Original Article

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Elevated alpha-fetoprotein affects the long-term prognosis after hepatectomy in patients with hepatitis B-related intrahepatic cholangiocarcinoma

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SUMMARY: This study investigates the prognostic significance of alpha-fetoprotein (AFP) in hepatitis B virus-related intrahepatic cholangiocarcinoma (HBV-ICC), given that AFP — though commonly used for hepatocellular carcinoma — is sometimes elevated in HBV-ICC, yet its clinical relevance remains unclear. The research retrospectively analyzed 839 HBV-ICC patients who underwent curative hepatectomy, categorizing them into AFP-positive (≥ 20 ng/mL) and AFP-negative groups. Using propensity score matching and inverse probability of treatment weighting to reduce bias, the study compared overall survival (OS) and time to recurrence (TTR). Results showed that AFP-positive patients had poorer liver function and more aggressive tumor characteristics, including higher rates of cirrhosis, microvascular invasion, and satellite nodules. Across both unadjusted and adjusted cohorts, elevated AFP was significantly associated with worse OS and earlier recurrence. Multivariate Cox analysis identified AFP as an independent predictor of poor prognosis. While CA19-9 alone demonstrated limited predictive value, its combination with AFP improved prognostic accuracy. The study concludes that elevated serum AFP independently predicts adverse survival and recurrence outcomes in HBV-ICC patients after curative resection, and combining AFP with CA19-9 enhances prognostic stratification, supporting AFP as a practical biomarker for postoperative risk assessment.

Keywords: alpha-fetoprotein (AFP), CA19-9, Intrahepatic cholangiocarcinoma (ICC), hepatitis B virus (HBV), hepatectomy

1. Introduction

Intrahepatic cholangiocarcinoma (ICC) originates from the intrahepatic secondary bile ducts and their branches, accounting for approximately 10–15% of primary liver cancers, and is the second most common primary malignant tumor of the liver (*1,2*). In recent years, both the incidence and mortality of ICC have been rapidly increasing worldwide (*1,3*). Radical hepatectomy remains the preferred curative treatment for patients with early- and intermediate-stage ICC; however, the 5-year postoperative survival rate remains below 40% (*4*). In Asia, particularly in China, hepatitis B virus (HBV) infection is an important etiological factor for ICC, it has been reported that HBV-related ICC accounts for about 27.7–77.2% of cases (*5,6*). Compared with non-HBV-related ICC, HBV-related ICC exhibits poorer prognosis and distinct heterogeneity (*7-9*). Song *et al*. suggested that patients with HBV-related ICC have shorter recurrence-free survival than those without HBV infection, and that HBV-related ICC may originate from hepatocytes (*9*). Therefore, HBV-related ICC may share certain biological characteristics with hepatocellular carcinoma (HCC).

Alpha-fetoprotein (AFP) is another important tumor marker for liver cancer and has been widely used to assess treatment efficacy, particularly in HBV-related HCC (*10,11*). In addition, AFP is also associated with germ cell tumors, gastric cancer, and colorectal cancer (*12*). For example, compared with AFP-negative gastric

cancer, AFP-positive gastric cancer demonstrates stronger invasiveness and worse prognosis (*13,14*). In Asia, a considerable proportion of ICC cases are associated with HBV infection (*8*). A previous study reported that approximately 25% of HBV-related ICC patients present with elevated AFP (*8*). However, the clinicopathological differences between AFP-positive and AFP-negative subgroups in HBV-related ICC, as well as the prognostic significance of AFP, remain unclear.

Based on a large clinical cohort, the present study aims to explore the clinicopathological characteristics of HBV-related ICC patients with different AFP levels, analyze the role of AFP in long-term prognosis and tumor recurrence, and evaluate the prognostic predictive value of combining AFP with CA19-9.

2. Materials and Methods

2.1. Study design

A total of 839 patients with HBV-related ICC who underwent hepatectomy at the Third Affiliated Hospital of Naval Medical University between January 2016 and December 2020 were retrospectively analyzed. Patients were stratified into AFP-positive and AFP-negative groups based on a serum AFP cutoff value of 20 ng/mL, comprising 237 and 602 patients, respectively. Eligibility criteria included: (1) Postoperative histopathological confirmation of ICC; (2) All patients were classified as Child–Pugh class A; (3) No prior antitumor therapy and no history of other malignancies; (4) All surgeries were performed as radical hepatectomies (R0); (5) Absence of major vascular or bile duct invasion, including bile duct, hepatic vein, or portal vein tumor thrombus; and (6) Eastern Cooperative Oncology Group (ECOG) performance status of ≤ 2. Exclusion criteria were: (1) Non-hepatitis B virus-related ICC; (2) Recurrence ICC; (3) Microscopic positive margins; (4) Tumor recurrence within 1 month postoperatively; (5) History of previous anticancer therapy; (6) Failure to recover within 1 month owing to severe postoperative complications; (7) Missing clinicopathological data or loss to follow-up withen 90 days after resection. The study was conducted in accordance with the Declaration of Helsinki, approved by the institutional ethics committee, and written informed consent was obtained from all participants.

2.2. Surgical procedures and pathological assessment

R0 resection was defined as complete macroscopic removal of the tumor with histologically negative margins (*15*). Major hepatectomy was defined as resection of three or more hepatic segments, while minor hepatectomy referred to resection of fewer than three segments (*16*). Surgical margin width was classified as wide (≥ 1 cm) and narrow (< 1 cm) according to the minimum distance from the tumor edge to the resection plane (*17*). The extent of lymph node dissection was standardized according to previously published criteria (*18*). Microvascular invasion (MVI) was defined as the presence of tumor cell nests within vascular spaces lined by endothelial cells, including the portal vein, hepatic vein, or capsular vessels (*19*).

2.3. Pathological criteria for ICC

All tumor specimens were sampled using a 7-point baseline sampling method (*20*). According to the 4th and 5th edition of the World Health Organization classification of liver tumors, ICC was defined as a tumor arising proximal to the second-order bile ducts and showing strong positivity for at least one cholangiocytic marker, such as cytokeratin 19 or mucin core protein 1. Patients diagnosed with hepatocellular carcinoma or combined hepatocellular-cholangiocarcinoma, characterized by the presence of distinct HCC and ICC components-regardless of whether transitional or transformation zones were present-or tumors with separate cells or regions expressing hepatocytic and cholangiocytic markers were excluded.

2.4. Postoperative surveillance

Patients were monitored every 2 months during the first 6 months after surgery, and every 6 months thereafter. Follow-up evaluations consisted of serum tumor marker (AFP, CEA, CA19-9), abdominal ultrasonography, and contrast-enhanced computed tomography (CT) or magnetic resonance imaging (MRI). In cases of suspected recurrence or metastasis, repeat enhanced CT or MRI of the upper abdomen was performed. Positron emission tomography–CT (PET-CT) or bone scintigraphy was conducted when clinically indicated. The primary endpoints were overall survival (OS) and time to recurrence (TTR). OS was defined as the interval from hepatectomy to death from any cause or last follow-up, while TTR was defined as the interval from hepatectomy to the first documented recurrence or last follow-up.

2.5. Statistical analysis

Statistical analyses were performed using R software, version 4.3.2 (*http://www.r-project.org*). Categorical variables were expressed as counts (*n*) or percentages (%) and compared using the χ^2 test or Fisher's exact test, as appropriate. To minimize baseline imbalances between groups, propensity score matching (PSM) with nearest-neighbor matching (1:1 ratio) and inverse probability of treatment weighting (IPTW) were applied. OS and TTR were estimated using the Kaplan–Meier method and compared by the log-rank test. Prognostic factors for OS and TTR were identified using univariate and multivariate Cox proportional hazards regression analyses. Variables with a p value < 0.05 in univariate

analysis were entered into multivariate analysis to determine independent predictors. A two-tailed p value < 0.05 was considered statistically significant.

3. Patient characteristics

The inclusion and exclusion process is shown in Figure 1. Among the 839 patients with HBV-related ICC, 237 were classified as AFP-positive and 602 as AFP-negative. Compared with AFP-negative patients, those in the AFP-positive group were more likely to be male (82.7% *vs*. 73.9%, $p = 0.009$), have concomitant cirrhosis (51.5% *vs*. 34.7%, $p < 0.001$), present with ascites (51.5% *vs*. 34.7%, $p < 0.001$), exhibit higher serum ALT levels (33.3% *vs*. 25.6%, $p = 0.030$), demonstrate poorer liver function (49.8% *vs*. 40.4%, $p = 0.016$), and have a higher proportion of elevated HBV-DNA load (54.0% *vs*. 40.0%, $p < 0.001$) (Table 1). To minimize bias due to baseline imbalances, propensity score matching (PSM) and inverse probability of treatment weighting (IPTW) were performed. In the PSM cohort, 222 AFP-positive patients were matched to 222 AFP-negative patients, while in the IPTW cohort, 238.2 AFP-positive and 600.3 AFP-negative patients were included (Table 2).

