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ORIGINAL RESEARCH

The Prognostic Values of Serum Liver Enzymes in Intrahepatic Cholangiocarcinoma Patients After Liver Resection: A Multi-Institutional Analysis of 605 Patients

Yang Huang[1,*], Anque Liao[2,*], Liangliang Xu[1], Hui Li[3], Mingqing Xu[1], Li Jiang[1]

[1]Division of Liver Surgery, Department of General Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, People's Republic of China; [2]Department of Operation Room, West China Hospital, Sichuan University/West China School of Nursing, Sichuan University, Chengdu, People's Republic of China; [3]Department of Hepatobiliary Pancreatic Tumor Center, Chongqing University Cancer Hospital, Chongqing, 400030, People's Republic of China

*These authors contributed equally to this work

Correspondence: Mingqing Xu; Li Jiang, Division of Liver Surgery, Department of General Surgery, West China Hospital, Sichuan University, Chengdu, Sichuan, 610041, People's Republic of China, Tel +86-15908373657, Email xmq_westchina@163.com; 1023987259@qq.com

Purpose: The value of liver enzymes, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT), in predicting the prognosis of intrahepatic cholangiocarcinoma (ICC) patients who underwent curative resection has not been elucidated. Therefore, we aimed to construct prognostic nomograms for surgically treated ICC patients.

Methods: The impact of liver enzymes on overall survival (OS) and recurrence-free survival (RFS) was analysed using Kaplan–Meier analysis and evaluated by univariate and multivariate analyses. Nomograms were constructed for predicting the probability of 1-, 3-, and 5-year OS and RFS and evaluated by receiver operating characteristic (ROC) curves, calibration curves and decision curve analysis (DCA).

Results: High ALT, AST, ALP and GGT levels were associated with worse prognoses in surgically treated ICC patients. Nomograms for OS and RFS were constructed based on five prognostic factors: number of high liver enzyme (No. HLE), CA19-9 ≥ 37 U/mL, multiple tumours, lymph node invasion and microvascular invasion (MVI). Compared with 8th edition TNM stage, these nomograms showed better predictive value. The C-index and 1-, 3- and 5-year areas under the curve (AUCs) of the nomograms for OS and RFS in the discovery and validation cohorts were higher than those of the 8th TNM stage. The calibration plots indicated that there was good agreement between the actual observations and predictions.

Conclusion: Preoperative ALT, AST, ALP and GGT levels could predict prognosis in surgically treated ICC patients. The nomograms showed good predictive ability for predicting the survival of ICC patients.

Keywords: liver enzyme, cholangiocarcinoma, hepatectomy, prognostic factor, nomogram

Introduction

Intrahepatic cholangiocarcinoma (ICC) accounts for approximately 10–15% of primary liver cancers.[1,2] The incidence and mortality of ICC rank behind only hepatocellular carcinoma (HCC) and have shown a significant upwards trend worldwide.[3] The prognosis of ICC is extremely poor owing to its high aggressiveness and malignant biological behaviour, as well as the lack of effective treatment, particularly for patients at an advanced stage.[4,5] For patients with early-stage ICC, surgical resection remains the optimal curative method.[5] However, prognosis after R0 resection is still unsatisfactory, with a 5-year survival rate of < 40% for ICC patients.[6] In addition, systematic chemotherapy of gemcitabine and cisplatin has become a standard strategy for advanced unresectable ICC, but the median survival time for patients is less than one year.[6] Therefore, it is of great importance to explore new prognostic factors that facilitate the identification of ICC patients with a high risk of survival.

