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*Original Article*

# Combined hepatocellular and cholangiocarcinoma: WHO classification-based analysis of long-term prognosis after surgery

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**Abstract:** Background and aims: As a rare hepatic malignancy, combined hepatocellular and cholangiocarcinoma (CHC) is poorly understood. Research into its clinicopathological characteristics and the factors that affect its prognosis may improve clinical outcomes in this condition. Methods: The clinicopathological data of 58 surgically treated CHC patients were retrospectively reviewed according to the 2010 WHO classification scheme. Univariate and multivariate analyses were performed for risk factors related to mortality and recurrence. Results: There were 7 patients with the classical subtype of CHC (12.1%) and 51 patients with the stem cell subtype (87.9%), 8 with typical subtype (TS) (13.7%), 22 with intermediate cell subtype (INT) (37.9%) and 21 with cholangiolocellular subtype (CLC) (36.2%). Multivariable analyses revealed that the disease-free survival (DFS) rates of patients with the classical subtype was lower than for patients with the stem cell subtype ($P = 0.032$). The overall survival (OS) and recurrence rates of patients with the TS, INT and CLC subtypes did not differ (all $P > 0.05$). Sex ($P = 0.003$), satellite nodules ($P = 0.003$) and lymph node metastasis ($P = 0.040$) were independent risk factors for overall survival of CHC. Preoperative serum carcinoembryonic antigen (CEA) levels were an independent risk factor for OS ($P = 0.017$) and DFS ($P = 0.020$) of CHC. Conclusions: The classical subtype of CHC exhibits earlier recurrence than the stem cell subtype after surgical treatment. Preoperative serum CEA levels were an independent predictor for CHC prognosis.

**Keywords:** Combined hepatocellular and cholangiocarcinoma, hepatectomy, tumor marker, prognosis

## Introduction

Combined hepatocellular and cholangiocarcinoma (CHC) is relatively rare, accounting for 1.0% to 6.3% of all primary liver carcinomas in Asia [1-7] and 2.4% to 14.3% in Western countries [8, 9]. In 1949, Allen and Lisa described this disease and divided it into 3 histological types (Type A, double tumor; Type B, combined type; Type C, mixed type) [8]. In 1985, Goodman further distinctly stratified this tumor into 3 groups (Type I, collision tumor; Type II, transitional tumor; Type III, fibrolamellar tumor) [9]. Subsequently, in the 7th American Joint Committee on Cancer (AJCC) TNM staging system, CHC was grouped into intrahepatic cholangiocarcinoma (ICC) [10]. However, CHC originates from cells with histological features of both hepatocellular carcinoma (HCC) and ICC and has distinct clinical features from either of these tumor types [5]. A retrospective analysis using Allen and Lisa's grouping method of 44 CHC patients who underwent hepatectomy (33 combined type, 11 mixed type) suggested that CHC had poor prognosis after liver resection regardless of the subtype [11]. Another study indicated that radical hepatectomy could provide a better prognosis for Allen type C CHC [12]. The controversy over the treatment and prognosis of CHC exists in previous studies is due to the small sample size of the available studies and disparate diagnostic standards [2, 3, 5, 7, 13, 14].

In 2010, the World Health Organization (WHO) Classification of Tumors of the Digestive Sys-

**Table 1.** Histopathological features of combined hepatocellular and cholangiocarcinoma according to the 2010 WHO classification scheme

| Subtypes | Stroma | Immunohistochemical staining | Histological features |
|---|---|---|---|
| Classical type | | | |
| HCC component | Scarce | HepPar-1 and/or CEA, AFP | Typical HCC, well, moderately, or poorly differentiated. |
| CC component | Prevalent | CK7 and CK19 | Typical biliary adenocarcinoma, well, moderately, or poorly differentiated. Mucin production may be present based on histochemistry. |
| Stem cell subtypes | | | |
| Typical subtype | Prevalent | CK7 and CK19, NCAM/CD56, Kit and/or EpCAM | Mature-looking hepatocytes in cancer nests, with peripheral clusters of small tumor cells that have hyperchromatic nuclei and a distinct nucleus and high nucleus: cytoplasm ratio. |
| Intermediate-cell subtype | Moderate-prevalent | HepPar-1 or AFP, CK19 or CEA | Tumor cells show histological features that are intermediate between hepatocytes and cholangiocytes. The cell show solid nests or strands and/or trabeculae of small, oval-shaped cells, with scant cytoplasm and hyperchromatic nuclei. |
| Cholangiolocellular subtype | Prevalent | CK19 and/or Kit, NCAM/CD56, EpCAM | Tumor show histological features with admixtures of small monotonous glands, reflecting so-called antler-like anastomosing patterns. The tumor cells are smaller in size than normal hepatocytes, with a high nucleus: cytoplasm ratio and hyperchromatic oval nuclei. |

Abbreviations: HCC, hepatocellular carcinoma; CC, cholangiocarcinoma; CEA, carcinoembryonic antigen; AFP, a-fetoprotein; CK7, keratin7; CK19, keratin19; NCAM/CD56, nuclear cell adhesion molecule; EpCAM, epithelial cell adhesion molecule.

tem provided a definite description of CHC, dividing it into classical subtype (CS) and stem cell subtype (SC) [15]. The SC subtype is further subdivided into the typical subtype (TS), intermediate cell subtype (INT) and cholangiolocellular subtype (CLC). The histopathological features of each subtype are shown in **Table 1** [15, 16].