3.2. Survival analysis

The median follow-up time for all patients was 35.1 months (95% CI: 30.9-39.2). In the primary cohort, the 1-, 3-, and 5-year recurrence rates were 53.0%, 64.2%, and 68.2% in the AFP-positive group, compared with 40.2%, 56.3%, and 60.8% in the AFP-negative group ($p < 0.001$, Figure 2A). Correspondingly, the 1-, 3-, and 5-year OS rates were 65.1%, 43.1%, and 37.1% in the AFP-positive group, and 78.1%, 54.8%, and 47.5% in the AFP-negative group ($p < 0.001$, Figure 2B). In the PSM cohort, recurrence rates at 1, 3, and 5 years were 52.4%, 63.4%, and 66.7% in the AFP-positive group, compared with 40.7%, 50.5%, and 59.5% in the AFP-negative group ($p = 0.005$, Figure 3A). OS rates were 67.4%, 44.2%, and 37.8% in the AFP-positive group, compared with 75.0%, 55.5%, and 47.9% in the AFP-negative group ($p = 0.008$, Figure 3B). In the IPTW cohort, the 1-, 3-, and 5-year recurrence rates were 50.3%, 63.0%, and 66.3% in the AFP-positive group, compared with 40.7%, 56.2%, and 61.1% in the AFP-negative group ($p = 0.004$). OS rates were 67.4%, 44.2%, and 37.8% in the AFP-positive group, compared with 77.6%, 55.5%, and 47.9% in the AFP-negative group ($p < 0.001$, Figure 4A and 4B).

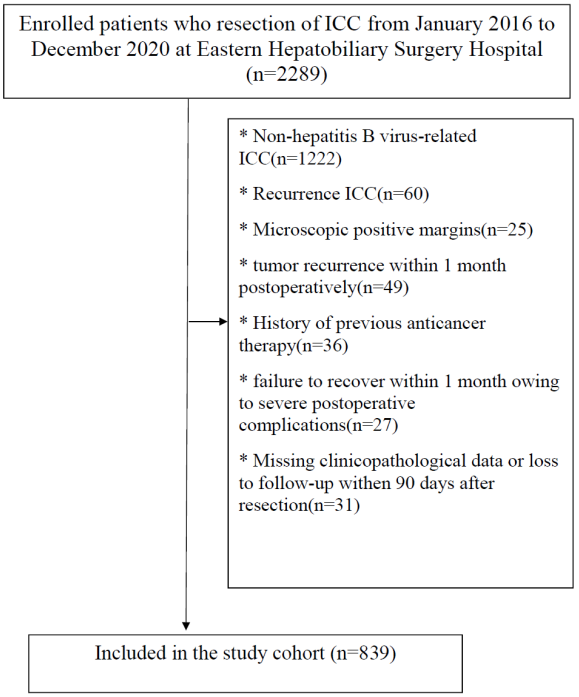

Figure 1. The flow chart of the study.

3.3. Independent risk Factors for tumor recurrence and OS

Results of univariate analysis are summarized in Tables 3–5. In the multivariate analysis of the primary cohort, independent predictors of OS included Cirrhosis (yes *vs*. no, HR = 1.471, 95%CI 1.174-1.842, $p < 0.001$), AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.294, 95%CI 1.037-1.615, $p = 0.023$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.570, 95%CI 1.239-1.990, $p < 0.001$), lymphadenectomy (yes *vs*. no, HR = 1.514, 95%CI 1.150-1.992, $p = 0.003$), tumor size (> 5 *vs*. ≤ 5 cm, HR = 1.577, 95%CI 1.246-1.995, $p < 0.001$), MVI (present *vs*. absent, HR = 1.433, 95%CI 1.106-1.857, $p = 0.007$), and satellite lesions (yes *vs*. no, HR = 1.329, 95%CI 1.056-1.673, $p = 0.016$). Independent predictors of recurrence were AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.363, 95%CI 1.113-1.670, $p = 0.003$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.376, 95%CI 1.103-1.716, $p = 0.005$), tumor size (> 5 *vs*. ≤ 5 cm, HR = 1.268, 95%CI 1.029-1.563, $p = 0.026$), MVI (present *vs*. absent, HR = 1.484, 95%CI 1.162-1.895, $p = 0.002$), satellite lesions (yes *vs*. no, HR = 1.381, 95%CI 1.112-1.716, $p = 0.004$), and lymph node metastasis (yes *vs*. no, HR = 1.492, 95%CI 1.063-2.094, $p = 0.021$). In the PSM cohort, independent predictors of OS included AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.589, 95%CI 1.188-2.216, $p = 0.002$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.520, 95%CI 1.077-2.145, $p = 0.017$), lymphadenectomy (yes *vs*. no, HR = 1.571, 95%CI 1.097-2.249, $p = 0.014$), tumor size (> 5 *vs*. ≤ 5 cm, HR = 1.566, 95%CI 1.154-2.216, $p = 0.004$), MVI (present *vs*. absent, HR = 1.689, 95%CI 1.217-2.345, $p = 0.002$), and satellite lesions (yes *vs*. no, HR = 1.491, 95%CI 1.102-2.017, $p = 0.010$). Independent predictors of recurrence were sex (male *vs*. female, HR = 1.502, 95%CI 1.036-2.177, $p = 0.032$), AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.471, 95%CI 1.144-1.890, $p = 0.003$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.373,

Table 1. Basal clinicopathological characteristics of ICC patients before propensity score analysis

Variables	AFP ≤ 20 ng/mL (*n* = 602)	AFP > 20 ng/mL (*n* = 237)	*p*-value	*SMD*
Sex			0.009	0.214
Female	157 (26.1)	41 (17.3)		
Male	445 (73.9)	196 (82.7)		
Age, years			0.165	0.117
≤ 60	484 (80.4)	201 (84.8)		
> 60	118 (19.6)	36 (15.2)		
BMI			1.000	0.006
≤ 22	194 (32.2)	77 (32.5)		
> 22	408 (67.8)	160 (67.5)		
Diabetes			0.359	0.088
No	571 (94.8)	229 (96.6)		
Yes	31 (5.2)	8 (3.4)		
Antivirus			0.649	0.045
No	530 (88.0)	212 (89.5)		
Yes	72 (12.0)	25 (10.5)		
Cirrhosis			0.001	0.270
No	387 (64.3)	121 (51.1)		
Yes	215 (35.7)	116 (48.9)		
Gastroesophageal Varices			0.739	0.034
No	535 (88.9)	208 (87.8)		
Yes	67 (11.1)	29 (12.2)		
Ascites			< 0.001	0.292
No	581 (96.5)	211 (89.0)		
Yes	21 (3.5)	26 (14.0)		
ALT, U/L			0.030	0.171
≤ 44	448 (74.4)	158 (66.7)		
> 44	154 (25.6)	79 (33.3)		
PLT, $*10^9$/mL			0.032	0.167
≤ 100	82 (13.6)	47 (19.831%)		
> 100	520 (86.4)	190 (80.169%)		
NLR			0.242	0.096
≤ 2.5	336 (55.8)	121 (51.1)		
> 2.5	266 (44.2)	116 (48.9)		
INR			0.176	0.110
≤ 1.1	542 (90.0)	205 (86.5)		
> 1.1	60 (10.0)	32 (13.5)		
ALBI Grade			0.016	0.063
Grade I	359 (59.6)	119 (50.2)		
Grade II	243 (40.4)	118 (49.8)		
HBV-DNA, IU/mL			< 0.001	0.283
≤ 2,000	361 (60.0)	109 (46.0)		
> 2,000	241 (40.0)	128 (54.0)		
CEA, ng/mL			0.184	0.109
≤ 10	555 (92.0)	211 (89.0)		
> 10	47 (7.8)	26 (11.0%)		
CA19-9, U/mL			0.065	0.151
≤ 37	436 (72.4)	187 (78.9)		
> 37	166 (27.6)	50 (21.2)		
ASA grade			0.311	0.086
II	146 (24.3)	49 (20.7)		
III	456 (75.7)	188 (79.3)		
Major Hepatectomy			0.138	0.112
No	460 (76.4)	193 (81.435%)		
Yes	142 (23.6)	44 (18.565%)		
Hilar Clamping, min			0.687	0.037
≤ 15	293 (48.7)	111 (46.8)		
> 15	309 (51.3)	126 (53.2)		
Surgical Margin, cm			0.187	0.039
≤ 1	502 (83.4)	207 (87.3)		
> 1	100 (16.6)	30 (12.7)		
Lymphadenectomy			0.691	0.178
No	432 (71.8)	174 (73.4)		
Yes	170 (28.2)	63 (26.6)		

Bold values indicate statistical significance ($p < 0.05$). PLT, platelet; AST, Aspartate transaminase; AFP, alpha fetoprotein; NLR, neutrophil-to-lymphocyte ratio; ALBI, Albumin-bilirubin; HBsAg, Hepatitis B surface antigen; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; ASA, American society of Aneshesiologists.