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Recently, the 8th edition of the American Joint Committee on Cancer (AJCC) Staging has undergone several significant modifications, particularly in the T category, providing more detailed information to help physicians predict prognosis and make treatment decisions.[7] Apart from tumour staging systems, an increasing number of parameter indicators have been explored in the assessment of ICC prognosis. Tumour markers, such as CEA (carcinoembryonic antigen) and CA19-9, have been confirmed to be closely correlated with the prognosis of ICC patients.[8,9] Inflammatory indicators, such as the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and lymphocyte-to-monocyte ratio (LMR), are also important prognostic factors.[10,11] Moreover, nutritional indicators, such as the albumin-globulin score and skeletal muscle index, can predict the long-term outcomes of ICC patients after curative resection.[12] Liver function indicators, such as albumin–bilirubin (ALBI) grade and albumin-to-alkaline phosphatase ratio (AAPR), also play important roles in prognostic outcomes.[13] To date, accumulating evidence has demonstrated that abnormal expression of liver enzymes may lead to poor prognosis in cancers. For instance, elevated ALT, AST, ALP and GGT levels are often observed in patients with gallbladder cancer, renal cancer and hepatocarcinoma.[14–18] However, the relationship between abnormal liver enzymes and the prognosis of ICC patients following curative resection has not been elucidated.

Therefore, we aimed to assess the prognostic significance of liver enzymes including ALT, AST, ALP and GGT in surgically treated ICC patients, and to propose feasible and user-friendly models to stratify ICC patients at different risk of postoperative outcomes and validate its predictive capacity.

Materials and Methods

Study Population

Patients with ICC who underwent radical resection at two participating institutions from 2011 to 2020 were retrospectively reviewed. A total of 605 patients were included in the study. The last follow-up date was June 1, 2023. The exclusion criteria were as follows: patients who underwent preoperative radiofrequency ablation (RFA), transarterial chemoembolization (TACE) or other anticancer therapies; those with extrahepatic metastasis; those who underwent liver transplantation; those with obstructive jaundice or biliary tract infection; and those with incomplete clinical data or intermittent follow-up time. Patient demographics, clinicopathologic characteristics, preoperative computed tomography (CT) images, and disease status at the end of follow-up were collected from the institutional electronic database and clinical correspondence. This study was performed in accordance with the guidelines of the 1975 Declaration of Helsinki and approved by the Biopharmaceutical Ethics Review Committee of West China Hospital of Sichuan University and the Ethics Review Committee of Chongqing University Cancer Hospital.

Clinical Data Collection

The demographic and clinical parameters upon first admission to the hospital were recorded. We randomly divided all the enrolled patients into discovery (n = 423) and validation (n = 182) cohorts at a ratio of 7:3.[19] In addition, according to the previously reported cut-offs of ALT, AST, ALP, GGT and CA19-9 (ALT: 45 IU/L, AST: 40 IU/L, GGT: 71 IU/L, ALP: 129 IU/L and CA19-9: 37 U/mL),[20] patients were divided into low- and high-ALT groups, low- and high-AST groups, low- and high-ALP groups, and low- and high-GGT groups in the discovery and validation cohorts (Figure 1). Then, according to the No. HLE, we divided patients into three groups: 0, 1–2 and 3–4. Tumour-related clinicopathological characteristics, including differentiation, tumour number, largest tumour size, MVI, cirrhosis, lymph node status, capsule invasion and perineural invasion, were also acquired. The TNM stages were assigned according to the 8th edition of the AJCC staging system.[7] Liver enzymes were measured using standard clinical chemistry methods on the Roche Cobas 8000 modular analyzer series (Roche Diagnostics, Basel, Switzerland) and CA19-9 levels were determined using an immunoassay on the Roche Cobas e602 analyzer by using the Elecsys CA 19–9 assay kit (Roche Diagnostics, Basel, Switzerland).

