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Citation	Journal of gastroenterology and hepatology, 34(6), 1074-1080 https://doi.org/10.1111/jgh.14547
Issue Date	2019-06
Doc URL	http://hdl.handle.net/2115/81696
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Title: Clinical and pathological features of combined hepatocellular-cholangiocarcinoma compared with other liver cancers

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Disclosure statement: The authors have no conflicts of interest to disclose, including financial disclosures, and no grants from any organizations.

Acknowledgment: We thank Susan Furness, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Title:

Clinical and pathological features of combined hepatocellular-cholangiocarcinoma compared with other liver cancers

Abstract:

Background and Aim: Combined hepatocellular-cholangiocarcinoma (CHC) is a primary liver cancer containing both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC) elements. Its reported clinicopathological features and prognoses have varied because of its low prevalence. This study aimed to clarify these aspects of CHC.

Methods: We enrolled 28 patients with CHC, 1050 with HCC, and 100 with ICC and compared the clinicopathological characteristics and prognosis of CHC with HCC and ICC. We also analyzed prognostic factors, recurrence patterns, and management in CHC patients.

Results: The incidences of hepatitis B virus (HBV), and high alpha-fetoprotein (AFP) and protein induced by vitamin K absence or antagonists-II (PIVKAII) levels were significantly higher among CHC compared with ICC patients. Multiple tumors were more frequent in CHC compared with the other groups, while vascular invasion and lymph node metastasis were more frequent in the CHC than the HCC group. The 5-year overall (OS) and disease-free survival (DFS) rates for CHC were 25.1% and 22.6%, respectively. OS was significantly lower than for HCC ($P<0.001$) but not ICC ($P=0.152$), while DFS was significantly lower than for HCC and ICC ($P=0.008$ and $P=0.005$, respectively). Multivariate analysis identified carcinoembryonic antigen (CEA) levels and tumor size as independent predictors in patients with CHC.

Conclusions: The clinical features of CHC, including sex, HBV infection, AFP and PIVKAI levels, were similar to HCC, while its prognosis and pathological features, including vascular invasion and lymph node metastasis, were similar to ICC. CEA levels and tumor size were independent prognostic factors in patients with CHC.

Key words: combined hepatocellular-cholangiocarcinoma, hepatocellular carcinoma, intrahepatic cholangiocarcinoma, resection, prognosis

Abbreviations

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; AFP, alpha-fetoprotein; PIVKAI, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9; OS, overall survival; DFS, disease-free survival; HBV, hepatitis B virus; HR, hazard ratio; CI, confidence interval; HPC, hepatic progenitor cell.

Introduction

Combined hepatocellular-cholangiocarcinoma (CHC) is a rare primary liver cancer containing elements of both hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC), and accounting for 1.0%–4.7% of all primary liver cancers.¹⁻⁶ The definition of CHC has changed over time. In 1949, Allen and Lisa classified CHC into double cancer (type A), combined type (type B), and mixed type (type C).⁷ In 1985, Goodman et al. modified the classification by dividing CHC into collision (type I), transitional (type II), and fibrolamellar tumors (type III).⁸ In 2010, the fourth edition of the World Health Organization (WHO) classification defined two types of CHC: classical type and subtypes with stem-cell features,⁹ the latter of which was subdivided into typical, intermediate-cell, and cholangiolocellular subtypes. However, the reported clinical and pathological features and prognoses of CHC have varied because of its low prevalence. In this study, we aimed to compare the clinicopathological features and prognosis of CHC with those of HCC and ICC, and to analyze the prognostic factors in patients with CHC.

Patients and Methods

A search of patients who underwent hepatic resection for primary liver cancer at our institution between January 1990 and March 2016 identified 28 patients with CHC, 1050 with HCC, and 100 with ICC. CHC was diagnosed according to the definition of type C in Allen and Lisa's classification; i.e., double cancer and collision cancer were excluded (Fig. 1).⁷

Patients were followed-up postoperatively on an outpatient basis by monitoring of tumor markers and abdominal dynamic computed tomography and/or magnetic resonance imaging every 3 months. Recurrence of CHC, HCC, or ICC was diagnosed by combined examination of imaging studies and tumor markers.

The medical records of all patients were reviewed for clinicopathological information, including sex, age, viral markers, serum alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonists-II (PIVKAII), carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), surgical procedure, and pathological reports. Pathological data included tumor number, tumor size, lymph node metastasis, vascular invasion, and the findings of non-cancerous liver. Tumor node metastasis (TNM) stage was determined according to the criteria of the Liver Cancer Study Group of Japan (6th edition).¹⁰ We compared the clinical and pathological characteristics, overall survival (OS), and disease-free survival (DFS) between patients with CHC and patients with HCC or ICC. We also investigated the prognostic factors for OS and DFS, recurrence patterns, and management among patients with CHC. This study was approved by the institutional review board of our institution.

Statistical analysis

Statistical analyses were performed using EZR version 1.35 (Saitama Medical Center, Jichi Medical University, Saitama, Japan), which is a graphical user interface for R (The R Foundation for Statistical Computing, Vienna, Austria).¹¹ Clinical and pathological variables were examined by Fisher's exact test and Student's *t* test. OS and DFS curves were plotted using the Kaplan–Meier method and compared using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards regression model. A *P* value of <0.05 was considered statistically significant.