In the present study, the clinicopathological and prognostic features of 58 patients with CHC who underwent hepatectomy were analyzed according to the latest 2010 WHO classification.

## Materials and methods

### *Patients and samples*

Fifty-eight CHC patients were admitted in the Department of Hepatobiliary Surgery of the First Affiliated Hospital of Sun Yat-sen University from April 2003 to August 2015 and underwent hepatic resection. Patients with CHC who received preoperative chemotherapy, radiofrequency ablation, percutaneous ethanol injection, or other anti-tumor treatment were excluded from this study. The following clinicopathological information was reviewed and analyzed: age, sex, preoperative symptom, hepatitis B virus status, surgical strategy, tumor size, tumor satellite nodules, cirrhosis, extent of resection, lymph node metastasis status, preoperative hematology parameters (monocyte, platelet, r-GT, ALP, LDH, AFP, CEA, CA125, and CA19-9) and follow-up information.

Fresh CHC tissues were collected within 30 min after hepatectomy. The samples were fixed with 10% formalin and embedded in paraffin. The diagnosis of CHC met the criteria of the World Health Organization (WHO) classification of Tumors of the Digestive System, 2010 [15]. This study was approved by the Ethics Committee of our hospital, and written informed consent was obtained from all the patients.

### *Pathological diagnosis*

To differentiate between the subtypes, formalin-embedded blocks were re-sectioned and stained via both H&E and immunohistochemistry for hepatocellular, biliary and hepatic stem/progenitor cells (HPC) markers. In the classical subtype, both typical HCC and cholangiocarcinoma were confirmed in the same tumor [15]. Mucin presence has been found to be of great significance in the diagnosis of the classical subtype [15]. In the stem cell subtypes, TS is characterized by mature-looking hepatocytes in cancer nests, with peripheral clusters of small tumor cells that have hyperchromatic nuclei and a high nucleus: cytoplasm ratio [15]. INT cells show histological features intermediate between those of hepatocytes and cholangiocytes, being small and oval-shaped,

with scant cytoplasm and hyperchromatic nuclei [15, 17]. CLC is characterized by a tubular, cordlike, antler-like anastomosing pattern, with abundant stroma [15, 18-20]. To confirm CHC, positive hepatocyte parafin1 (Hep-Par1) expression was used for the HCC component, and positive cytokeratin 19 (CK19) expression was used for the cholangiocarcinoma component [15]. Moreover, CD56 was used as a stem/progenitor cell marker.

*Immunohistochemistry*

Immunohistochemical studies were performed using a three-step immunoperoxidase technique with the following primary antibodies to identify biliary or hepatocellular differentiation of each tumor subtype: for CS and INT, Hep-Par1 (anti-hepatocyte marker) and CK19; for TS and CLC, CD56 and CK19. Four-millimeter-thick sections were prepared from the paraffin-embedded blocks. The sections were deparaffinized in xylene and rehydrated through graded alcohol washes, followed by antigen retrieval by heating in sodium citrate buffer (10 mm, pH 6.0) for 15 min. Endogenous peroxidase activity was blocked using 3% $H_2O_2$ for 15 min. The slides were then incubated in PBS (pH 7.4) containing normal goat serum (dilution 1:10) and were subsequently incubated with the prediluted primary monoclonal antibody [(CK19, b170; Novocastra, UK, dilution 1:100); (Hep-Par1, OC-H1E5; Novocastra, UK, dilution 1:50); (CD56, 1B6; Novocastra, UK, dilution 1:50)] at 4°C overnight. Subsequently, the sections were incubated with biotin-labeled anti-IgG and incubated with avidin-biotin peroxidase complex. The reaction products were visualized via diaminobenzidine staining and Meyer's hematoxylin counterstaining. A positive result was defined as staining of > 10% of the tumor cells.

*Follow-up*

Postoperative follow-up of CHC patients was performed every month for the first six months, every 3 months for the following 2 years, and then twice a year thereafter. At each follow-up appointment in the outpatient clinic, the patients received a physical examination; tests for the levels of serum AFP, CA19-9, and CEA; abdominal ultrasound or computed tomography (CT); and a chest X-ray. The endpoint of follow-up was December 2015. Recurrence was defined as a new lesion identified by an imaging examination, such as contrast-enhanced CT, magnetic resonance imaging (MRI) or positron emission tomography-CT (PET-CT). Recurrence should not be identified by elevated serum AFP, CA19-9 or CEA levels alone. Patients with confirmed CHC recurrence received a repeat hepatectomy, transcatheter arterial chemoembolization (TACE), radiofrequency ablation (RFA), or supportive care only.

*Statistical analysis*

Continuous normal distribution variables were compared using analysis of variance (ANOVA) or an independent samples T-test. Non-normally distributed numerical variables were tested using the Kruskal-Wallis test or the Mann-Whitney U test. The Pearson chi-square test was used to test for differences between categorical variables. Survival curves were calculated using the Kaplan-Meier method and compared using a log-rank test. The Cox proportional hazards model (Backward stepwise) was used to determine the independent prognostic factors, and the results were expressed as hazard ratios (HRs) with 95% confidence intervals (95% CIs). Differences were considered significant when $P < 0.05$ using two-tailed tests. The statistical analyses were performed using SPSS version 20.0 software (IBM SPSS Inc., Chicago, IL, USA).