Table 1. Basal clinicopathological characteristics of ICC patients before propensity score analysis (continued)

Variables	AFP ≤ 20 ng/mL (*n* = 602)	AFP > 20 ng/mL (*n* = 237)	*p*-value	*SMD*
Blood loss,mL	200.0 [150.0;400.0]	210.0 [200.0;400.0]	0.216	0.047
Transfusion			0.601	0.049
No	521 (86.5)	209 (88.2)		
Yes	81 (13.5)	28 (11.8)		
Tumor Size, cm			0.012	0.201
≤ 5	296 (49.2)	93 (39.2)		
> 5	306 (50.8)	144 (60.8)		
Tumor Number			0.176	0.105
Single	564 (93.7)	215 (90.7)		
Multiple	38 (6.3)	22 (9.3)		
Microvascular Invasion			0.029	0.024
Absent	525 (87.2)	192 (81.0)		
Present	77 (12.8)	45 (19.0)		
Satellite			0.042	0.160
No	464 (77.1)	166 (70.0)		
Yes	138 (22.9)	71 (30.0)		
Capsule			0.099	0.119
Incomplete	526 (87.4)	196 (82.7)		
Complete	76 (12.6)	41 (17.3)		
Differentiation			0.180	0.116
Poor	69 (11.5)	19 (8.0)		
Moderate/Well	533 (88.5)	218 (92.0)		
Lymph node metastasis			0.643	0.044
No	532 (88.4)	206 (86.9)		
Yes	70 (11.6)	31 (13.1)		
Adjuvant Therapy			0.101	0.131
No	447 (74.3)	162 (68.4)		
Yes	155 (25.7)	75 (31.6)		

Bold values indicate statistical significance ($p < 0.05$). PLT, platelet; AST, Aspartate transaminase; AFP, alpha fetoprotein; NLR, neutrophil-to-lymphocyte ratio; ALBI, Albumin-bilirubin; HBsAg, Hepatitis B surface antigen; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; ASA, American society of Aneshesiologists.

95%CI 1.011-1.865, $p = 0.042$), MVI (present *vs*. absent, HR = 1.674, 95%CI 1.231-2.276, $p = 0.001$), and satellite lesions (yes *vs*. no, HR = 1.476, 95%CI 1.103-1.976, $p = 0.009$). In the IPTW cohort, independent predictors of OS were ALBI grade (II *vs*. I, HR = 1.293, 95%CI 1.030-1.624, $p = 0.027$), AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.314, 95%CI 1.038-1.663, $p = 0.023$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.570, 95%CI 1.193-2.064, $p = 0.001$), lymphadenectomy (yes *vs*. no, HR = 1.574, 95%CI 1.185-2.091, $p = 0.011$), tumor size (> 5 *vs*. ≤ 5 cm, HR = 1.469, 95%CI 1.162-1.857, $p = 0.001$), MVI (present *vs*. absent, HR = 1.387, 95%CI 1.028-1.870, $p = 0.032$), and satellite lesions (yes *vs*. no, HR = 1.351, 95%CI 1.056-1.729, $p = 0.017$). Independent predictors of recurrence were AFP level (> 20 *vs*. ≤ 20 ng/mL, HR = 1.360, 95%CI 1.090-1.696, $p = 0.006$), CA19-9 level (> 37 *vs*. ≤ 37 U/mL, HR = 1.459, 95%CI 1.148-1.853, $p = 0.002$), MVI (present *vs*. absent, HR = 1.482, 95%CI 1.147-1.915, $p = 0.003$), and satellite lesions (yes *vs*. no, HR = 1.386, 95%CI 1.106-1.736, $p = 0.004$).

3.4. Prognostic prediction of AFP and CA19-9

Receiver operating characteristic (ROC) analysis showed that CA19-9 alone yielded 1-, 3-, and 5-year areas under the curve (AUCs) of 0.604, 0.589, and 0.583 for OS prediction, which improved to 0.658, 0.623, and 0.620 when combined with AFP. For recurrence prediction, CA19-9 alone yielded AUCs of 0.570, 0.543, and 0.593, which improved to 0.658, 0.623, and 0.619 when combined with AFP (Figure 5A and 5B).

4. Discussion

Elevated AFP predicts poor prognosis in HCC patients, but there are relatively few studies evaluating the prognostic impact of marked AFP elevation in ICC patients, particularly in those with HBV-related ICC. Huang *et al*. enrolled 119 HBV-ICC patients and reported an AFP > 20 ng/mL prevalence of 25.2% (30/119) (*8*). Their multivariable Cox analysis indicated that elevated AFP was an independent risk factor for poor prognosis in HBV-related ICC, but a similar association was not observed in non-HBV-related ICC; however, that retrospective study had a small sample size and did not perform an in-depth analysis of AFP (*8*).

In the present study we evaluated the prognostic significance of AFP in a substantially larger HBV-ICC cohort. We first observed that patients with different AFP levels differed with respect to sex, cirrhosis, ascites, ALT, platelet count, ALBI grade, HBV-DNA load, tumor diameter, MVI, and satellite nodules. These findings

Table 2. Basal clinicopathological characteristics of ICC patients after propensity score analysis

Variables	PSM Cohort				Stable IPTW Cohort			
	AFP ≤ 20 ng/mL (*n* = 222)	AFP > 20 ng/mL (*n* = 222)	*p*-value	*SMD*	AFP ≤ 20 ng/mL (*n* = 600.3)	AFP > 20 ng/mL (*n* = 238.3)	*p*-value	*SMD*
Sex			0.612	0.060			0.990	0.001
Female	35 (15.8)	40 (18.0)			142.1 (23.7)	56.5 (23.47)		
Male	187 (84.2)	182 (82.0)			458.2 (76.3)	181.8 (76.3)		
Age, years			0.442	0.085			0.262	0.101
≤ 60	182 (82.0)	189 (85.1)			486.1 (81.0)	202.0 (84.8)		
> 60	40 (18.0)	33 (14.9)			114.2 (19.0)	36.3 (15.2)		
BMI			0.919	0.019			0.703	0.032
≤ 22	70 (31.5)	72 (32.4)			195.4 (32.5)	81.2 (34.1)		
> 22	152 (68.5)	150 (67.6)			404.9 (7.5)	157.1 (65.9)		
Diabetes			0.371	0.106			0.578	0.050
No	209 (94.1)	214 (96.4)			571.0 (95.1)	229.1 (96.2)		
Yes	13 (5.9)	8 (3.6)			29.3 (4.9)	9.2 (3.8)		
Antivirus			0.640	0.059			0.669	0.037
No	201 (90.5)	197 (88.7)			530.6 (88.4)	207.7 (87.2)		
Yes	21 (9.5)	25 (11.3)			69.7 (11.6)	30.5 (12.8)		
Cirrhosis			0.084	0.174			0.746	0.026
No	136 (61.3)	117 (52.7)			363.6 (60.6)	141.2 (59.3)		
Yes	86 (38.7)	105 (47.3)			236.7 (39.4)	97.0 (40.7)		
Gastroesophageal Varices			0.317	0.109			0.099	0.129
No	190 (85.6)	198 (89.2)			523.2 (87.2)	217.2 (91.2)		
Yes	32 (14.4)	24 (10.8)			77.1 (12.8)	21.0 (8.8)		
Ascites			0.582	0.070			0.981	0.002
No	204 (91.9)	208 (93.7)			568.7 (94.7)	225.8 (94.8)		
Yes	18 (8.1)	14 (6.3)			31.6 (5.3)	12.4 (5.2)		
ALT,U/L			0.143	0.149			0.908	0.010
≤ 44	165 (74.3)	150 (67.6)			435.4 (72.5)	171.8 (72.1)		
> 44	57 (25.7)	72 (32.4)			164.9 (27.5)	66.5 (27.9)		
PLT, $*10^9$/mL			0.804	0.035			0.728	0.028
≤ 100	38 (17.1)	41 (18.5)			91.9 (15.3)	38.9 (16.3)		
> 100	184 (82.9)	181 (81.5)			508.4 (84.7)	199.4 (83.7)		
NLR			0.776	0.036			0.865	0.014
≤ 2.5	118 (53.2)	114 (51.4)			333.0 (55.5)	130.5 (54.8)		
> 2.5	104 (46.8)	108 (48.6)			267.3 (44.5)	107.8 (45.2)		
INR			0.773	0.041			0.571	0.043
≤ 1.1	196 (88.3)	193 (86.9)			532.6 (88.7)	214.6 (90.1)		
> 1.1	26 (11.7)	29 (13.1)			67.7 (11.3)	23.7 (9.9)		

Bold values indicate statistical significance ($p < 0.05$). AFP, alpha fetoprotein; BMI, body mass index; ALT, alanine aminotransferase; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; INR, international normalized ratio; ALBI, Albumin-bilirubin; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; CEA, carcinoembryonic antigen; CA19-9, Carbohydrate antigen19-9; ASA, American society of Aneshesiologists.