Results

Clinical and pathological characteristics

The 28 patients with CHC included 23 males and five females, with a mean age of 62.5 ± 11.8 years (range, 32–78 years). There was no significant difference in sex ratio or age between the CHC group and the HCC/ICC group. Eleven patients (39.3%) were positive for hepatitis B virus (HBV) and five (17.9%) were positive for hepatitis C virus (HCV). Significantly more patients in the CHC group had HBV infection compared with the ICC group. All patients in the CHC group were classified as Child-Pugh A. Above normal levels of preoperative AFP (≥ 10 ng/ml), PIVKAI (≥ 40 mAU/ml), CEA (≥ 6.5 ng/ml), and CA19-9 (≥ 37 U/ml) were found in 18 (64.3%), 14 (50.0%), seven (25.0%), and 14 (50.0%) CHC patients, respectively. Significantly more patients in the CHC group had elevated AFP and PIVKAI levels compared with the ICC group. Regarding the surgical procedures, anatomical resection was performed in 28 (87.5%) CHC patients, 691 (65.8%) HCC patients, and 95 (95.0%) ICC patients. Anatomical resection was performed significantly more frequently in patients with CHC than HCC. The resection surface was histologically free of tumor in all patients. Multiple tumors were seen in 16 patients (57.1%) with CHC, which was significantly more than in the other groups. Tumor size was not significantly different among the groups. Vascular invasion, especially portal vein invasion, was more frequent in the CHC compared with the HCC group but not the ICC group; however, hepatic vein invasion or bile duct invasion was significantly less frequent in the CHC compared with the ICC group. All patients with CHC underwent lymph node dissection, and lymph node metastasis was more frequent in the CHC compared with the HCC group. TNM stage was worse in the CHC compared with the HCC group, but there was no significant difference between the CHC and ICC

groups. Pathologically, patients with CHC had significantly less cirrhosis than patients with HCC, but more than patients with ICC (Table 1).

Survival analysis

The survival analysis data are summarized in Table 2. The median OS in the CHC group was 25 months, and the 1-, 3-, and 5-year OS rates were 57.9%, 33.4%, and 25.1%, respectively. OS in the CHC group was significantly lower than in the HCC group ($P<0.001$) but not significantly different from the ICC group ($P=0.152$) (Fig. 2A). The median DFS was 5 months, and the 1-, 3-, and 5- DFS rates were 30.2%, 22.6%, and 22.6%, respectively. DFS was significantly lower in the CHC group compared with both the HCC and ICC groups ($P=0.008$ and $P=0.005$, respectively) (Fig. 2B). We compared the prognoses of CHC and HCC according to stage, and showed that OS tended to be lower in the CHC compared with the HCC group among stage III and IVa patients, but there was no significant difference for any stage. Similarly, there was no significant difference in DFS between the CHC and HCC groups at any stage (Fig. S1).

Prognostic factor analysis

Univariate analysis showed that CEA level, tumor size, and vascular invasion were predictive factors for lower OS in patients with CHC, while CEA level and tumor size were also predictive factors for lower DFS in CHC patients. Multivariate analysis showed that CEA level and tumor size were independent predictors of OS (CEA: hazard ratio (HR) 3.35, 95% confidence interval (CI) 1.06–10.56, $P=0.039$; tumor size: HR 2.69, 95% CI 1.04–6.92, $P=0.041$) and recurrence (CEA: HR 3.17, 95% CI 1.13–8.88, $P=0.028$; tumor size: HR 3.14, 95% CI 1.30–7.59, $P=0.011$) in patients with CHC (Table 3).

Recurrence of CHC and management

The recurrence pattern of CHC and its management are summarized in Table 4. At the date of our investigations, 22 of the 28 patients with CHC (78.6%) had experienced recurrences, with a median time to recurrence of 5 months (range 0.2–67). Twelve of the 22 patients had extrahepatic recurrences, including lymph node metastasis in seven patients, lung metastasis in six, bone metastasis in one, and brain metastasis in one. The management strategies for the first recurrence varied, including resection for intrahepatic recurrence or solitary lymph node metastasis, resection, radiofrequency ablation, transcatheter arterial chemoembolization, or chemotherapy for intrahepatic recurrence, and radiotherapy for bone metastasis. Extrahepatic recurrence was mainly treated with chemotherapy, including fluorouracil and cisplatin (n=4), tegafur/uracil (n=2), tegafur/gimeracil/oteracil (n=2), fluorouracil (n=1), gemcitabine (n=1), tegafur/gimeracil/oteracil and gemcitabine (n=1), and sorafenib (n=1).

The clinicopathological features in patients with intrahepatic and extrahepatic recurrence are summarized in Table 5. Recurrence only in the liver occurred in 10 patients and liver recurrence together with extrahepatic recurrence occurred in 12 patients. Extrahepatic recurrence was significantly correlated with tumor size, lymph node metastasis, and stage ($P=0.002$, $P=0.015$, and $P=0.012$, respectively). OS rates after intrahepatic and extrahepatic recurrence are shown in Fig. S2. The 1-year OS rate in patients with extrahepatic recurrence tended to be poorer than in those with intrahepatic recurrence, but there was no significant difference ($P=0.562$).