## Results

*Demographic and clinical characteristics*

The demographic and clinical characteristics of the patients are summarized in **Table 2**. One patient was excluded due to death from hepatic failure within 30 days of the operation. Fifty-eight patients were included in the final study, with 45 males (77.6%) and 13 females (22.4%). The mean age of the cohort was 53.5 ± 11.3 years (range: 27-78 years). There were 38 patients (65.5%) who were symptomatic at the time of diagnosis, including 35 patients with upper abdominal pain and 3 patients with jaundice. Forty-one patients (70.7%) had viral hepatitis [40 patients (69.0%) with hepatitis B virus (HBV), 1 patient (1.7%) with hepatitis C virus]. Before the operation, 18 patients (31.0%) had elevated serum AFP (≥ 200 ng/ml), 12 patients (20.7%) had elevated serum CEA (> 5 ng/ml), and 27 patients (46.6%) had elevated serum CA19-9 (> 35 IU/ml). In addition, 6

**Table 2.** Demographic and baseline characteristics of 58 patients diagnosed with combined hepatocellular and cholangiocarcinoma (CHC) according to 2010 WHO classification

| Factors* | Total CHC (n = 58) | CS (n = 7) | Stem cell subtypes (SC) | | | *P*† | |
|---|---|---|---|---|---|---|---|
| | | | TS (n = 8) | INT (n = 22) | CLC (n = 21) | a | b |
| Age (years) | 53.5 ± 11.3 | 55.1 ± 14.4 | 48.9 ± 7.8 | 55.2 ± 9.3 | 52.9 ± 13.2 | 0.370 | 0.684 |
| Sex (male, %) | 45 (77.6) | 7 (100) | 7 (87.5) | 14 (63.6) | 17 (81.0) | 0.281 | 0.331 |
| Symptom (yes, %) | 38 (65.5) | 6 (85.7) | 5 (62.5) | 14 (63.6) | 13 (62.0) | 0.993 | 0.403 |
| HBsAg (yes, %) | 40 (69.0) | 5 (71.4) | 6 (75.0) | 13 (59.1) | 16 (76.2) | 0.441 | 0.187 |
| Tumor size (cm) | 6.0 (4.8-10.0) | 5.6 (5.0-12.0) | 7.5 (6.0-10.7) | 5.5 (4.0-10.5) | 7.0 (4.0-8.5) | 0.482 | 0.527 |
| Satellite nodules (yes, %)‡ | 9 (15.5) | 2 (28.6) | 3 (37.5) | 2 (9.1) | 2 (9.5) | 0.104 | 0.296 |
| Capsule (no, %) | 33 (56.9) | 3 (42.9) | 6 (75.0) | 11 (50.0) | 13 (61.9) | 0.437 | 0.450 |
| Cirrhosis (yes, %) | 35 (60.3) | 3 (42.9) | 6 (75.0) | 14 (63.7) | 12 (57.1) | 0.669 | 0.418 |
| Resection margin | | | | | | | |
| R0 (%) | 47 (81.0) | 5 (71.4) | 5 (62.5) | 19 (86.4) | 18 (85.7) | 0.276 | 0.607 |
| R1 (%) | 11 (19.0) | 2 (28.6) | 3 (37.5) | 3 (13.6) | 3 (14.3) | | |
| LNM (yes, %) | 12 (20.7) | 1 (14.3) | 3 (37.5) | 5 (22.7) | 3 (14.3) | 0.391 | 0.656 |
| Monocytes ($10^9$/L) | 0.6 (0.4-0.9) | 0.6 (0.4-1.0) | 0.7 (0.4-0.9) | 0.5 (0.4-0.7) | 0.5 (0.4-0.9) | 0.473 | 0.761 |
| Platelet ($10^9$/L) | 188.5 (148.3-220.0) | 206.0 (143.0-215.0) | 191.0 (135.8-287.5) | 188.5 (158.3-255.3) | 187.0 (129.0-213.5) | 0.640 | 0.870 |
| r-GT (IU/L) | 98.0 (41.8-202.0) | 129.0 (121.0-214.0) | 102.5 (61.0-289.3) | 80.5 (32.8-134.3) | 64.0 (40.0-290.0) | 0.553 | 0.148 |
| ALP (IU/L) | 104.0 (79.0-154.8) | 95.0 (79.0-158.0) | 114.5 (89.8-211.8) | 98.0 (76.3-147.3) | 106.0 (79.0-140.5) | 0.579 | 0.744 |
| LDH (IU/L) | 189.0 (164.3-228.0) | 181.0 (152.0-227.0) | 203.5 (163.5-377.8) | 185.5 (162.8-225.1) | 195.0 (169.0-236.0) | 0.829 | 0.797 |
| AFP (ng/mL) | 24.4 (4.8-372.0) | 42.8 (20-123.7) | 531.5 (8.2-6242.6) | 20.3 (3.3-191.2) | 31.1 (4.1-320.8) | 0.192 | 0.623 |
| CEA (ng/ml) | 2.3 (1.5-3.7) | 3.0 (1.4-6.6) | 3.59 (2.0-6.3) | 2.1 (1.2-2.5) | 3.2 (1.5-4.7) | 0.057 | 0.744 |
| CA125 (IU/ml) | 13.8 (9.8-40.8) | 14.1 (9.8-41.5) | 32.0 (15.3-66.1) | 12.9 (9.2-30.6) | 13.4 (6.9-72.2) | 0.181 | 0.852 |
| CA19-9 (IU/ml) | 23.8 (12.3-301.5) | 203.0 (13.0-1095.6) | 62.2 (16.8-367.7) | 16.8 (10.0-204.2) | 31.3 (10.7-317.9) | 0.590 | 0.290 |

