AFP, α-Fetoprotein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen; ASA, American Society of Anesthesiologists.

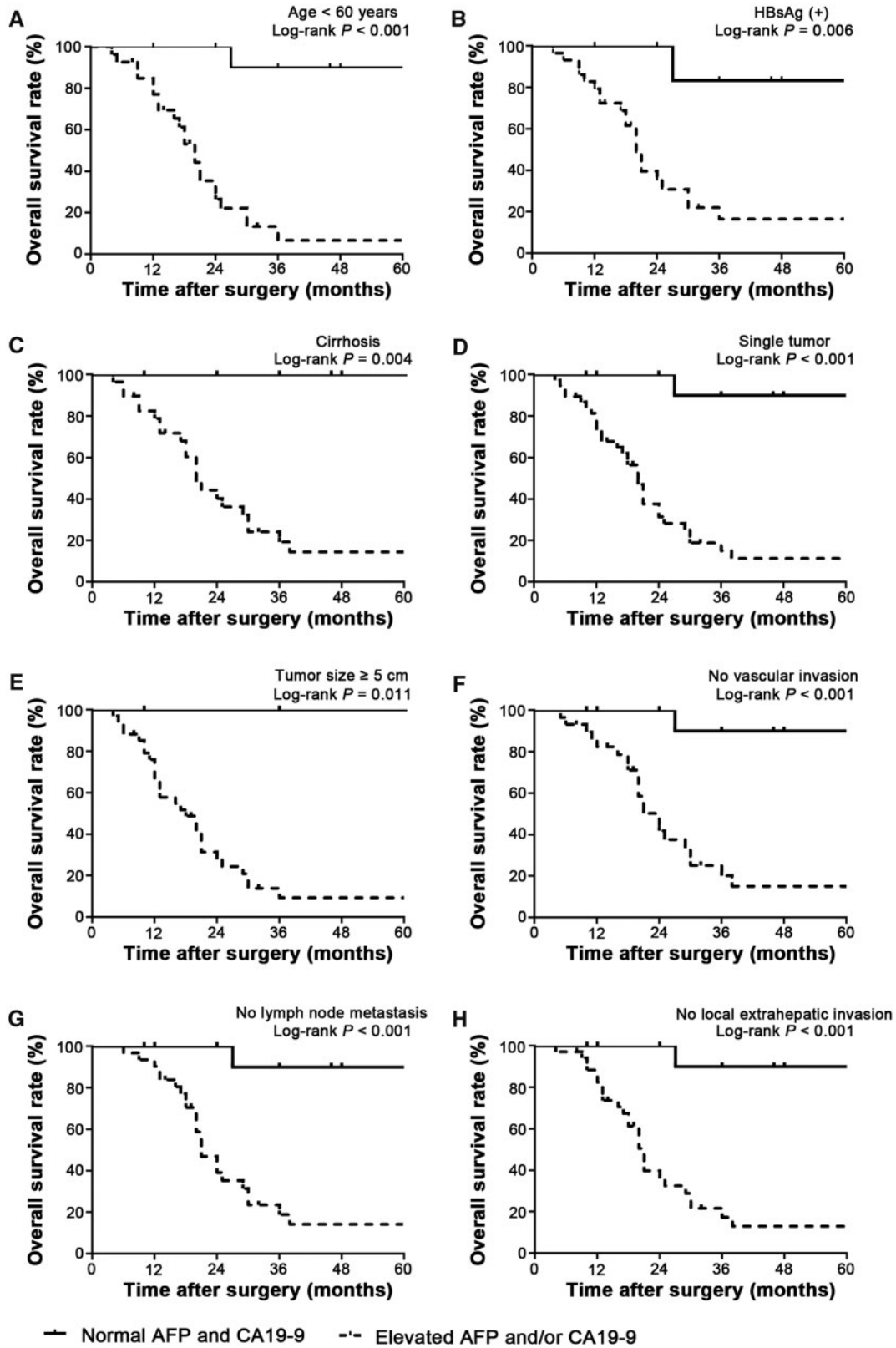


Figure 3. The predictive value of elevated AFP and/or CA19-9 for OS in different subgroups of cHCC-CC patients after curative resections. (A) Age <60 years; (B) positive HBsAg; (C) cirrhosis; (D) single tumor; (E) tumor size ≥ 5 cm; (F) without vascular invasion; (G) without lymph-node metastasis; (H) without local extra-hepatic invasion. cHCC-CC, combined hepatocellular carcinoma and cholangiocarcinoma; AFP, alpha-fetal protein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen.