Discussion

CHC is a rare type of primary liver cancer, accounting for 1.0%–4.7% of primary liver cancers.¹⁻⁶ In our institution, 28 of 1,178 patients (2.4%) who underwent resection for primary liver cancer had CHC, which was similar to the incidence in previous reports.¹⁻⁶ However, the definition of CHC has changed, with both the Allen and Lisa classification and Goodman classification being used in clinical practice. The current definition of CHC is Allen and Lisa type C or Goodman type II.^{7,8} Although the latest WHO classification has made the definition of CHC clearer, it has not been easily accepted by clinicians because of its complexities regarding subtypes with stem-cell features.⁹

Although the origin of CHC cells remains unclear, CHC is widely considered to be derived from hepatic progenitor cells (HPCs).^{5,12-15} HPCs have bipotential differentiation into either hepatocytes or cholangiocytes, which are thought to be located near the canal of Hering.^{12,16} HPCs are induced and expanded during liver injury, such as hepatitis or cirrhosis, leading to malignant transformation, which is thought to be a mechanism for carcinogenesis of CHC.¹⁷

The clinical and pathological features of CHC are similar to those of HCC or ICC. The current study found no difference in age at diagnosis among the three groups. Male patients were predominant in all groups, and although the male-to-female ratios in CHC and HCC differed from that in ICC, the difference was not significant. HBV infection was significantly more common among patients with CHC or HCC compared with ICC. These results were largely in accord with previous reports indicating similar tumorigenic backgrounds for CHC and HCC.^{18,19} The incidences of elevated AFP, PIVKAI, CEA, and CA19-9 in patients with CHC were 64.3%, 50.0%, 25.0%, 50.0%,

respectively, which were similar to those reported in previous studies.¹⁸⁻²¹ The rates of elevated AFP and PIVKAI were similar in CHC and HCC patients, but were significantly lower in patients with ICC, while the rates of elevated CEA were similar in CHC and ICC patients and lower in HCC patients. The levels of these tumor markers support the idea that CHC includes both HCC and ICC components. The frequency of lymph node metastasis in CHC was 21.4%, which was in accord with incidences reported in previous studies (12%–33%).^{19,22,23}

CHC is difficult to diagnose preoperatively due to its heterogeneous imaging characteristics, with features that overlap both HCC and ICC.²⁴ Thus most CHC cases are initially misdiagnosed as either HCC or ICC, and the correct diagnosis is only reached after examination of surgical specimens. Some authors have suggested that CHC should be considered preoperatively in patients with tumors showing characteristic imaging features of HCC but elevated CA19-9 levels, tumors with characteristic ICC imaging features and elevated AFP levels, or in patients with elevated levels of both serum markers.^{25,26}

Minor or major hepatic resection, with or without lymph node dissection, is currently the consensus recommended treatment for CHC.^{24,27} The outcomes following liver transplantation are consistently poorer in patients with CHC compared with HCC,^{28,29} and one study found no survival benefit of transplant over resection in patients with CHC.³⁰ Although some studies have reported a good response of CHC to transarterial chemoembolization and sorafenib, these treatments require further evaluation.^{27,31,32}

Survival of patients with CHC has been reported in several previous studies, with a 5-year OS rate of 7.9%–36.4%.^{1,21-23} Previous studies consistently found that

CHC had a poorer prognosis than HCC,^{1,2,21-23,33} while the prognosis with regard to ICC varied, with some reports showing a poorer or similar prognosis and others showing a better prognosis.^{1,2,21-23,33} Our results showed that the OS of patients with CHC was significantly poorer than that of patients with HCC but not significantly different from patients with ICC, while CHC was associated with a significantly poorer DFS than either HCC or ICC. Adverse clinicopathological prognostic factors associated with tumor recurrence and survival have been reported in several studies, and included large tumor size (>5 cm), presence of satellite nodules, lymph node involvement, multifocality, vascular invasion, portal vein invasion, high tumor stage, high levels of CA19-9, decreased capsule formation, and free surgical margins <2 cm.^{18,24,34,35} However, many of these factors were not significant according to multivariate analysis. Our results identified tumor size ≥ 5 cm and high levels of CEA as significant prognostic factors for OS and DFS according to multivariate analysis.

Few studies have reported on the recurrence of CHC after resection. One study found that CHC recurred frequently in the liver, while another found that extrahepatic recurrence was commonly seen in lymph nodes.^{1,19,21} The present study showed that extrahepatic metastasis was the most frequent form of recurrence, and was managed by chemotherapy. Furthermore, extrahepatic recurrence was correlated with tumor size, lymph node metastasis, and stage. There are currently no definitive guidelines for the treatment of recurrent CHC, and chemotherapy regimens were chosen according to the treatment of HCC or ICC; however, the outcomes after recurrence were poor. The prognosis of CHC after resection is thus poor due to both the high recurrence rate, including extrahepatic recurrence, and ineffective treatment after recurrence. In practice, adjuvant postoperative chemotherapy might be a useful strategy in patients

with CHC, especially in patients at high risk of extrahepatic recurrence due to tumor size ≥ 5 cm, lymph node metastasis, and poor stage.

This study had some limitations. First, it was a retrospective, single-center study, and second, the sample size for CHC was relatively small. Further, high-volume, multicenter studies are therefore necessary to validate our results.

In conclusion, the clinical features of patients with CHC, including sex, HBV infection, AFP levels, and PIVKAI levels, were more similar to HCC than ICC, while its prognosis and pathological features, including vascular invasion and lymph node metastasis, were more similar to ICC. Multivariate analysis identified high CEA levels and tumor size ≥ 5 cm as independent prognostic factors for both OS and recurrence in patients with CHC.