Abbreviations: CS, Classical subtype; TS: Typical subtype; INT: Intermediate cell subtype; CLC: Cholangiolocellular subtype; HBsAg, Hepatitis B surface antigen; LNM, Lymph node metastasis; r-GT, Gamma-glutamyl transpeptidase; ALP, Alkaline phosphatase; LDH, Lactate dehydrogenase; AFP, Alpha fetal protein; CEA, carcinoembryonic antigen; CA125, Carbohydrate antigen 125; CA19-9, Carbohydrate antigen 19-9. *Continuous variables are presented as mean ± standard deviation or median (interquartile); number (percent) was used for categorical variables. †*P*-value for (a): TS *vs.* INT *vs.* CLC, tested with analysis of variance (ANOVA) or independent samples Kruskal-Wallis test or Pearson chi-square test; (b): CS *vs.* SC, tested with independent samples T test or Mann-Whitney U test or Pearson chi-square test. ‡Satellite nodules diagnosed by preoperative image finding.

**Table 3.** Treatment outcomes of patients with combined hepatocellular and cholangiocarcinoma

| Items | Results (total patients = 58) |
|---|---|
| Surgery strategy | |
| Right hepatectomy | 7 (12.1%) |
| Left hepatectomy | 10 (17.2%) |
| Trisegmentectomy | 6 (10.3%) |
| Bisegmentectomy | 16 (27.6%) |
| Segmentectomy | 13 (22.4%) |
| Subsegmentectomy | 6 (10.3%) |
| No evidence of tumor recurrence | 9 (15.5%) |
| Tumor recurrence | 49 (84.5%) |
| Intrahepatic recurrence | 32 (65.3%) |
| Extra-hepatic recurrence | 17 (34.7%) |
| Management after tumor recurrence (n = 49) | |
| TACE | 10 (20.4%) |
| Repeated hepatectomy | 2 (4.1%) |
| RFA | 4 (8.2%) |
| Supportive care only | 34 (69.4%) |
| Disease free survival, months | 5.0 mo (3.98-6.02) |
| Overall survival, months | 10.0 mo (7.64-12.36) |

Results are expressed as n (%) or median value (95% confidence interval). Abbreviations: TACE, trans-arterial chemoembolization; RFA, radiofrequency ablation.

patients had simultaneously elevated AFP and CA19-9 levels. Cirrhosis was present in 35 patients (60.3%). A preoperative biopsy was performed in 4 patients: the diagnosis could not be determined in 2 cases, and the other 2 patients were diagnosed with ICC. There were no significant differences in the demographic and clinical characteristics between patients with CS and SC; this was also the case for patients with TS, INT, and CLC (all $P > 0.05$) (**Table 2**).

*Treatments and histopathological findings*

The surgical strategies were designed based on a multidisciplinary team meeting. The operative procedures included right hepatectomy (n = 7); left hepatectomy (n = 10); trisegmentectomy (n = 6); bisegmentectomy (n = 16); segmentectomy (n = 13); and subsegmentectomy (n = 6). The combined bile duct, pancreas or stomach operation was based on surgical need. The surgical characteristics of each subtype and the histopathological findings are summarized in **Tables 2** and **3**, respectively. There were 9 patients (15.5%) with satellite nodules. Tumor dissemination to regional lymph nodes metastasis was detected in 12 patients (20.7%). The median maximum tumor size of all 58 patients was 6.0 cm. The pathological diagnosis and immunohistochemical staining were confirmed by two experienced pathologists. Hematoxylin and eosin and immunohistochemistry images of representative cases of the various CHC subtypes are presented in **Figure 1**. In total, 7 (12.1%), 8 (13.8%), 22 (37.9%), and 21 (36.2%) cases were diagnosed as CS, TS, INT, and CLC, respectively (**Table 2**).

*Disease-free survival (DFS) of CHC patients*

The results indicated that 49 patients (84.5%) experienced tumor recurrence during the observation period. The Kaplan-Meier analysis showed that the median DFS for the total group was 5.0 months (**Figure 2A**), while this figure was 3.0 months for patients with CS, 4.0 months for patients with TS, 6.0 months for patients with INT and 5.0 months for patients with CLC. In addition, the median DFS for patients with the stem cell subtype was 5.0 months.

The six-, 12-, and 36-month DFS rates for the entire cohort were 36.3%, 13.4%, and 13.4%, respectively (**Figure 2A**). There were no differences in DFS for the different stem cell subtypes ($P = 0.800$, **Figure 4A**). Multivariate analysis indicated that the DFS rates for the classical subtype were lower than for the stem cell subtype (HR 0.391, 95% CI 0.166-0.922; $P = 0.032$) (**Figure 3A**; **Table 4**). Moreover, preoperative serum CEA > 5 IU/mL was an independent risk factor for early disease recurrence in CHC (HR 2.373, 95% CI 1.146-4.915; $P = 0.020$) (**Table 4**).