Table 2. Basal clinicopathological characteristics of ICC patients after propensity score analysis (continued)

Variables	PSM Cohort				Stable IPTW Cohort			
	AFP ≤ 20 ng/mL (*n* = 222)	AFP > 20 ng/mL (*n* = 222)	*p*-value	*SMD*	AFP ≤ 20 ng/mL (*n* = 600.3)	AFP > 20 ng/mL (*n* = 238.3)	*p*-value	*SMD*
ALBI Grade			0.569	0.063			0.733	0.028
Grade I	109 (49.1)	116 (52.3)			343.0 (57.1)	139.4 (58.5)		
Grade II	113 (50.9)	106 (47.7)			257.3 (42.9)	98.8 (41.5)		
HBV-DNA, IU/mL			0.776	0.036			0.825	0.018
≤ 2,000	110 (49.550)	106 (47.748)			339.4 (56.5)	136.8 (57.4)		
> 2,000	112 (50.450)	116 (52.252)			260.9 (43.5)	101.4 (42.6)		
CEA, ng/mL			0.180	0.144			0.056	0.165
≤ 10	207 (93.2)	198 (89.2)			554.1 (92.3)	208.1 (87.3)		
> 10	15 (6.8)	24 (10.8)			46.2 (7.7)	30.2 (12.7)		
CA19-9, U/mL			0.177	0.139			0.877	0.014
≤ 37	164 (73.9)	177 (79.7)			445.5 (74.2)	178.2 (74.8)		
> 37	58 (26.1)	45 (20.3)			154.8 (25.8)	60.0 (25.2)		
ASA grade			0.145	0.149			0.903	0.010
II	59 (26.6)	45 (20.3)			139.7 (23.3)	54.4 (22.8)		
III	163 (73.4)	177 (79.7)			460.6 (76.7)	183.9 (77.2)		
Major Hepatectomy			0.158	0.145			0.144	0.122
No	170 (76.6)	183 (82.4)			459.6 (76.6)	194.2 (81.5)		
Yes	52 (23.4)	39 (17.6)			140.7 (23.4)	44.0 (18.5)		
Hilar Clamping, min			0.634	0.054			0.766	0.025
≤ 15	100 (45.0)	106 (47.7)			290.2 (48.3)	112.2 (47.1)		
> 15	122 (55.0)	116 (52.3)			310.1 (51.7)	126.1 (52.9)		
Surgical Margin, cm			0.786	0.039			0.332	0.082
≤ 1	189 (85.1)	192 (86.5)			504.9 (84.1)	207.3 (87.0)		
> 1	33 (14.9)	30 (13.5)			95.3 (15.9)	31.0 (13.0)		
Lymphadenectomy			0.078	0.178			0.584	0.047
No	147 (66.2)	165 (74.3)			434.8 (72.4)	177.5 (74.5)		
Yes	75 (33.8)	57 (25.7)			165.5 (27.6)	60.8 (25.5)		
Blood loss, mL	250.000 [150.0;400.0]	200.000 [150.0;400.0]	0.902	0.098	405.05 ± 1864.78	334.26 ± 323.70	0.329	0.053
Transfusion			0.668	0.054				
No	192 (86.5)	196 (88.3)			521.4 (86.9)	207.8 (87.2)	0.904	0.011
Yes	30 (13.5)	26 (11.7)			78.9 (13.1)	30.5 (12.8)		
Tumor Size, cm			0.441	0.082			0.734	0.029
≤ 5	97 (43.7)	88 (39.6)			278.3 (46.4)	113.9 (47.8)		
> 5	125 (56.3)	134 (60.4)			321.9 (53.6)	124.4 (52.2)		
Tumor Number			0.359	0.105			0.358	0.070
Single	209 (94.1)	203 (91.4)			561.5 (93.5)	218.5 (91.7)		
Multiple	13 (5.9)	19 (8.6)			38.8 (6.5)	19.7 (8.3)		

Bold values indicate statistical significance ($p < 0.05$). AFP, alpha fetoprotein; BMI, body mass index; ALT, alanine aminotransferase; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; INR, international normalized ratio; ALBI, Albumin-bilirubin; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; CEA, carcinoembryonic antigen; CA19-9, Carbohydrate antigen19-9; ASA, American society of Aneshesiologists.

Table 2. Basal clinicopathological characteristics of ICC patients after propensity score analysis (continued)

Variables	PSM Cohort				Stable IPTW Cohort			
	AFP ≤ 20 ng/mL (n = 222)	AFP > 20 ng/mL (n = 222)	p-value	*SMD*	AFP ≤ 20 ng/mL (n = 600.3)	AFP > 20 ng/mL (n = 238.3)	p-value	*SMD*
Microvascular Invasion			0.900	0.024			0.989	0.001
Absent	183 (82.4)	185 (83.3)			513.2 (85.5)	203.6 (85.5)		
Present	39 (17.6)	37 (16.7)			87.1 (14.5)	34.6 (14.5)		
Satellite			1.000	0.010			0.680	0.032
No	161 (72.5)	162 (73.0)			452.5 (75.4)	182.9 (76.8)		
Yes	61 (27.5)	60 (27.0)			147.8 (24.6)	55.4 (23.2)		
Capsule			0.356	0.100			0.467	0.059
Incomplete	192 (86.5)	184 (82.9)			522.2 (87.0)	202.4 (85.0)		
Complete	30 (13.5)	38 (17.2)			78.1 (13.0)	35.9 (15.0)		
Edmondson-Steiner Grade			0.512	0.078			0.014	0.201
I-II	23 (10.4)	18 (8.1)			73.0 (12.2)	15.1 (6.4)		
III-VI	199 (89.6)	204 (91.9)			527.3 (87.8)	223.1 (93.6)		
Lymph node metastasis			0.581	0.065			0.938	0.006
No	189 (85.1)	194 (87.4)			532.7 (88.7)	211.0 (88.5)		
Yes	33 (14.9)	28 (12.6)			67.6 (11.3)	27.3 (11.5)		
Adjuvant Therapy			0.681	0.049			0.099	0.139
No	151 (68.0)	156 (70.3)			446.8 (74.4)	162.4 (68.2)		
Yes	71 (32.0)	66 (29.3)			153.5 (25.6)	31.8 (31.8)		

Bold values indicate statistical significance ($p < 0.05$). AFP, alpha fetoprotein; BMI, body mass index; ALT, alanine aminotransferase; PLT, platelet; NLR, neutrophil-to-lymphocyte ratio; INR, international normalized ratio; ALBI, Albumin-bilirubin; HBV-DNA, hepatitis B virus-deoxyribonucleic acid; CEA, carcinoembryonic antigen; CA19-9, Carbohydrate antigen19-9; ASA, American society of Aneshesiologists.

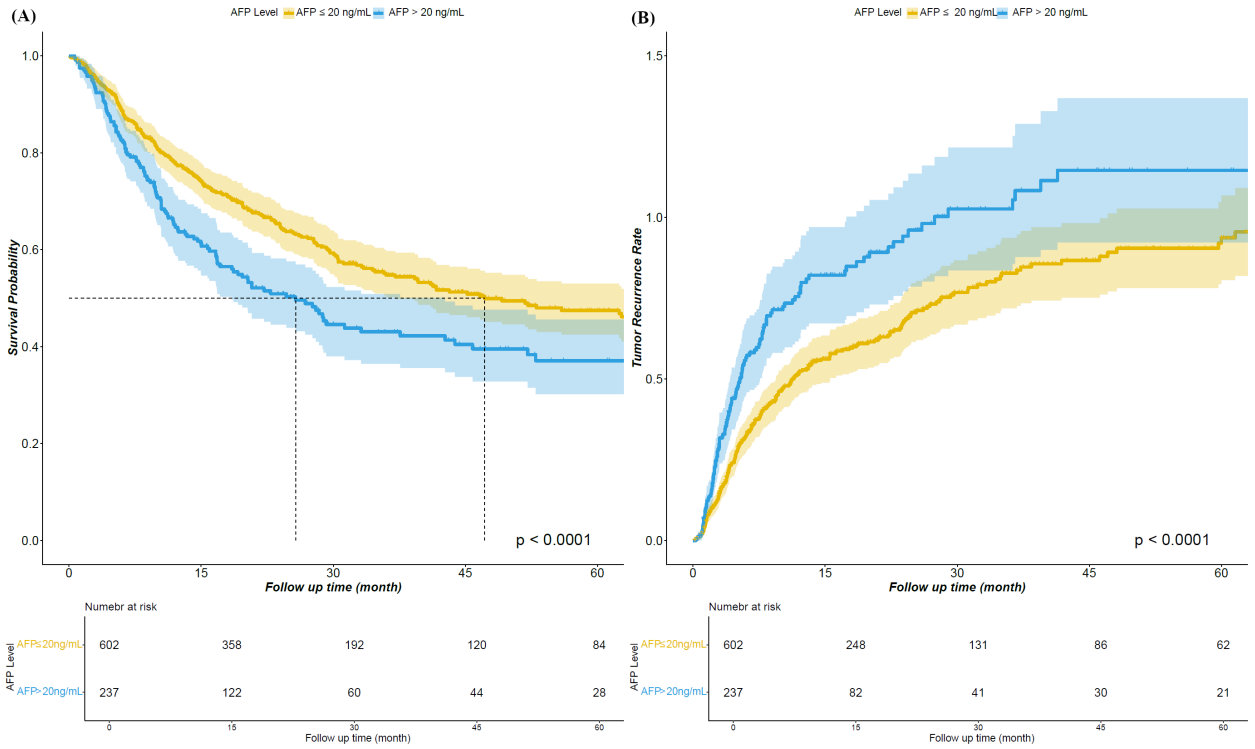

Figure 2. Kaplan-Meier analysis for OS in HBV-ICC patients with different alpha-fetoprotein levels in primary cohort. (A) Overall Survival; **(B)** Time to recurrence.