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Supporting information

Additional supporting information may be found online in the supporting information section of this article.

Figure S1. Overall survival (OS) (A) and disease-free survival (DFS) rates (B) in patients with combined hepatocellular-cholangiocarcinoma (CHC) and hepatocellular carcinoma (HCC) according to Liver Cancer Study Group of Japan (6th edition) stage. OS tended to be lower in patients with stage III and IVa CHC compared with HCC, but there was no significant difference at any stage. There was no significant difference in DFS between the CHC and HCC groups at any stage.

Figure S2. Overall survival (OS) after recurrence in patients with intrahepatic and extrahepatic recurrence of combined hepatocellular-cholangiocarcinoma. The 1-year OS rate in patients with extrahepatic recurrence tended to be poorer than in those with intrahepatic recurrence, but the difference was not significant ($P=0.562$).

Table 1 Clinical and pathological characteristics of patients with CHC, HCC, and ICC (n, %)

Characteristics	CHC (n=28)	HCC (n=1050)	ICC (n=100)	<i>P</i> value	
				CHC vs HCC	CHC vs ICC
Sex					
Male	23 (82.1)	864 (82.3)	62 (62.0)	1.000	0.069
Female	5 (17.5)	186 (17.7)	38 (38.0)		
Age (yr)	62.5±11.8 (32-78)	62.0±10.6 (18-90)	63.5±11.0 (36-87)	0.813	0.660
Viral infection					
HBV	11 (39.3)	407 (38.8)	15 (15.0)	1.000	0.008*
HCV	5 (17.9)	369 (35.1)	12 (12.0)	0.070	0.528
Child-Pugh classification					
A	28 (100.0)	1015 (96.7)	99 (99.0)	1.000	1.000
B	0 (0.0)	35 (3.3)	1 (1.0)		
AFP (ng/ml)					
<10	10 (35.7)	413 (39.6)	57 (80.3)	0.845	<0.001*
≥10	18 (64.3)	631 (60.4)	14 (19.7)		
PIVKaII (mAU/ml)					
<40	14 (50.0)	294 (35.5)	61 (95.3)	0.159	<0.001*
≥40	14 (50.0)	535 (64.5)	3 (4.7)		
CEA (ng/ml)					
<6.5	21 (75.0)	672 (87.8)	54 (73.0)	0.073	1.000
≥6.5	7 (25.0)	93 (12.2)	20 (27.0)		
CA199 (U/ml)					
<37	14 (50.0)	468 (62.9)	27 (37.0)	0.170	0.263
≥37	14 (50.0)	276 (37.1)	46 (63.0)		
Surgical procedure					
Anatomical resection	28 (87.5)	691 (65.8)	95 (95.0)	0.012*	0.219
Non-anatomical resection	4 (12.5)	359 (34.2)	5 (5.0)		
Tumor number					
Solitary	12 (42.9)	668 (63.6)	76 (76.0)	0.030*	0.002*
Multiple	16 (57.1)	382 (36.4)	24 (24.0)		
Tumor size (cm)					
6.2±4.2		5.7±4.5	6.1±3.5	0.608	0.923
<2	4 (14.3)	104 (9.9)	6 (6.0)		
2-5	10 (35.7)	503 (47.9)	34 (34.0)		
5-10	9 (32.1)	284 (27.0)	46 (46.0)		

≥10	5 (17.9)	159 (15.1)	14 (14.0)		
Vascular invasion [†]	19 (67.9)	336 (32.0)	81 (81.0)	<0.001*	0.194
Portal vein invasion	18 (64.3)	295 (28.1)	50 (50.0)	<0.001*	0.204
Hepatic vein invasion	2 (7.1)	115 (11.0)	34 (34.0)	0.760	0.004*
Hepatic artery invasion	0 (0.0)	4 (0.4)	5 (5.0)	1.000	0.585
Bile duct invasion	2 (7.1)	43 (4.1)	57 (57.0)	0.328	<0.001*
Lymph node metastasis	6 (21.4)	15 (1.4)	40 (40.0)	<0.001*	0.079
pStage [†]					
I	2 (7.1)	93 (8.9)	2 (2.0)	<0.001*	0.157
II	2 (7.1)	433 (41.2)	22 (22.0)		
III	11(39.3)	316 (30.1)	26 (26.0)		
IVa	13 (46.5)	178 (17.0)	43 (43.0)		
IVb	0 (0.0)	30 (2.9)	7 (7.0)		
Non-cancerous liver					
Cirrhosis	5 (17.9)	321 (33.1)	2 (2.5)	0.008*	0.013*
Non-cirrhosis	23 (82.1)	648 (66.9)	77 (97.5)		

**P*value <0.05. [†]Liver Cancer Study Group of Japan, 6th edition.

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; HBV, hepatitis B virus; HCV hepatitis C virus; AFP, alpha-fetoprotein; PIVKALII, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 2 Median survival time and cumulative survival rates in patients with CHC, HCC, and ICC

Groups	Median OS (months)	1-year OS rate	3-year OS rate	5-year OS rate	Median DFS (months)	1-year DFS rate	3-year DFS rate	5-year DFS rate
CHC (n=28)	25	57.9%	33.4%	25.1%	5	30.2%	22.6%	22.6%
HCC (n=1050)	82	87.6%	70.8%	57.8%	23	64.0%	39.7%	30.1%
ICC (n= 100)	37	75.9%	50.9%	41.9%	18	57.7%	41.8%	36.9%

CHC, combined hepatocellular-cholangiocarcinoma; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; OS, overall survival; DFS, disease-free survival.