*Overall survival (OS) of CHC patients*

Forty-nine patients (84.5%) died of tumor recurrence and disease progression during the follow-up period. The median OS of the total group was 10.0 months (**Figure 2B**), while this figure

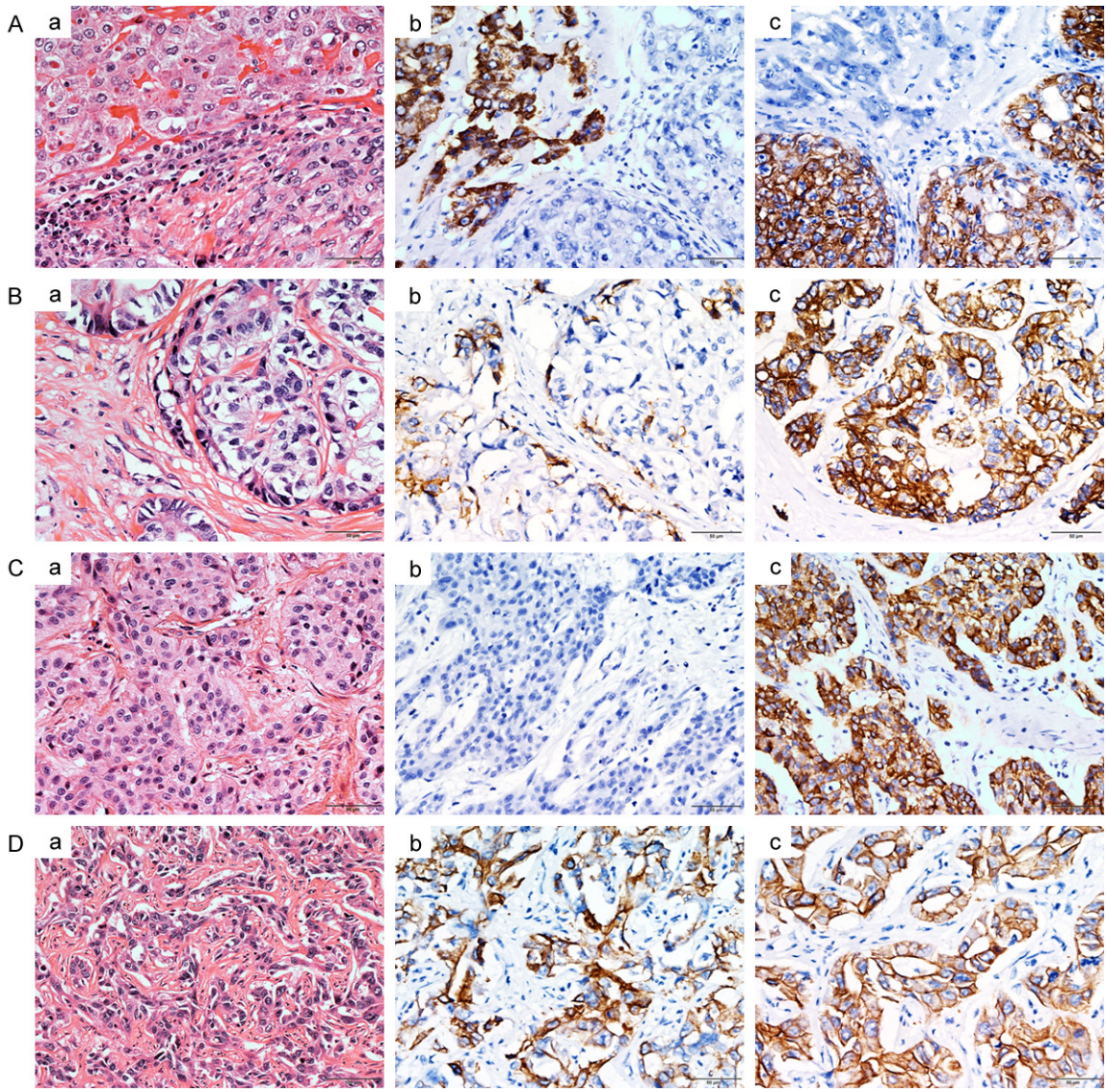



**Figure 1.** Representative microscopic images of various subtypes of combined hepatocellular and cholangiocarcinoma (CHC). A. Classical subtype. (a) The HCC (upper left) and ICC (lower right) components were contiguous with the transitional region at the boundary; (b) Immunohistochemical staining for HerPar-1 was positive only in the HCC component; (c) CK19 was positive only in the ICC component. B. Stem cell features, typical subtype. (a) The tumor shows a nested growth pattern, with peripheral clusters of small cells exhibiting a high nucleus: cytoplasm ratio in the sclerotic stroma; (b) CD56 exhibits a circumferential staining pattern; (c) CK19-positive tumor cells. C. Stem cell features, intermediate cell subtype. (a) The tumor was composed of small, oval-shaped cells with a trabecular, solid nested pattern. HerPar-1 expression was negative (b) while CK19 expression was positive (c). D. Stem cell features, cholangiolocellular subtype. (a) The tumor cells show a tubular structure with marked fibrous stroma. Diffuse expression of CD56 (b) and CK19 (c) were observed in the membranes of the tumor cells. Magnification: D-a × 200, all other × 400.

was 6.0 months for patients with CS, 7.0 months for patients with TS, 12.0 months for patients with INT and 11.0 months for patients with CLC. The six-month, 1-, 3- and 5-year OS rates for the entire CHC group were 68.7%, 37.2%, 13.0%, and 10.8%, respectively (**Figure 2B**). There were no differences in the OS rates for patients with the classical subtype and stem cell subtype ($P = 0.302$) (**Figure 3B**; **Table 4**). The overall survival rates for patients with TS, INT and CLC did not differ significantly ($P = 0.608$) (**Figure 4B**). Multivariate analysis showed that male sex (HR 3.878, 95% CI 1.594-9.434; $P = 0.003$), the presence of satellite nodules (HR 4.042, 95% CI 1.619-10.088; $P = 0.003$), lymph node metastasis (HR 2.042,