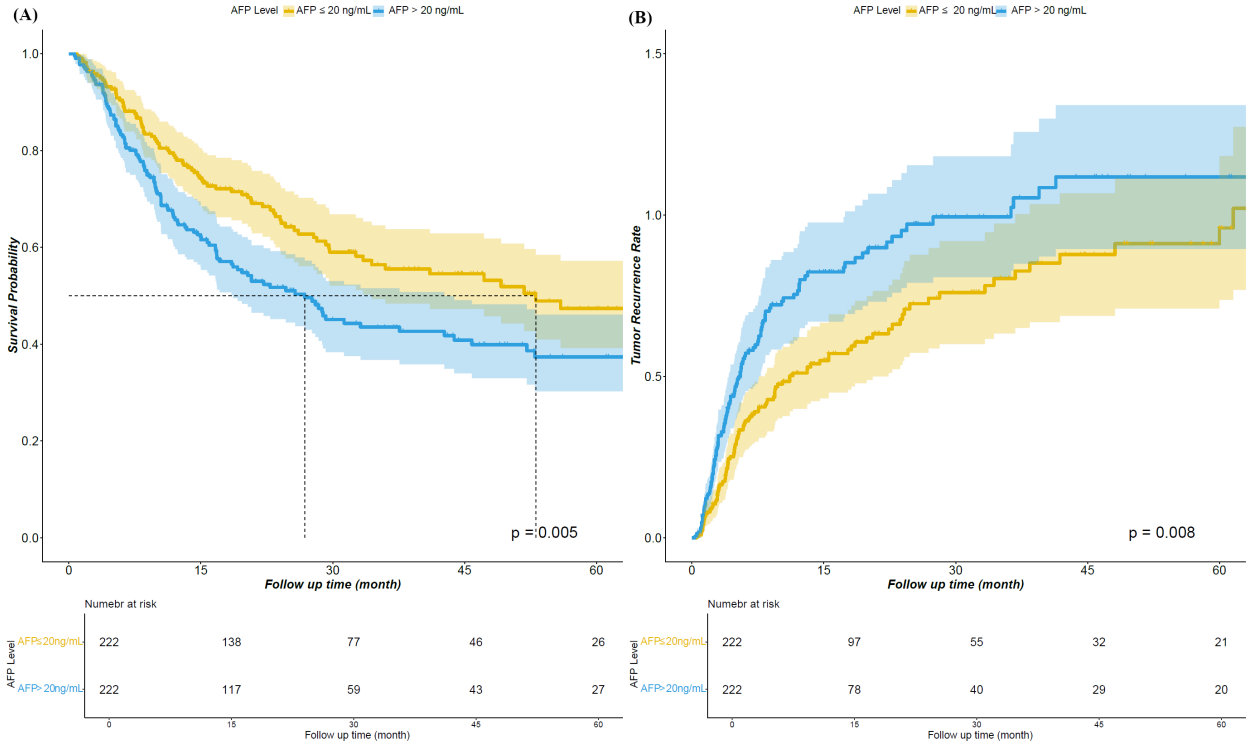

Figure 3. Kaplan-Meier analysis for OS in HBV-ICC patients with different alpha-fetoprotein levels in PSM cohort. (A) Overall Survival; **(B)** Time to recurrence.

suggest that AFP-positive patients have worse liver function and more aggressive tumor features. In the unmatched cohort, the 1-, 3-, and 5-year OS rates and recurrence rates in the AFP-positive group were 65.1%, 43.1%, 37.1% and 53.0%, 64.2%, 68.2%, respectively. The AFP-positive group exhibited poorer overall survival and higher recurrence compared with the AFP-negative group. After adjustment for potential confounders, both

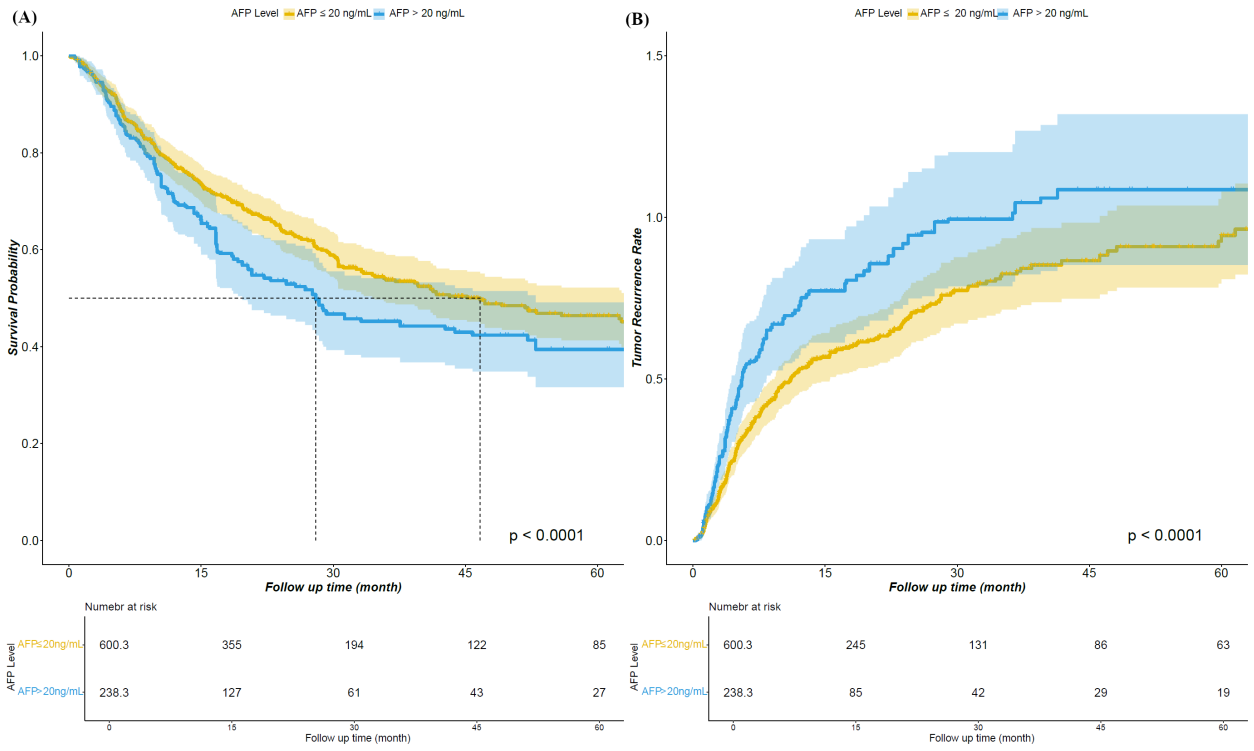

Figure 4. Kaplan-Meier analysis for OS in HBV-ICC patients with different alpha-fetoprotein levels in IPTW cohort. (A) Overall Survival; **(B)** Time to recurrence.

the PSM and IPTW cohorts produced concordant results. Subsequent Cox regression analyses demonstrated that serum AFP level was an independent prognostic factor in the original, PSM, and IPTW cohorts. Additionally, we found that CA19-9 alone had limited prognostic performance (OS AUCs at 1, 3, 5 years: 0.604, 0.589, 0.583; recurrence AUCs: 0.570, 0.543, 0.593), whereas combining CA19-9 with AFP improved predictive accuracy.

The association between AFP and ICC tumor biology has been explored previously. Prior studies reported correlations between higher AFP and cirrhosis and worse liver function (*21*). A multicenter retrospective study found that higher AFP was strongly associated with formation of massive tumor nodules (OR 25.000, $p < 0.001$) (*22*). At the molecular level, AFP expression in cholangiocarcinoma has been reported to correlate positively with eIF3C expression, which may promote ICC progression by enhancing cholangiocyte proliferation (*23*). Established independent prognostic factors for recurrence and survival-such as CA19-9 level, MVI, and tumor size-were also confirmed in our study, consistent with previous reports (*24*). The lower discriminatory power of CA19-9 for ICC prognosis is in part due to its elevation in various benign and malignant biliary disorders, which reduces specificity. Given this limitation, several investigators have proposed combining CA19-9 with other circulating markers; for example, Wang *et al*. combined CA19-9 with circulating cell-free DNA (cfDNA) for prognostic assessment (*25*). AFP is routinely measured in clinical practice, is inexpensive, and readily available. Until other serum biomarkers are validated at scale, combining AFP with CA19-9 may offer a pragmatic improvement in prognostic stratification for HBV-ICC.