Table 3 Univariate and multivariate analyses of prognostic factors in patients with CHC

Variables	Overall survival			Disease-free survival		
	Univariate analysis	Multivariate analysis		Univariate analysis	Multivariate analysis	
	<i>P</i> value	HR (95%CI)	<i>P</i> value	<i>P</i> value	HR (95%CI)	<i>P</i> value
Gender	0.205			0.132		
Age ≥60 yr	0.083			0.150		
Viral infection	0.346			0.203		
AFP ≥10 ng/ml	0.311			0.605		
PIVKAI ≥40 mAU/ml	0.315			0.240		
CEA ≥6.5 ng/ml	0.008*	3.35 (1.06-10.56)	0.039*	0.009†	3.17 (1.13-8.88)	0.028*
CA19-9 ≥37 U/ml	0.319			0.221		
Surgical procedure	0.644			0.886		
Tumor number	0.633			0.915		
Tumor size ≥5cm	0.006*	2.69 (1.04-6.92)	0.041*	0.004†	3.14 (1.30-7.59)	0.011*
Lymph node metastasis	0.104			0.310		
Vascular invasion	0.023*	2.11 (0.73-6.08)	0.168	0.066		
Pathological cirrhosis	0.219			0.358		

**P* value <0.05.

CHC, combined hepatocellular-cholangiocarcinoma; HR, hazard ratio; CI, confidence interval; AFP, alpha-fetoprotein; PIVKAI, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

Table 4. Recurrence patterns and management of first recurrence after resection of CHC

Variables	n (%)
Number of recurrence	22 (78.6)
Location	
Intrahepatic	10 (45.5)
Extrahepatic	6 (27.3)
Both	6 (27.3)
Management	
Chemotherapy	12 (54.6)
Resection	3 (13.6)
TACE	3 (13.6)
BSC	2 (9.1)
RFA	1 (4.6)
Radiotherapy	1 (4.6)

CHC, combined hepatocellular-cholangiocarcinoma; TACE, transcatheter arterial chemoembolization; BSC, best supportive care; RFA, radiofrequency ablation.

Table 5. Recurrence patterns and characteristics of patients with CHC (n, %)

Characteristics	Recurrence patterns		Pvalue
	Intrahepatic (n=10)	Extrahepatic (n=12)	
Sex			
Male	8 (80.0)	11 (91.7)	0.571
Female	2 (20.0)	1 (8.3)	
Age (yr)	62.3±(13.9)	60.3±(10.0)	0.692
Viral infection			
HBV	7 (70.0)	6 (50.0)	0.415
HCV	1 (10.0)	2 (16.7)	1.000
AFP (ng/ml)			
<10	3 (30.0)	5 (41.7)	0.675
≥10	7 (70.0)	7 (58.3)	
PIVKaII (mAU/ml)			
<40	6 (60.0)	4 (33.3)	0.391
≥40	4 (40.0)	8 (66.7)	
CEA (ng/ml)			
<6.5	7 (70.0)	8 (66.7)	1.000
≥6.5	3 (30.0)	4 (33.3)	
CA199 (U/ml)			
<37	2 (20.0)	7 (58.3)	0.099
≥37	8 (80.0)	5 (41.7)	
Surgical procedure			
Anatomical resection	9 (90.0)	10 (83.3)	1.000
Non-anatomical resection	1 (10.0)	2 (16.7)	
Tumor number			
Solitary	5 (50.0)	4 (33.3)	0.666
Multiple	5 (50.0)	8 (66.7)	
Tumor size (cm)			
<5	8 (80.0)	1 (8.3)	0.002*
≥5	2 (20.0)	11 (91.7)	
Vascular invasion [†]			
No	4 (40.0)	2 (16.7)	0.348
Yes	6 (60.0)	10 (83.3)	
Lymph node metastasis			
No	10 (100.0)	6 (50.0)	0.015*

Yes	0 (0.0)	6 (50.0)	
pStage†			
I	1 (10.0)	0 (0.0)	0.012*
II	1 (10.0)	0 (0.0)	
III	6 (60.0)	2 (16.7)	
Iva	2 (20.0)	10 (83.3)	
Non-cancerous liver			
Cirrhosis	1 (10.0)	2 (16.7)	1.000
Non-cirrhosis	9 (90.0)	10 (83.3)	

**P*value <0.05. †Liver Cancer Study Group of Japan, 6th edition.

CHC, combined hepatocellular-cholangiocarcinoma; HBV, hepatitis B virus; HCV hepatitis C virus; AFP, alpha-fetoprotein; PIVKAI, protein induced by vitamin K absence or antagonists-II; CEA, carcinoembryonic antigen; CA19-9, carbohydrate antigen 19-9.