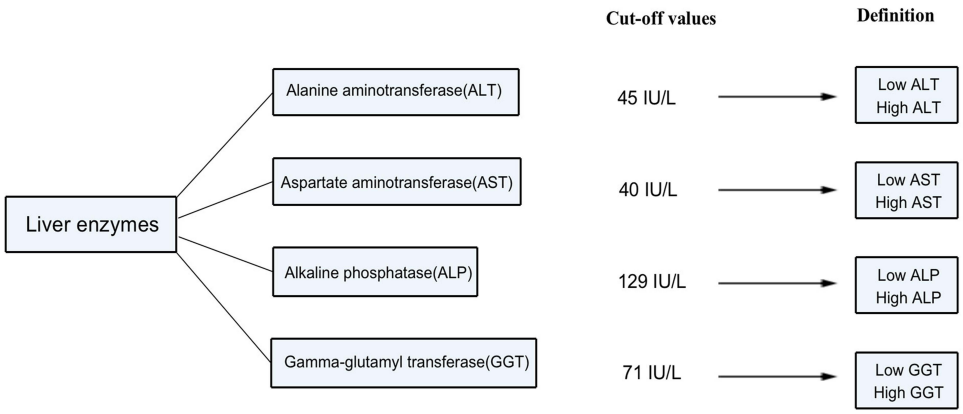

Figure 1 Definitions of the high liver enzymes according to the cut-off values of ALT, AST, ALP and GGT.
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase.

Follow-Up

Patients were followed up according to the National Comprehensive Cancer Network (NCCN) with regular contrast-enhanced ultrasonography every month for the first year, every 3 months for 2 years, and every 6 months thereafter. In addition, we contacted patients who chose not to go back to the hospital for reexamination through a telephone follow-up survey. A total of 25 patients were excluded due to incomplete clinical records or intermittent follow-up time.

Statistical Analysis

Continuous variables are presented as the median (interquartile range, IQR), and categorical variables are presented as percentages. The Mann–Whitney *U*-test was used to determine the difference in continuous variables between groups, and the chi-square test or Fisher's exact test was used for categorical variables as appropriate. The endpoints were OS and RFS. Survival curves were generated by the Kaplan–Meier method and compared with the Log rank test. Risk factors associated with OS and RFS in univariable and multivariable analyses were explored using the Cox proportional hazards regression model. The variables with P values < 0.1 in univariable analyses were incorporated into multivariable analyses. Hazard ratios (HRs) were calculated together with their 95% confidence intervals (CIs).

The independent prognostic indicators identified in the discovery group were integrated to construct two nomograms for predicting the probability of 1-, 3-, and 5-year OS and RFS, and the nomograms were validated in the validation cohort. The discriminating ability of the nomograms was evaluated using the ROC and Harrell's concordance index (C-index). In addition, calibration curves were plotted by visualizing the relationship between the actual outcomes and the predicted probability of outcomes (1000 internal tests by bootstrap). Finally, DCA was used to evaluate the clinical usefulness of the nomogram. All statistical analyses were performed using GraphPad Prism (version 8.0, San Diego, California, USA) and R 4.1.1 software (The R Foundation for Statistical Computing, Vienna, Austria). A two-tailed P value less than 0.05 was considered statistically significant.

Results

Patient Characteristics

In this study, we analysed two cohorts of ICC patients, the discovery and validation cohorts (Table 1). Four hundred and twenty-three patients [213 (50.4%) male; median (IQR) age, 59 (51, 65) years; median (IQR) BMI, 22.66 (20.77–24.87)] were randomly assigned to the discovery cohort. Serum hepatitis B surface antigen (HBsAg) was positive in 122 patients (28.8%), and 71 patients (16.8%) had hepatolithiasis. A total of 136 patients (32.2%) had a history of smoking, and 57 patients (13.5%) had diabetes. CA19-9 < 37 U/mL was observed in 159 patients (37.6%). The median (IQR) ALT, AST, ALP and GGT levels were 31 (22–44), 32 (25–40), 111 (85–157) and 70 (35–145) IU/L, respectively. The numbers of patients classified by No. HLE into the 0, 1 or 2, and 3 or 4 groups were 154 (36.4%), 135 (31.9%) and 134 (31.7%), respectively. The basic pathological characteristics are provided in Table 1. The numbers of patients who were diagnosed

Table 1 Baseline Characteristics and Concomitant Conditions of Included ICC Patients