Regarding potential biological mechanisms linking HBV, AFP, and ICC: the canonical "inflammation-to-carcinoma" pathway explains HBV-driven hepatocarcinogenesis and the associated AFP elevation in HCC (*26,27*). HBV is a hepatotropic virus that primarily infects hepatocytes and does not readily persist in cholangiocytes (*28*). The mechanism by which HBV contributes to ICC remains unclear. Stem/progenitor-cell theories of cholangiocarcinogenesis may reconcile the observed relationships among HBV, HCC-like biology, and AFP. Hepatic progenitor cells located in the Hering canal can, under injurious or oncogenic stimuli, differentiate along hepatocytic or cholangiocytic lineages (*29*). HBV can integrate fragments of its genome (*e.g.*, HBx) into the genomes of adult hepatic progenitor cells, potentially driving transformation toward malignant hepatocytic or cholangiocytic phenotypes (*30*). AFP is a marker of cellular dedifferentiation and immaturity and is expressed in hepatic progenitor cells. ICCs that synthesize and secrete substantial AFP may therefore retain progenitor-like or hepatocytic features (*31*). Beyond effects on progenitor cells, Song *et al*. reported that HBV can induce transdifferentiation of mature hepatocytes into ICC-like cells, which in hepatocyte-origin ICCs continue to proliferate and

Table 3. Univariate cox regression analysis of OS and recurrence in HBV-related ICC patients before propensity score analysis

Variable	OS		Recurrence	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Sex		0.597		0.131
Male *vs*. Female	1.068 (0.837 - 1.362)		1.191 (0.949 - 1.495)	
Age, years		0.133		0.453
> 60 *vs*. ≤ 60	1.215 (0.943 - 1.565)		0.910 (0.712 - 1.164)	
BMI		0.182		0.545
> 22 *vs*. ≤ 22	0.863 (0.696 - 1.071)		0.940 (0.770 - 1.148)	
Diabetes		0.551		0.168
Yes *vs*. No	1.155 (0.719 - 1.855)		1.343 (0.883 - 2.045)	
Antivirus		0.160		0.174
Yes *vs*. No	0.771 (0.536 - 1.108)		0.806 (0.590 - 1.101)	
Cirrhosis		0.035		0.998
Yes *vs*. No	1.247 (1.015 - 1.532)		1.000 (0.826- 1.211)	
Gastroesophageal Varices		0.725		0.235
Yes *vs*. No	1.058 (0.772 - 1.450)		0.826 (0.603 - 1.132)	
Ascites		0.133		0.720
Yes *vs*. No	1.374 (0.908 - 2.079)		0.923 (0.595 - 1.431)	
ALT,U/L		0.262		0.106
> 44 *vs*. ≤ 44	1.135 (0.910 - 1.415)		1.183 (0.965 - 1.450)	
PLT, $*10^9$/mL		0.408		0.554
> 100 *vs*. ≤ 100	0.892 (0.680 - 1.170)		1.082 (0.834 - 1.403)	
NLR		< 0.001		0.040
> 2.5 *vs*. ≤ 2.5	1.466 (1.195 - 1.798)		1.127 (0.798 - 1.593)	
INR		0.024		0.712
> 1.1 *vs*. ≤ 1.1	1.397 (1.044 - 1.869)		0.944 (0.694 - 1.283)	
ALBI Grade		< 0.001		0.351
II *vs*. I	1.449 (1.181 - 1.777)		1.094 (0.906 - 1.322)	
HBV-DNA, IU/mL		0.588		0.861
> 2,000 *vs*. ≤ 2,000	1.058 (0.862 - 1.300)		1.017 (0.842 - 1.228)	
AFP, ng/mL		0.001		< 0.001
> 20 *vs*. ≤ 20	1.449 (1.1681 -1.798)		1.430 (1.171 - 1.746)	
CEA, ng/mL		0.086		0.925
> 10 *vs*. ≤ 10	1.362 (0.957 - 1.940)		0.983 (0.689 - 1.402)	
CA19-9, U/mL		< 0.001		< 0.001
> 37 *vs*. ≤ 37	1.981 (1.588 - 2.471)		1.599 (1.296 - 1.971)	
ASA grade		0.188		0.766
III *vs*. II	0.852 (0.670 - 1.0816)		0.967 (0.775 - 1.207)	
Major Hepatectomy		0.218		0.036
Yes *vs*. No	1.164 (0.914 - 1.482)		1.264 (1.015 - 1.575)	
Hilar Clamping, min		0.556		0.045
> 15 *vs*. ≤ 15	1.063 (0.867 - 1.305)		1.213 (1.005 - 1.464)	
Surgical Margin, cm		0.054		0.089
> 1 *vs*. ≤ 1	0.744 (0.551 - 1.006)		0.796 (0.613 - 1.035)	
Lymphadenectomy		< 0.001		< 0.001
Yes *vs*. No	1.892 (1.529 - 2.341)		1.551 (1.268 - 1.898)	
Blood loss,mL	1.000 (0.999 - 1.000)	0.931	1.000 (0.999 - 1.000)	0.637
Transfusion		0.021		0.685
Yes *vs*. No	1.389 (1.052 - 1.835)		1.060 (0.800 - 1.403)	
Tumor Size, cm		< 0.001		< 0.001
> 5 *vs*. ≤ 5	1.913 (1.547 - 2.366)		1.545 (1.278 - 1.868)	
Tumor Number		0.319		0.496
Multiple *vs*. Single	1.200 (0.839 - 1.715)		1.217 (1.009 - 1.469)	
Microvascular Invasion		< 0.001		< 0.001
Present *vs*. Absent	1.754 (1.362 - 2.257)		1.780 (1.404 - 2.256)	
Satellite		< 0.001		< 0.001
Yes *vs*. No	1.764 (1.417 - 2.195)		1.686 (1.374 - 2.069)	
Capsule		0.039		0.923
Complete *vs*. Incomplete	0.719 (0.526 - 0.983)		1.013 (0.780 - 1.315)	
Diferentiation		0.243		0.153
Moderate/Well *vs*. Poor	0.834 (0.615 - 1.131)		0.815 (0.615 - 1.079)	
Lymph node metastasis		< 0.001		< 0.001
Yes *vs*. No	2.062 (1.572 - 2.704)		1.950 (1.504 - 2.529)	
Adjuvant Therapy		0.140		0.804
Yes *vs*. No	0.838 (0.662 - 1.060)		0.974 (0.792 - 1.198)	

Table 4. Univariate cox regression analysis of OS and recurrence in HBV-related ICC patients after PSM

Variable	OS		Recurrence	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Sex		0.056		0.020
Male *vs*. Female	1.450 (0.990 - 2.122)		1.528 (1.070 - 2.183)	
Age, years		0.863		0.297
> 60 *vs*. ≤ 60	1.032 (0.723 - 1.473)		0.833 (0.590 - 1.175)	
BMI		0.139		0.423
> 22 *vs*. ≤ 22	0.808 (0.610 - 1.072)		0.897 (0.688 - 1.170)	
Diabetes		0.383		0.722
Yes *vs*. No	0.692 (0.303 - 1.583)		0.885 (0.453 - 1.731)	
Antivirus		0.232		0.176
Yes *vs*. No	0.743 (0.457 - 1.209)		0.752 (0.498 - 1.136)	
Cirrhosis		0.115		0.642
Yes *vs*. No	1.254 (0.946 - 1.662)		1.060 (0.828 - 1.358)	
Gastroesophageal Varices		0.594		0.222
Yes *vs*. No	0.884 (0.561 - 1.392)		0.771 (0.508 - 1.170)	
Ascites		0.487		0.287
Yes *vs*. No	1.198 (0.720 - 1.992)		0.702 (0.366 - 1.346)	
ALT,U/L		0.713		0.757
> 44 *vs*. ≤ 44	0.947 (0.707 - 1.268)		1.044 (0.796 - 1.368)	
PLT, $*10^9$/mL		0.512		0.988
> 100 *vs*. ≤ 100	0.890 (0.628 - 1.261)		0.988 (0.718 - 1.360)	
NLR		0.016		0.027
> 2.5 *vs*. ≤ 2.5	1.414 (1.066 - 1.877)		1.330 (1.034 - 1.711)	
INR		0.322		0.686
> 1.1 *vs*. ≤ 1.1	1.226 (0.819 - 1.834)		0.914 (0.591 - 1.414)	
ALBI Grade		0.116		0.540
II *vs*. I	1.241 (0.948 - 1.623)		1.081 (0.843 - 1.386)	
HBV-DNA, IU/mL		0.931		0.488
> 2,000 *vs*. ≤ 2,000	1.012 (0.765 - 1.339)		0.914 (0.710 - 1.177)	
AFP, ng/mL		0.006		0.008
> 20 *vs*. ≤ 20	1.477 (1.120 - 1.948)		1.404 (1.094 - 1.802)	
CEA, ng/mL		0.146		0.760
> 10 *vs*. ≤ 10	1.465 (0.875 - 2.452)		1.081 (0.656 - 1.781)	
CA19-9, U/mL		< 0.001		0.003
> 37 *vs*. ≤ 37	1.840 (1.326 - 2.553)		1.576 (1.167 - 2.128)	
ASA grade		0.205		0.727
III *vs*. II	0.813 (0.590 - 1.120)		0.950 (0.712 - 1.268)	
Major Hepatectomy		0.212		0.041
Yes *vs*. No	1.223 (0.892 - 1.678)		1.370 (1.012 - 1.855)	
Hilar Clamping, min		0.734		0.328
> 15 *vs*. ≤ 15	1.045 (0.811 - 1.347)		1.126 (0.888 - 1.430)	
Surgical Margin, cm		0.744		0.516
> 1 *vs*. ≤ 1	1.062 (0.739 - 1.528)		0.896 (0.644 - 1.248)	
Lymphadenectomy		< 0.001		< 0.001
Yes *vs*. No	2.117 (1.559 - 2.877)		1.667 (1.254 - 2.217)	
Blood loss,mL	1.000 (0.999 - 1.000)	0.606	1.000 (0.999 - 1.000)	0.340
Transfusion		0.235		0.399
Yes *vs*. No	1.316 (0.837 - 2.070)		1.204 (0.782 - 1.855)	
Tumor Size, cm		< 0.001		0.002
> 5 *vs*. ≤ 5	1.942 (1.467 - 2.570)		1.463 (1.144 - 1.870)	
Tumor Number		0.773		0.961
Multiple *vs*. Single	0.931 (0.571 - 1.516)		1.011 (0.660 - 1.548)	
Microvascular Invasion		< 0.001		< 0.001
Present *vs*. Absent	1.892 (1.395 - 2.568)		1.925 (1.457 - 2.544)	
Satellite		< 0.001		< 0.001
Yes *vs*. No	1.763 (1.314 - 2.366)		1.638 (1.249 - 2.148)	
Capsule		0.160		0.656
Complete *vs*. Incomplete	0.752 (0.505 - 1.119)		1.072 (0.789 - 1.457)	
Diferentiation		0.503		0.221
Moderate/Well *vs*. Poor	0.851 (0.530 - 1.365)		0.774 (0.513 - 1.166)	
Lymph node metastasis		< 0.001		< 0.001
Yes *vs*. No	2.303 (1.610 - 3.2957)		1.757 (1.254 - 2.460)	
Adjuvant Therapy		0.161		0.458
Yes *vs*. No	0.798 (0.582 - 1.094)		0.903 (0.690 - 1.182)	