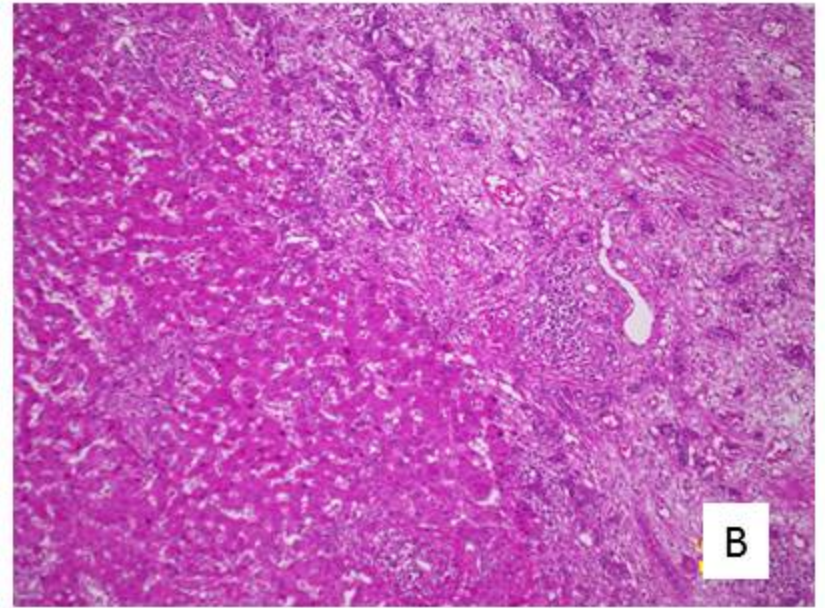
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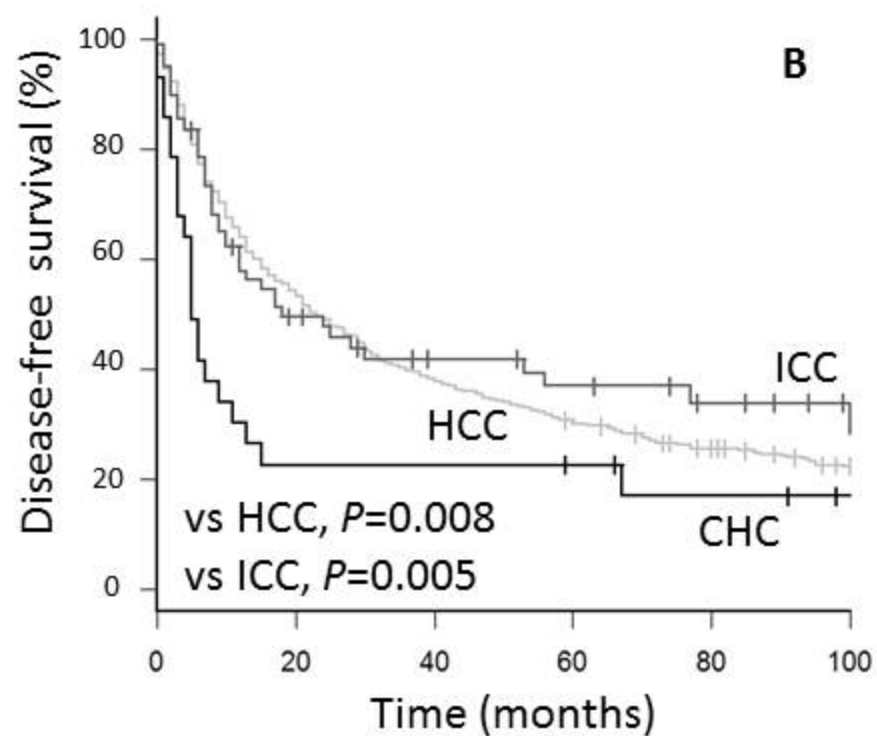
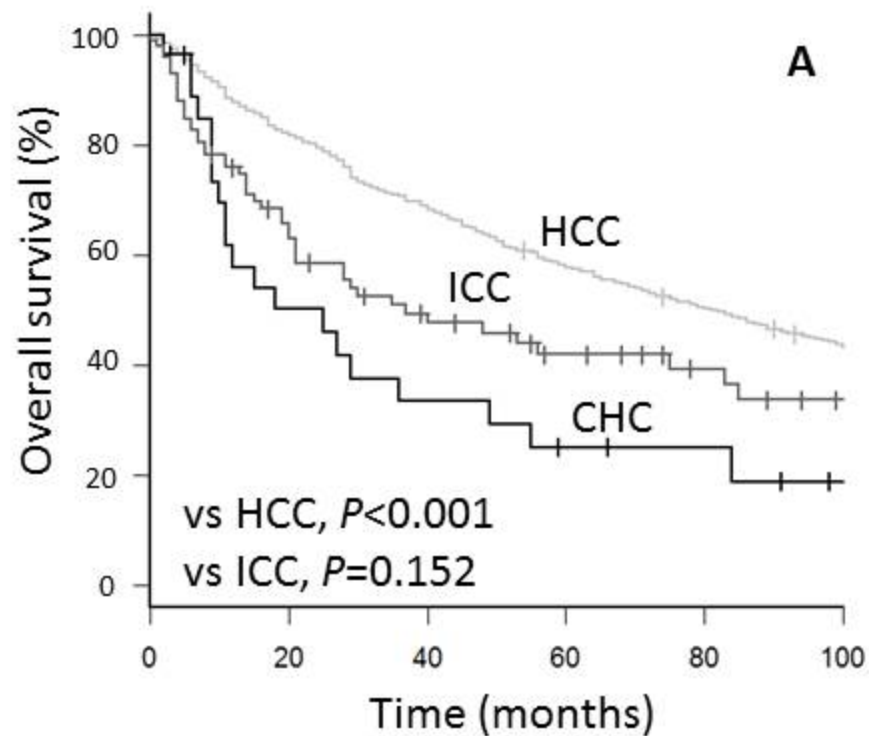
Figure 1