Variables	Discovery Cohort (n=423)	Validation Cohort (n=182)	P value
Age, year, median (IQR)	59 (51–65)	58 (49–66)	0.797
Male gender, n (%)	213 (50.4)	88 (48.4)	0.651
Body mass index, kg/m2, median (IQR)	22.66 (20.77–24.87)	22.89 (20.64–24.80)	0.878
Smoking status, +, n (%)	136 (32.2)	59 (32.4)	0.949
HBsAg positive, n (%)	122 (28.8)	58 (31.9)	0.455
Hepatolithiasis, n (%)	71 (16.8)	32 (17.6)	0.811
Diabetes, n (%)	57 (13.5)	19 (10.4)	0.301
ALT, IU/L, median (IQR)	31 (22–44)	28 (21–49)	0.515
AST, IU/L, median (IQR)	32 (25–40)	30 (24–41.25)	0.596
ALP, IU/L, median (IQR)	111 (85–157)	106 (82–156.5)	0.356
GGT, IU/L, median (IQR)	70 (35–145)	73 (33.75–153.25)	0.956
No. HLE = 0, n (%)	154 (36.4)	70 (38.5)	0.631
0 < No. HLE < 3, n (%)	135 (31.9)	58 (31.9)	0.991
No. HLE ≥ 3, n (%)	134 (31.7)	54 (29.7)	0.625
CA19-9 < 37 U/mL, n (%)	159 (37.6)	69 (37.9)	0.940
Maximum tumor size, cm, median (IQR)	5.8 (4.2–7.9)	5.5 (4–7.525)	0.670
Solitary tumor, n (%)	303 (71.6)	140 (76.9)	0.178
Tumor differentiation, n (%)			
Well	21 (5.0)	8 (4.4)	0.764
Moderate	112 (26.5)	56 (30.8)	0.280
Poor	290 (68.6)	118 (64.8)	0.370
Liver cirrhosis, n (%)	106 (25.1)	49 (26.9)	0.630
Capsule invasion, n (%)	278 (65.7)	114 (62.6)	0.466
Perineural invasion, n (%)	69 (16.3)	25 (13.7)	0.423
Lymph node invasion, n (%)	107 (25.3)	37 (20.3)	0.188
MVI, n (%)	48 (11.3)	19 (10.4)	0.744
TNM stage, n (%)			
I	71 (16.8)	37 (20.3)	0.296
II	38 (9.0)	22 (12.1)	0.241
III	314 (74.2)	123 (67.6)	0.094

Abbreviations: ICC, intrahepatic cholangiocarcinoma; HBsAg, hepatitis B surface antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; No. HLE, number of high liver enzymes; CA19-9, carbohydrate antigen 19–9; MVI, microvascular invasion.

with TNM stages I, II and III were 71 (16.8%), 38 (9.0%) and 314 (74.2%), respectively. There were no differences in the above clinicopathologic characteristics between the discovery and validation cohorts (all $P > 0.05$).

Association Between Liver Enzymes and ICC Patient Characteristics

Next, we analysed four liver enzyme indicators, ALT, AST, ALP and GGT, in the cohorts using laboratory examinations performed at the first admission to the hospital. The cut-off values reported in the literature were ALT 45 UI/L, AST 40 UI/L, ALP 129 UI/L, and GGT 71 UI/L. Four hundred and twenty-three patients in the discovery cohort were divided into low (n = 265) and high (n = 158) ALT groups, low (n = 290) and high (n = 133) AST groups, low (n = 262) and high (n = 161) ALP groups, and low (n = 212) and high (n = 211) GGT groups (Table 2). The low liver enzyme groups had a higher incidence rate of CA19-9 < 37 U/mL (all $p < 0.05$) and a lower proportion of perineural invasion and lymph node invasion (all $p < 0.05$) than the high liver enzyme groups. In addition, the low ALT, ALP and GGT groups had fewer patients with hepatolithiasis than the corresponding high groups (all $p < 0.05$). Moreover, patients in the low ALP and GGT groups had significantly smaller maximum tumour sizes than those in the corresponding high groups ($p = 0.005$ and $p < 0.001$, respectively). The other characteristics were comparable between the low and high liver enzyme groups.