Table 5. Univariate cox regression analysis of OS and recurrence in HBV-related ICC patients after IPTW

Variable	OS		Recurrence	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Sex		0.729		0.335
Male *vs*. female	1.046 (0.813 - 1.345)		1.125 (0.886 - 1.429)	
Age, years		0.047		0.836
> 60 *vs*. ≤ 60	1.292 (1.004 - 1.663)		0.974 (0.756 - 1.253)	
BMI		0.246		0.889
> 22 *vs*. ≤ 22	0.876 (0.701 - 1.095)		0.985 (0.795 - 1.219)	
Diabetes		0.994		0.605
Yes *vs*. No	0.998 (0.581 - 1.713)		1.140 (0.693 - 1.876)	
Antivirus		0.180		0.264
Yes *vs*. No	0.785 (0.551 - 1.118)		0.840 (0.618 - 1.141)	
Cirrhosis		0.156		0.522
Yes *vs*. No	1.169 (0.942 - 1.452)		0.936 (0.765 - 1.146)	
Gastroesophageal Varices		0.893		0.270
Yes *vs*. No	1.025 (0.718 - 1.463)		0.823 (0.582 - 1.163)	
Ascites		0.424		0.405
Yes *vs*. No	1.206 (0.762 - 1.910)		0.779 (0.433 - 1.402)	
ALT,U/L		0.373		0.166
> 44 *vs*. ≤ 44	1.111 (0.881 - 1.401)		1.162 (0.940 - 1.436)	
PLT, $*10^9$/mL		0.654		0.470
> 100 *vs*. ≤ 100	0.937 (0.705 - 1.246)		1.106 (0.842 - 1.451)	
NLR		0.001		0.058
> 2.5 *vs*. ≤ 2.5	1.428 (1.153 - 1.769)		1.213 (0.994 - 1.480)	
INR		0.048		0.403
> 1.1 *vs*. ≤ 1.1	1.366 (1.003 - 1.862)		0.866 (0.619 - 1.213)	
ALBI Grade		0.001		0.529
II *vs*. I	1.434 (1.159 - 1.775)		1.066 (0.873 - 1.303)	
HBV-DNA, IU/mL		0.979		0.893
> 2,000 *vs*. ≤ 2,000	1.003 (0.808 - 1.244)		0.986 (0.809 - 1.202)	
AFP, ng/mL		0.044		0.010
> 20 *vs*. ≤ 20	1.275 (1.006 - 1.616)		1.342 (1.073 - 1.678)	
CEA, ng/mL		0.024		0.528
> 10 *vs*. ≤ 10	1.536 (1.057 - 2.232)		1.137 (0.764 - 1.691)	
CA19-9, U/mL		< 0.001		< 0.001
> 37 *vs*. ≤ 37	2.028 (1.599 - 2.573)		1.703 (1.354 - 2.142)	
ASA grade		0.105		0.628
III *vs*. II	0.815 (0.637 - 1.044)		0.945 (0.752 - 1.188)	
Major Hepatectomy		0.229		0.053
Yes *vs*. No	1.166 (0.908 - 1.498)		1.261 (0.997 - 1.596)	
Hilar Clamping, min		0.712		0.070
> 15 *vs*. ≤ 15	1.041 (0.841 - 1.288)		1.201 (0.985 - 1.464)	
Surgical Margin, cm		0.104		0.106
> 1 *vs*. ≤ 1	0.774 (0.568 - 1.054)		0.809 (0.626 - 1.046)	
Lymphadenectomy		< 0.001		< 0.001
Yes *vs*. No	2.038 (1.640 - 2.532)		1.663 (1.351 - 2.047)	
Blood loss, mL	1.000 (0.999 - 1.000)	0.925	1.000 (0.999 - 1.000)	0.682
Transfusion		0.023		0.270
Yes *vs*. No	1.430 (1.050 - 1.948)		1.188 (0.874 - 1.614)	
Tumor Size, cm		< 0.001		< 0.001
> 5 *vs*. ≤ 5	1.858 (1.492 - 2.314)		1.516 (1.243 - 1.849)	
Tumor Number		0.398		0.460
Multiple *vs*. Single	1.160 (0.823 - 1.636)		1.141 (0.804 - 1.619)	
Microvascular Invasion		< 0.001		< 0.001
Present *vs*. Absent	1.647 (1.244 - 2.180)		1.732 (1.350 - 2.221)	
Satellite		< 0.001		< 0.001
Yes *vs*. No	1.725 (1.370 - 2.172)		1.653 (1.325 - 2.063)	
Capsule		0.023		0.789
Complete *vs*. Incomplete	0.676 (0.482 - 0.948)		1.038 (0.790 - 1.364)	
Diferentiation		0.723		0.292
Moderate/Well *vs*. Poor	0.939 (0.662 - 1.331)		0.856 (0.640 - 1.143)	
Lymph node metastasis		< 0.001		< 0.001
Yes *vs*. No	2.157 (1.635 - 2.846)		1.963 (1.510 - 2.553)	
Adjuvant Therapy		0.171		0.657
Yes *vs*. No	0.842 (0.658 - 1.077)		1.051 (0.844 - 1.308)	