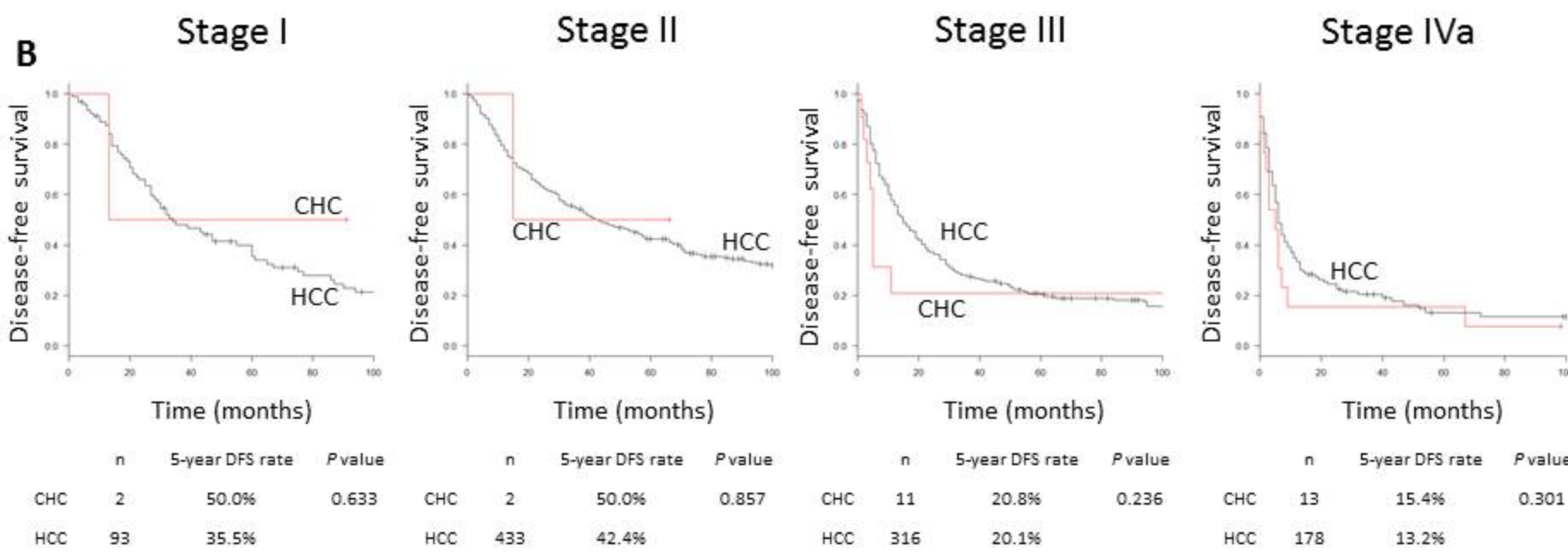
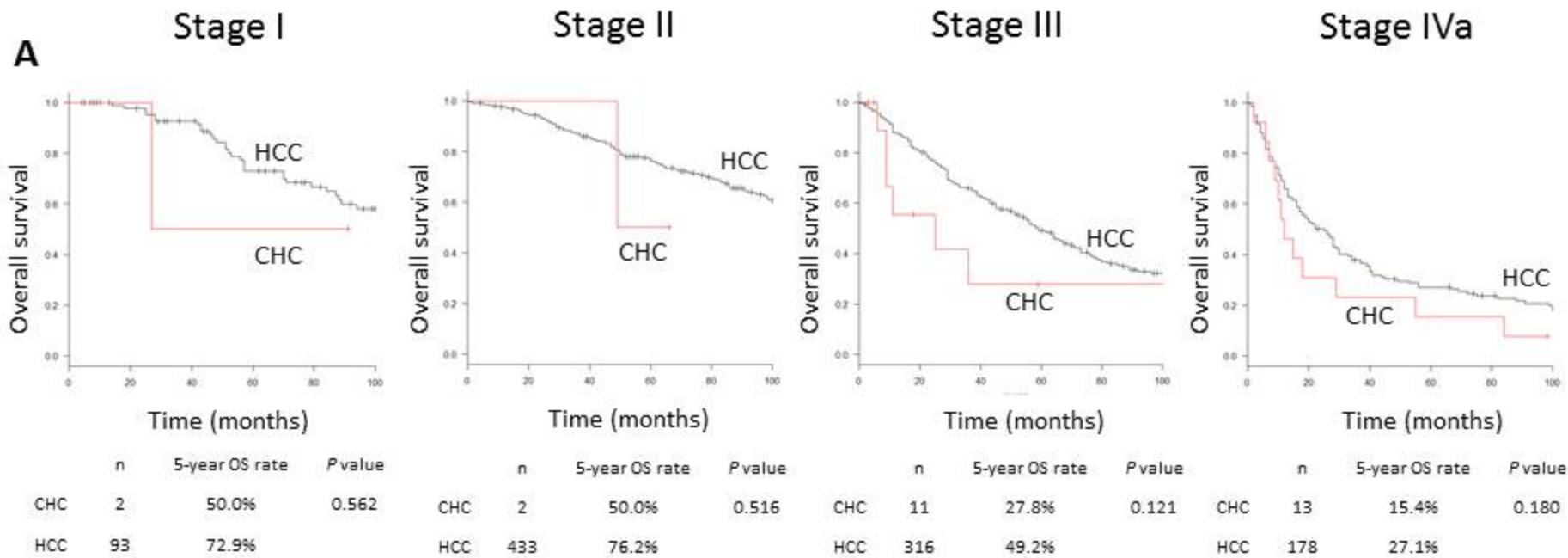
Gross specimen (A) and light microscopy (B; magnification $\times 40$) of combined hepatocellular-cholangiocarcinoma. Homogenous tumor was shown by macroscopic examination, but hepatocellular carcinoma (B, left) and intrahepatic cholangiocarcinoma components (B, right) were shown to coexisted by microscopy.