Table 2 Perioperative and Long-Term Outcomes of Included ICC Patients Undergoing Curative Resection in Discovery Cohort

Variables	ALT		P value	AST		P value	ALP		P value	GGT		P value
	Low	High		Low	High		Low	High		Low	High	
Number	265	158		290	133		262	161		212	211	
Age, year, median (IQR)	59 (51–65)	58 (50–64)	0.764	59 (51–65)	58 (50–63)	0.581	58 (50–65.25)	59.5 (52–63.5)	0.575	59.5 (51–66)	58 (51–64)	0.133
Male gender, n (%)	125 (47.2)	88 (55.7)	0.090	148 (51.0)	65 (48.9)	0.680	141 (53.8)	72 (44.7)	0.069	101 (47.6)	112 (53.1)	0.263
Body mass index, kg/m^2, median (IQR)	22.5 (20.68–24.89)	22.94 (20.96–25.12)	0.234	22.76 (20.7–25.33)	22.3 (20.96–24.55)	0.123	22.14 (20.21–24.46)	22.68 (20.96–24.54)	0.175	22.32 (20.28–25.30)	22.77 (21.17–24.77)	0.262
Smoking status, n (%)	86 (32.5)	50 (31.6)	0.863	92 (31.7)	44 (33.1)	0.781	89 (34.0)	47 (29.2)	0.307	66 (31.1)	70 (33.2)	0.653
HBsAg positive, n (%)	74 (27.9)	48 (30.4)	0.590	78 (26.9)	44 (33.1)	0.192	73 (27.9)	49 (30.4)	0.571	57 (26.9)	65 (30.8)	0.374
Hepatolithiasis, n (%)	34 (12.8)	37 (23.4)	0.005	42 (14.5)	29 (21.8)	0.061	32 (12.2)	39 (24.2)	0.001	26 (12.3)	45 (21.3)	0.013
Diabetes, n (%)	36 (13.6)	21 (13.3)	0.932	42 (14.5)	15 (11.3)	0.370	33 (12.6)	24 (14.9)	0.499	29 (13.7)	28 (13.3)	0.902
CA19-9 < 37 U/mL, n (%)	116 (43.8)	43 (27.2)	0.001	121 (41.7)	38 (28.6)	0.010	115 (43.9)	44 (27.3)	0.001	94 (44.3)	65 (30.8)	0.004
Maximum tumor size, cm, median (IQR)	6 (4.35–7.8)	5.35 (4–7.92)	0.255	5.55 (4–7.2)	6 (4.45–8.5)	0.474	5.4 (4–7)	6.4 (4.4–8.5)	0.005	5 (3.85–7)	6.4 (4.5–8.5)	< 0.001
Solitary tumor, n (%)	187 (70.6)	116 (73.4)	0.529	209 (72.1)	94 (70.7)	0.768	188 (71.8)	115 (71.4)	0.942	159 (75)	144 (68.2)	0.123
Cirrhosis, n (%)	63 (23.8)	43 (27.2)	0.429	66 (22.8)	40 (30.1)	0.107	70 (26.7)	36 (22.4)	0.315	60 (28.3)	46 (21.8)	0.123
Poor tumor differentiation, n (%)	175 (66.0)	115 (72.8)	0.148	192 (66.2)	98 (73.7)	0.124	180 (68.7)	110 (68.3)	0.935	136 (64.2)	154 (73.0)	0.050
Capsule invasion, n (%)	180 (67.9)	98 (62.0)	0.216	197 (67.9)	81 (60.9)	0.157	178 (67.9)	100 (62.1)	0.220	141 (66.5)	137 (64.9)	0.732
Perineural invasion, n (%)	31 (11.7)	38 (24.1)	0.001	40 (13.8)	29 (21.8)	0.038	28 (10.7)	41 (25.5)	< 0.001	23 (10.8)	46 (21.8)	0.002
Lymph node invasion, n (%)	57 (21.5)	50 (31.6)	0.020	65 (22.4)	42 (31.6)	0.044	52 (19.8)	55 (34.2)	0.001	38 (17.9)	69 (32.7)	< 0.001
MVI, n (%)	31 (11.7)	17 (10.8)	0.768	34 (11.7)	14 (10.5)	0.718	30 (11.5)	18 (11.2)	0.932	20 (9.4)	28 (13.3)	0.214
TNM stage, n (%)												
I	43 (16.2)	28 (17.7)	0.691	48 (16.6)	23 (17.3)	0.850	48 (18.3)	23 (14.3)	0.281	41 (19.3)	30 (14.2)	0.159
II	22 (8.3)	16 (10.1)	0.526	21 (7.2)	17 (12.8)	0.064	20 (7.6)	18 (11.2)	0.216	14 (6.6)	24 (11.4)	0.086
III	200 (75.5)	114 (72.2)	0.450	221 (76.2)	93 (69.9)	0.170	194 (74.0)	120 (74.5)	0.911	153 (72.2)	161 (76.3)	0.331