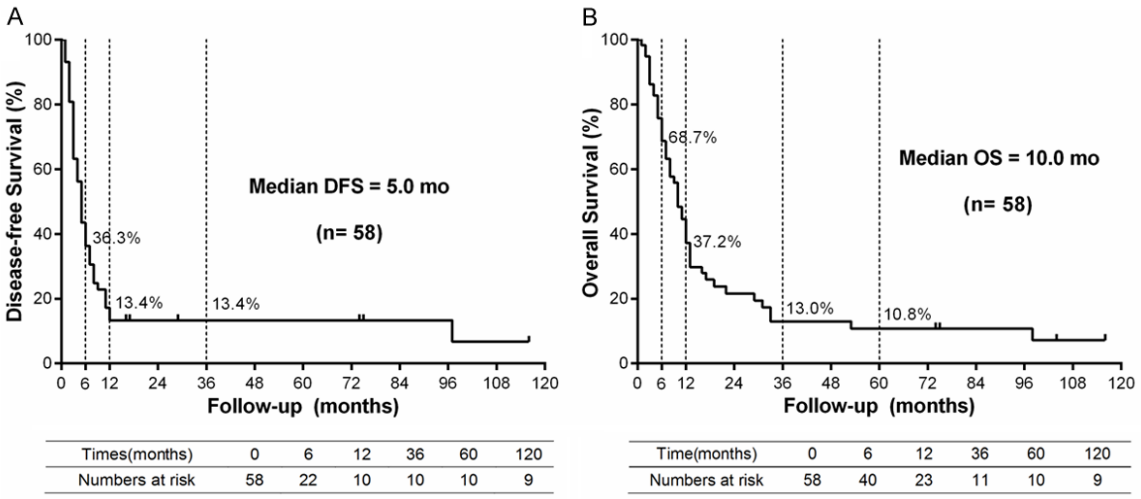



**Figure 2.** Kaplan-Meier survival curves of patients with combined hepatocellular and cholangiocarcinoma (CHC). A. The six-, 12-, and 36-month disease-free survival (DFS) rates of patients with CHC were 36.3%, 13.4%, and 13.4%, respectively. B. The six-month, 1-, 3- and 5-year overall survival (OS) rates of patients with CHC were 68.7%, 37.2%, 13.0%, and 10.8%, respectively.