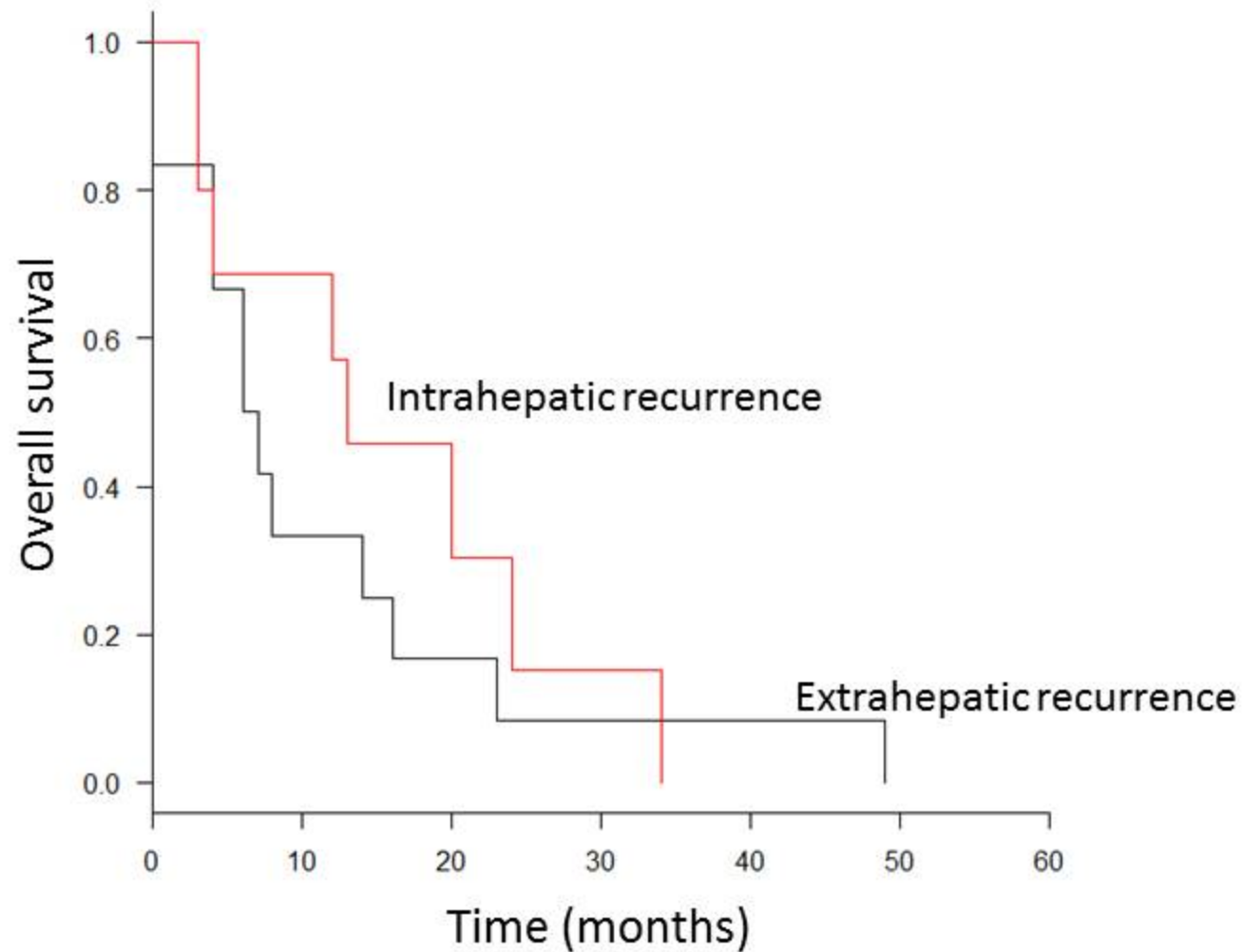
Figure 2

Overall survival (A) and disease-free survival rates (B) of patients with combined hepatocellular-cholangiocarcinoma (CHC), hepatocellular carcinoma (HCC), and intrahepatic cholangiocarcinoma (ICC). Overall survival in the CHC group was significantly lower compared with the HCC group ($P < 0.001$) but not compared with the ICC group ($P = 0.152$). Disease-free survival was significantly lower in the CHC compared with both the HCC and ICC groups ($P = 0.008$, $P = 0.005$, respectively).









	n	1-year OS rate	<i>P</i> value
Intrahepatic recurrence	10	57.1%	0.562
Extrahepatic recurrence	12	33.3%	