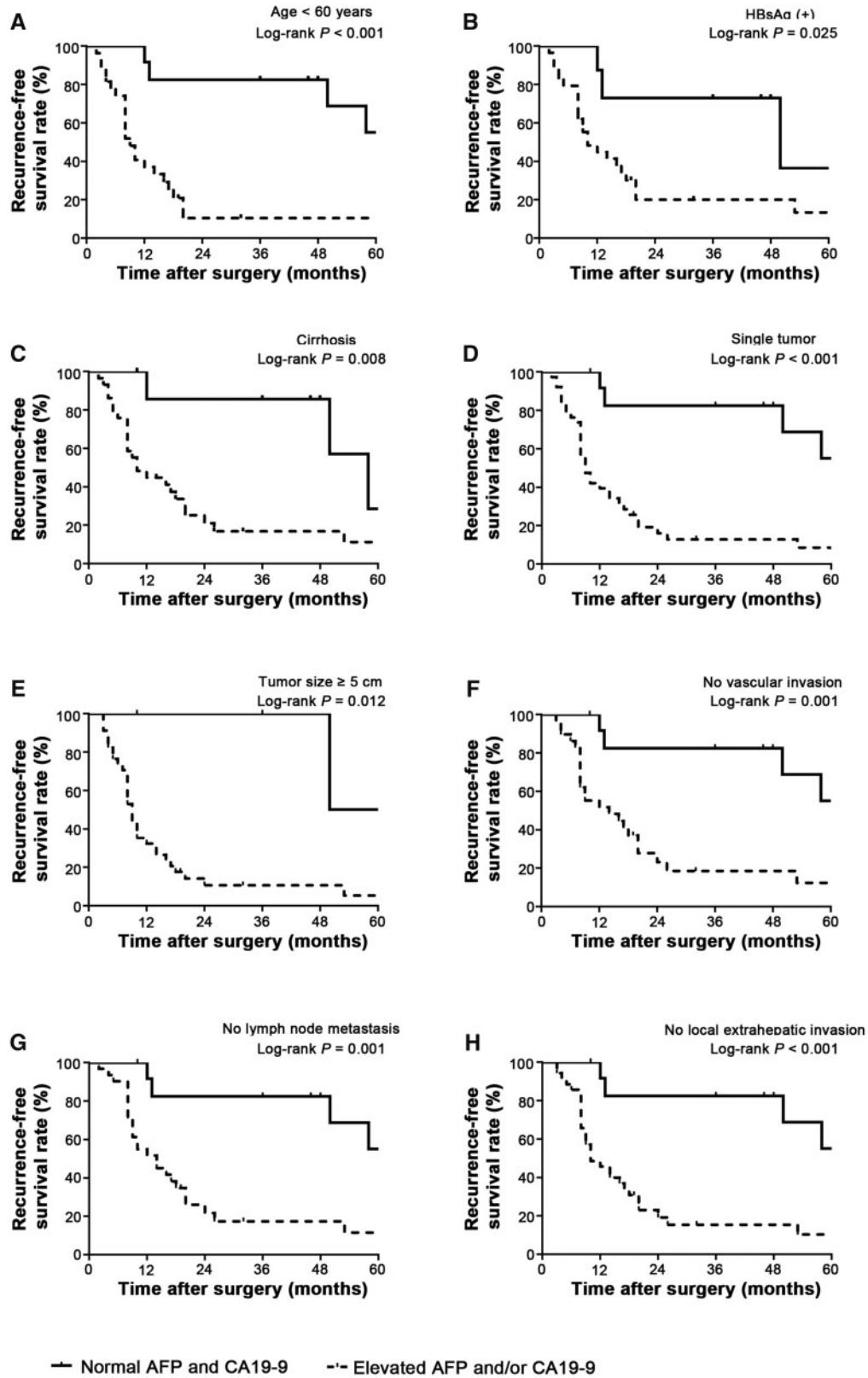


Figure 4. The predictive value of elevated AFP and/or CA19-9 for RFS in different subgroups of cHCC-CC patients after curative resections. (A) Age <60 years; (B) positive HBsAg; (C) cirrhosis; (D) single tumor; (E) tumor size ≥ 5 cm; (F) without vascular invasion; (G) without lymph-node metastasis; (H) without local extra-hepatic invasion. cHCC-CC, combined hepatocellular carcinoma and cholangiocarcinoma; AFP, alpha-fetal protein; CA19-9, carbohydrate antigen 19-9; HBsAg, hepatitis B surface antigen.

post-operative follow-up should be shortened, since its recurrence rate was higher than for those with normal AFP and CA19-9. Moreover, it could be used to guide the post-operative adjuvant therapies in cHCC-CC patients. Since the patients with elevated AFP and/or CA19-9 would have a higher likelihood of recurrence and shorter overall survival, post-operative adjuvant therapies, including chemotherapy, would be more strongly recommended in those patients.

The ratio of the males to females in patients with cHCC-CC in the present study was 2.93:1. It was comparable to the results of the previous studies [3]. The reasons for its predominance in males still remained unclear. In addition, the vast majority of patients admitted to our hospital belong to the Han ethnicity and all patients in the present study were of Han ethnicity. The ethnic features of patients in our hospital are similar to those in other centers around. Therefore, we believed that the results of this study can be generalized to other regions and more global populations.

There were some limitations for the present study. First, it is a retrospective study. It inevitably has some bias and confounding factors. In this study, we used multivariate analysis to control the interference of confounding factors as much as possible, making the results more convincing. Second, the number of patients enrolled is not big enough and all patients come from one single center. We plan to conduct a large prospective study to validate the results of this study by including more patients. Admittedly, the predictive values of combining AFP and CA19-9 still await validation in larger multicenter prospective studies.

Authors' contributions

X.Y.Y. developed the study concept and conducted statistical analyses. X.X.J., X.T.H., C.S.H., L.H.C., and L.J.L. contributed to developing the study concept, and analysis and interpretation of the data. X.X.J. and X.T.H. wrote the first draft of the manuscript. C.S.H., L.H.C., L.J.L., and X.Y.Y. critically revised the work. All authors read and approved the final manuscript.

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Conflicts of interest

None declared.

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