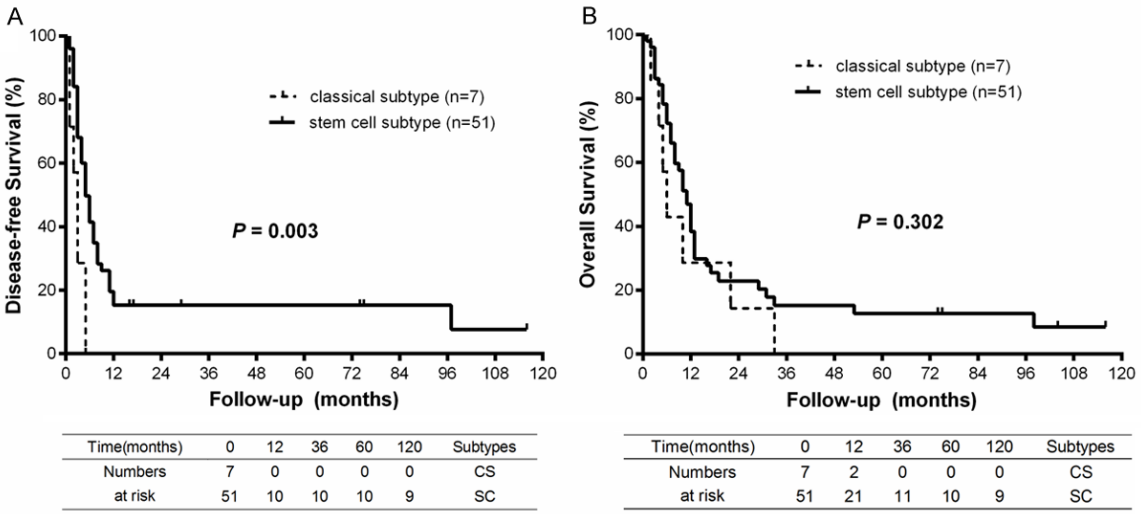


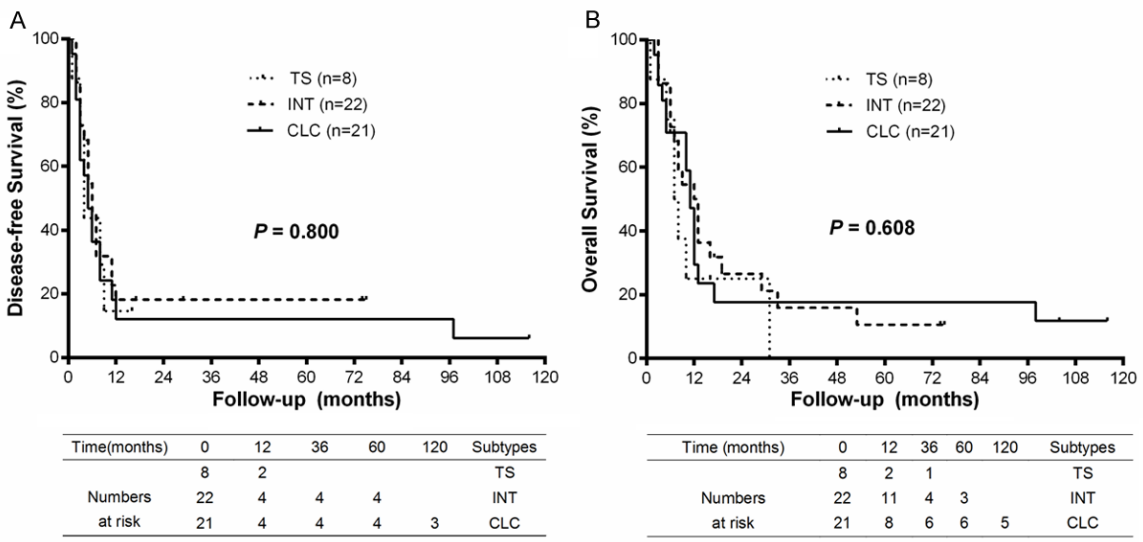

**Figure 3.** Kaplan-Meier estimate of disease-free survival and overall survival rates among patients with the classical subtype and the stem cell subtype of CHC.

95% CI 1.032-4.042; *P* = 0.040) and preoperative serum CEA > 5 IU/mL (HR 2.344, 95% CI 1.162-4.728; *P* = 0.017) were independent risk factors for mortality from CHC (**Table 4**).

Among the 49 patients with recurrence (including 32 patients with intrahepatic recurrence), 15 patients (30.6%) received therapies, including TACE (10 patients), repeat hepatectomy (2 patients), and RFA (4 patients); 1 patient underwent two palliative treatments. Other patients received supportive care only (34 patients) (**Table 3**). The median OS of 10 patients who received TACE for recurrent CHC was 17.0 months. The median OS of patients who received RFA for recurrent CHC was 13.0 months. No serious complications occurred during these treatments.

## Discussion

In this study, we discussed the clinicopathological features and prognosis of each CHC subtype according to the 2010 WHO classification




| Time(months) | 0 | 12 | 36 | 60 | 120 | Subtypes |
|---|---|---|---|---|---|---|
| | 8 | 2 | | | | TS |
| Numbers | 22 | 4 | 4 | 4 | | INT |
| at risk | 21 | 4 | 4 | 4 | 3 | CLC |

| Time (months) | 0 | 12 | 36 | 60 | 120 | Subtypes |
|---|---|---|---|---|---|---|
| | 8 | 2 | 1 | | | TS |
| Numbers | 22 | 11 | 4 | 3 | | INT |
| at risk | 21 | 8 | 6 | 6 | 5 | CLC |

**Figure 4.** Kaplan-Meier estimate of disease-free survival and overall survival rates of patients with the typical subtype (TS), intermediate-cell subtype (INT) and cholangiolocellular subtype (CLC) of CHC.

scheme [15]. The results indicated that the classical subtype of CHC is characterized by an earlier recurrence than the stem cell subtype after surgical treatment. Preoperative elevated serum CEA was an independent risk factor for the OS and DFS rates of CHC. Male sex, the presence of satellite nodules, and lymph node metastasis were independent indicators for OS only.

The 1, 3-, 5-year OS rates of the entire cohort of patients were 37.2%, 13.0% and 10.8%, respectively, and the median OS was 10.0 months. The OS was not correlated with the degree of radical surgery, tumor capsulation or cirrhosis conditions. It has been reported that the prognosis for CHC does not vary by the pathological type or the predominant tumor components [11]. The OS rates for all the CHC subtypes were not significantly different, which was consistent with Akiba's study [16]. Several studies have reported that CHC originates from hepatic stem/progenitor cells (HPCs) [21, 22]. Background HPCs were strongly correlated with multifocal occurrence and tumor recurrence after resection of CHC, and HPCs may be potential therapeutic targets for the prevention and control of CHC recurrence [23]. We found that the classical CHC subtype recurred earlier than the stem cell subtypes. In addition, the HPC marker CD56 was expressed at various levels in the different subtypes of CHC. This phenomenon may indicate that HPCs are more common in the classical CHC subtype than in the stem cell subtypes. Nevertheless, the relationship between the presence of HPCs and the prognosis of each of the CHC subtypes according to the 2010 WHO classification scheme requires further clarification.

Tumor heterogeneity and the proportion of HCC and ICC components are highly variable in CHC. The imaging characteristics of CHC have almost no specificity. Previous studies have suggested that the diagnostic value of preoperative contrast enhancement CT, MRI and PET-CT for CHC are limited [24-27]. These difficulties may be due to the low incidence of CHC and the lack of typical imaging characteristics. Recently, a study of Li et al. showed that contrast-enhanced ultrasound and CT had similar enhancement patterns for CHC tumors and that elevated serum AFP and/or CA19-9 levels, when combined with inconsistent imaging findings on the above modalities, may improve diagnostic accuracy [28]. Portolani et al. showed that diagnostic accordance rate of preoperative percutaneous liver biopsy was only 11.1% (1/9), and the remaining patients were misdiagnosed with metastatic carcinoma, HCC or ICC [29]. A preoperative biopsy was performed for 4 patients in the present study. The diagnosis of two patients could not be determined, and the other two patients were diagnosed with ICC. All the CHC patients in this study were diagnosed via postoperative routine pathology. Therefore, the