Abbreviations: ICC, intrahepatic cholangiocarcinoma; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; HBsAg, hepatitis B surface antigen; CA-199, carbohydrate antigen 19–9; MVI, microvascular invasion.

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In the validation cohort, 182 patients were divided into low (n =118) and high (n = 64) ALT groups, low (n = 128) and high (n = 54) AST groups, low (n = 115) and high (n = 67) ALP groups, and low (n = 88) and high (n = 94) GGT groups (Supplementary Table 1). Interestingly, the low liver enzyme groups had more patients with CA19-9 < 37 U/mL (all $p < 0.05$). The low ALP and GGT groups had fewer patients with hepatolithiasis than the corresponding high groups ($p = 0.035$ and $p = 0.033$, respectively). In addition, patients in the low-GGT group had a smaller maximum tumour size ($p = 0.009$) and less lymph node invasion ($p = 0.030$) than those in the high-GGT group. The other characteristics were comparable between the low and high liver enzyme groups.

Impacts of Liver Enzymes on Survival

To confirm the validity of these cut-off values, we analysed the OS and RFS of groups stratified by each cut-off point using a Kaplan–Meier analysis in the discovery cohort. As shown in Figure 2, patients with low ALT, low AST, low ALP and low GGT levels showed significantly higher OS rates than those with high liver enzymes ($p = 0.0001$, $p = 0.0003$, $p < 0.0001$ and $p < 0.0001$, respectively) (Figure 2A, C, E and G). Similarly, patients with low ALT, low AST, low ALP and low GGT levels also showed significantly higher RFS rates than those with high liver enzymes ($p = 0.0113$, $p = 0.0002$, $p = 0.0004$ and $p = 0.0008$, respectively) (Figure 2B, D, F and H). In the validation cohort, patients with low ALT, low AST, low ALP and low GGT levels also showed significantly higher OS and RFS rates than those with high liver enzyme levels (all $p < 0.05$) (Supplementary Figure 1a–h). These results suggested that liver enzymes may be associated with the long-term outcomes of ICC.