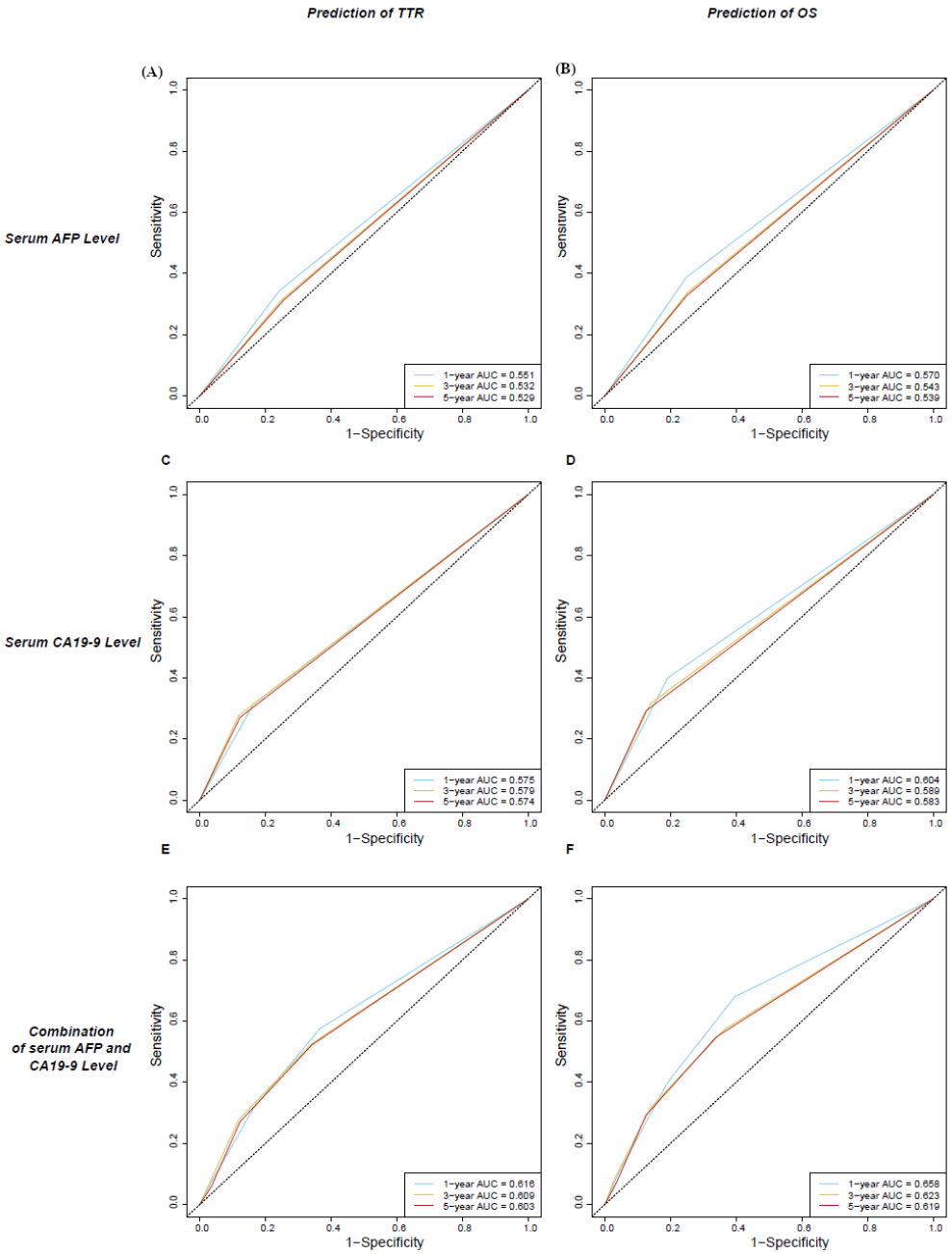

Figure 5. Receiver operating characteristic curve for prediction of TTR and OS. (A) Predition of TTR; **(B)** Predition of OS.

produce alterations in tumor markers and hematologic indices (*32*). Taken together, these findings support the hypothesis that AFP may act as an independent prognostic marker in HBV-ICC because HBV promotes cell-type reprogramming and preserves progenitor-like characteristics in tumors (*9*). Nevertheless, this proposed mechanism requires further tumor-biological investigation.

In conclusion. This retrospective single-center study of a large HBV-related ICC cohort, we demonstrated that elevated serum AFP was independently associated with worse overall survival and higher recurrence across the original, PSM, and IPTW cohorts. Lymph node dissection was identified as a risk factor irrespective of AFP level. Combining AFP with CA19-9 improved prognostic assessment for HBV-ICC. These results suggest that AFP, a routine and accessible biomarker, may help refine risk stratification and guide postoperative surveillance and management in HBV-related ICC; however, multicenter prospective studies and mechanistic research are needed to cnfoirm and further elucidate these observations.

This study has limitations. It is a single-center, retrospective analysis; therefore, our findings require external validation in multicenter cohorts. Residual confounding that was not accounted for in propensity-score methods may still exist.

Table 6. Multivariate cox regression analysis of recurrence and OS in HBV-related ICC patients

Variable	OS		Recurrence	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Primary Cohort				
NLR		0.240		0.870
> 2.5 *vs*. ≤ 2.5	1.140 (0.916 - 1.417)		1.017 (0.833 - 1.241)	
INR		0.306		-
> 1.1 *vs*. ≤ 1.1	1.175 (0.863 - 1.600)		-	
ALBI Grade		0.074		-
II *vs*. I	1.218 (0.981 - 1.512)		-	
Cirrhosis		< 0.001		-
Yes *vs*. No	1.471 (1.174 - 1.842)		-	
AFP, ng/mL		0.023		0.003
> 20 *vs*. ≤ 20	1.294 (1.037 - 1.615)		1.363 (1.113 - 1.670)	
CA19-9, U/mL		< 0.001		0.005
> 37 *vs*. ≤ 37	1.570 (1.239 - 1.990)		1.376 (1.103 - 1.716)	
Lymphadenectomy		0.003		0.424
Yes *vs*. No	1.514 (1.150 - 1.992)		1.114 (0.855 - 1.451)	
Transfusion		0.955		-
Yes *vs*. No	0.992 (0.738 - 1.333)		-	
Tumor Size, cm		< 0.001		0.026
> 5 *vs*. ≤ 5	1.577 (1.246 - 1.995)		1.268 (1.029 - 1.563)	
Microvascular Invasion		0.007		0.002
Present *vs*. Absent	1.433 (1.106 - 1.857)		1.484 (1.162 - 1.895)	
Satellite		0.016		0.004
Yes *vs*. No	1.329 (1.056 - 1.673)		1.381 (1.112 - 1.716)	
Capsule		0.050		-
Complete *vs*. Incomplete	0.727 (0.528 - 0.999)		-	
Lymph node metastasis		0.246		0.021
Yes *vs*. No	1.227 (0.869 - 1.733)		1.492 (1.063 - 2.094)	
PSM Cohort				
Sex		-		0.032
Male *vs*. female	-		1.502 (1.036 - 2.177)	
NLR		0.947		0.533
> 2.5 *vs*. ≤ 2.5	1.010 (0.750 - 1.361)		1.091 (0.829 - 1.436)	
AFP, ng/mL		0.002		0.003
> 20 *vs*. ≤ 20	1.589 (1.188 - 2.126)		1.471 (1.144 - 1.890)	
CA19-9, U/mL		0.017		0.042
> 37 *vs*. ≤ 37	1.520 (1.077 - 2.145)		1.373 (1.011 - 1.865)	
Major Hepatectomy		-		0.874
Yes *vs*. No	-		1.026 (0.744 - 1.417)	
Lymphadenectomy		0.014		0.077
Yes *vs*. No	1.571 (1.097 - 2.249)		1.387 (0.965 - 1.993)	
Tumor Size, cm		0.004		0.150
> 5 *vs*. ≤ 5	1.566 (1.154 - 2.126)		1.231 (0.928 - 1.633)	
Microvascular Invasion		0.002		0.001
Present *vs*. Absent	1.689 (1.217 - 2.345)		1.674 (1.231 - 2.276)	
Satellite		0.010		0.009
Yes *vs*. No	1.491 (1.102 - 2.017)		1.476 (1.103 - 1.976)	
Lymph node metastasis		0.180		0.587
Yes *vs*. No	1.350 (0.871 - 2.094)		1.125 (0.736 - 1.719)	
IPTW Cohort				
Age,years		0.148		-
> 60 *vs*. ≤ 60	1.222 (0.932 - 1.602)		-	
NLR		0.489		-
> 2.5 *vs*. ≤ 2.5	1.084 (0.863 - 1.360)		-	
INR		0.131		-
> 1.1 *vs*. ≤ 1.1	1.299 (0.925 - 1.824)		-	
ALBI Grade		0.027		-
II *vs*. I	1.293 (1.030 - 1.624)		-	
AFP, ng/mL		0.023		0.006
> 20 *vs*. ≤ 20	1.314 (1.038 - 1.663)		1.360 (1.090 - 1.696)	
CEA, ng/mL		0.996		-
> 10 *vs*. ≤ 10	1.001 (0.672 - 1.492)		-	
CA19-9, U/mL		0.001		0.002
> 37 *vs*. ≤ 37	1.570 (1.193 - 2.064)		1.459 (1.148 - 1.853)	
Lymphadenectomy		0.002		0.074
Yes *vs*. No	1.574 (1.185 - 2.091)		1.281 (0.976 - 1.680)	

Table 6. Multivariate cox regression analysis of recurrence and OS in HBV-related ICC patients (continued)

Variable	OS		Recurrence	
	HR (95% CI)	*p*	HR (95% CI)	*p*
Transfusion		0.982		-
Yes *vs*. No	0.996 (0.705 - 1.406)		-	
Tumor Size, cm		0.001		0.024
> 5 *vs*. ≤ 5	1.469 (1.162 - 1.857)		1.272 (1.032 - 1.566)	
Microvascular Invasion		0.032		0.003
Present *vs*. Absent	1.387 (1.028 - 1.870)		1.482 (1.147 - 1.915)	
Satellite		0.017		0.004
Yes *vs*. No	1.351 (1.056 - 1.729)		1.386 (1.106 - 1.736)	
Capsule		0.119		-
Complete *vs*. Incomplete	0.740 (0.507 - 1.081)		-	
Lymph node metastasis		0.485		0.143
Yes *vs*. No	1.144 (0.785 - 1.666)		1.298 (0.916 - 1.839)	

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