**Table 4.** Survival analyses of patients with combined hepatocellular and cholangiocarcinoma after surgical treatment (n = 58)

| Category | Overall survival (OS) | | | Disease-free survival (DFS) | | |
|---|---|---|---|---|---|---|
| | Univariate | Multivariate | | Univariate | Multivariate | |
| | *P* | HR (95% CI) | *P* | *P* | HR (95% CI) | *P* |
| Age (> 50 vs. ≤ 50 years) | 0.381 | | | 0.351 | | |
| Sex (male vs. female) | 0.004 | 3.878 (1.594-9.434) | 0.003 | 0.077 | | |
| Tumor size (> 5 vs. ≤ 5 cm) | 0.179 | | | 0.104 | | |
| Satellite nodules (yes vs. no) | 0.030 | 4.042 (1.619-10.088) | 0.003 | 0.709 | | |
| Cirrhosis (yes vs. no) | 0.581 | | | 0.787 | | |
| Capsulation (yes vs. no) | 0.535 | | | 0.942 | | |
| Extent of resection (R1 vs. R0) | 0.660 | | | 0.958 | | |
| Lymph node metastasis (yes vs. no) | 0.039 | 2.042 (1.032-4.042) | 0.040 | 0.138 | | |
| HBsAg (Positive vs. Negative) | 0.741 | | | 0.661 | | |
| AFP (> 200 vs. ≤ 200 ng/mL) | 0.925 | | | 0.967 | | |
| CEA (> 5 vs. ≤ 5 IU/mL) | 0.046 | 2.344 (1.162-4.728) | 0.017 | 0.002 | 2.373 (1.146-4.915) | 0.020 |
| CA125 (> 35 vs. ≤ 35 IU/mL) | 0.048 | | | 0.383 | | |
| CA19-9 (> 35 vs. ≤ 35 IU/mL) | 0.013 | | | 0.014 | | |
| Subtypes (CS vs. SC) | 0.302 | | | 0.003 | 0.391 (0.166-0.922) | 0.032 |

Abbreviations: HR, hazard ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen; AFP, alpha fetal protein; CEA, carcinoembryonic antigen; CA125, carbohydrate antigen 125; CA19-9, carbohydrate antigen 19-9; CS, classical subtype; SC, stem cell subtype.

significance of preoperative liver biopsy for CHC tends to be limited. Due to the possibilities of bleeding and needle tract seeding, in addition to the low accordance rate between the biopsy results and the pathological diagnosis, we do not recommend routine preoperative needle biopsy in patients with resectable CHC.

Significant differences have been observed in the clinicopathologic characteristics of CHC in the literature. The risk factors of HBV infection, male sex, microtubule tumor thrombus and elevated AFP levels are similar to the risk factors for HCC [3, 12, 30], while incomplete capsules and earlier lymph node metastasis are similar to those for ICC [12]. The benefit of intra-operative lymph node dissection for CHC prognosis is controversial [30, 31]. In our study, the survival analysis showed regional lymph node metastasis was related to the overall survival of CHC patients. We speculate that lymph node metastasis may not be a contraindication for surgical treatment, which is consistent with the viewpoint of Portolani et al. [29]. In addition, the therapeutic effect of liver transplantation for CHC was poor [32, 33]. It was also reported that elevated serum CEA levels were predictive of poor prognoses for HCC [34] and cholangiocarcinoma [35]. In the present study, the results showed that preoperative elevated serum CEA levels were an independent predictor for OS and DFS in CHC. Serum CEA levels may be a good tumor marker for stratifying patients with CHC to receive individual therapy.

Few reports have evaluated the therapeutic outcomes of nonsurgical treatment for CHC. TACE was recommended for palliative therapy to prolong the survival of unresectable HCC patients [36, 37]. TACE was able to improve survival to a greater degree than supportive treatment for unresectable ICC (median OS 12.2 months vs. 3.3 months, $P < 0.001$) [38]. However, the treatment effect of TACE for CHC patients remains unclear. In our study, because of poor CHC prognosis, 10 patients with recurrent CHC received TACE after the initial hepatectomy without severe complications. Previous studies have reported that RFA can improve the survival rates of patients with unresectable ICC [39] and HCC [40]. In this study, 4 patients with recurrent CHC received RFA treatment. Due to small sample size, there was clear selection bias in terms of which patient was offered TACE or RFA; for this reason, we were not able to study the effects of TACE or RFA for recurrent CHC. Large randomized controlled clinical trials should be carried out to confirm the efficacy of TACE and RFA for CHC. There is currently no consensus for the treatment of advanced or recurring CHC. As mentioned above, CHC tumors have a mixed HCC and ICC nature. Given

that HCC patients may benefit from the molecular target drug sorafenib [41] and ICC is relatively sensitive to chemotherapy [42], the combined use of these two methods might be considered in selected CHC patients.

There were some limitations in this study. First, this was a retrospective study, and the sample size was relatively small, which may have influenced the power of the statistical analysis. Second, the efficacy of adjuvant therapies, such as TACE, RFA or both, could not be verified due to the nonrandomized controlled nature of the study. Third, although we found that CHC cases with more HPC marker expression (classical subtype) tended to have poorer prognoses, we were not able to conclusively determine whether a precise therapy exists for targeting HPC and thereby preventing CHC recurrence. This question should be further studied.

In conclusion, patients with CHC showed a poor prognosis and rapid disease progress. In addition, patients with the classical subtype experienced an earlier recurrence after surgical treatment than did those patients with the stem cell subtypes of CHC. Preoperative serum CEA levels were an independent risk factor for both OS and DFS in CHC. Research into HPCs and the tumor characteristics of CHC may contribute to a more accurate understanding of the prognosis of this problematic malignant tumor and the effectiveness of interventions. Furthermore, randomized clinical trials of the efficacy of adjuvant therapies to prolong survival and delay relapse should be carried out.

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## Disclosure of conflict of interest

None.

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