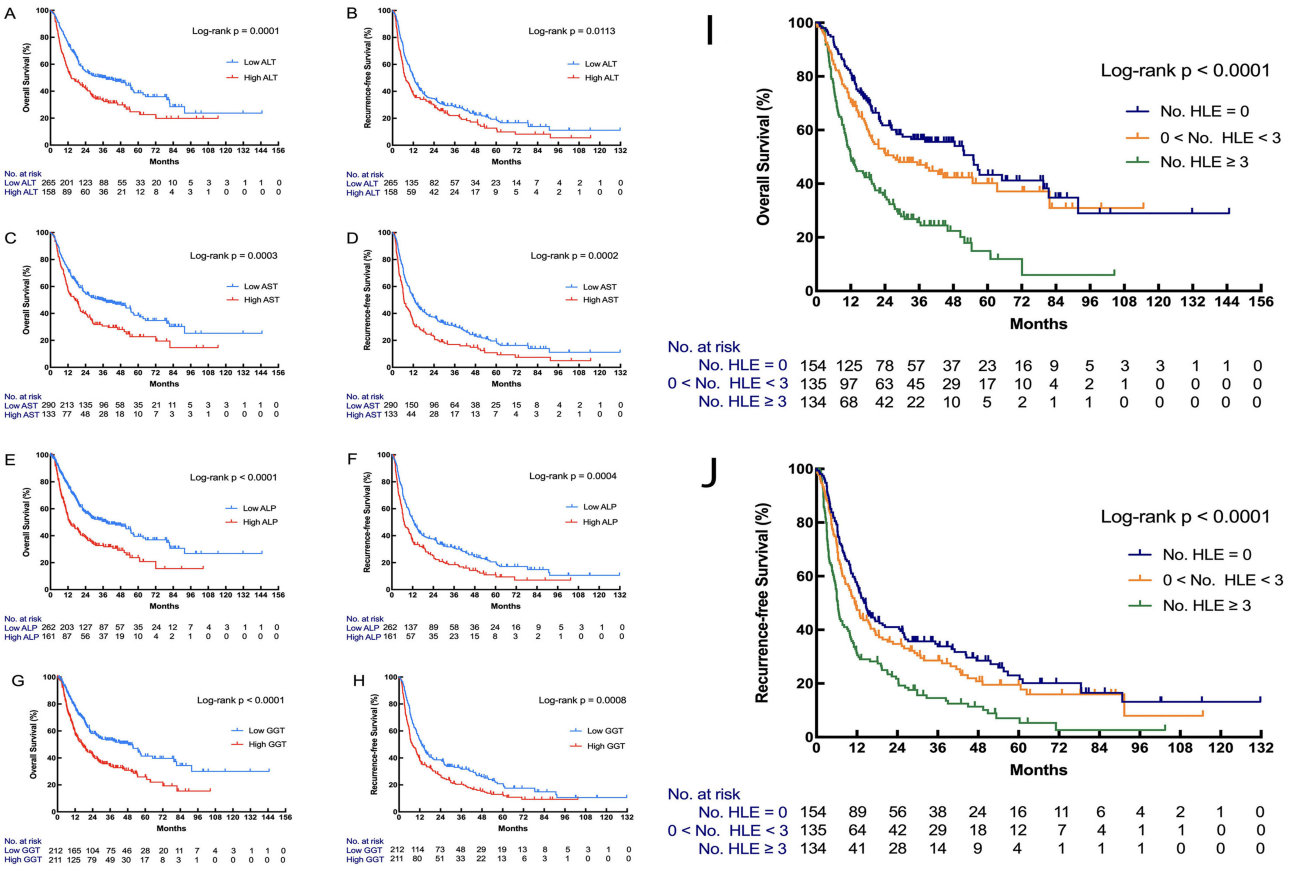

Figure 2 Kaplan–Meier survival curves in patients with high or low ALT, AST, ALP and GGT (**A**–**H**). Survival risk increased based on the increase in the NO. HLE (**I** and **J**).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; NO. HLE, number of high liver enzymes.

To further explore the impact of liver enzymes on the survival of ICC patients, we divided the discovery cohort into three subgroups according to the previously defined No. HLE: No. HLE = 0, 0 < No. HLE < 3, and No. HLE ≥ 3. As shown in Figure 2I and J, patients in the No. HLE = 0 group had the longest OS and RFS. Similarly, in the validation cohort, the three subgroups also exhibited significantly different OS and RFS rates ($p < 0.0001$ and $p = 0.0054$, respectively), as shown in Supplementary Figure 1i and j. These four liver enzyme indicators contributed to increasing mortality risk in an additive manner, suggesting that they are complementary predictors for poor prognosis in patients with ICC.

Univariable and Multivariable Analyses

In the discovery cohort, univariable analyses demonstrated that hepatolithiasis, 0 < No. HLE < 3, No. HLE ≥ 3, CA19-9 ≥ 37 U/mL, multiple tumours, tumour differentiation, perineural invasion, lymph node invasion, MVI and TNM stage II/III were risk factors associated with OS. HBsAg positivity, 0 < No. HLE < 3, No. HLE ≥ 3, CA19-9 ≥ 37 U/mL, maximum tumour size ≥ 5 cm, multiple tumours, tumour differentiation, perineural invasion, lymph node invasion, MVI and TNM stage II/III were risk factors associated with RFS (Table 3). In the validation cohort, the factors related to OS were the same as those in the discovery cohort, and those related to RFS were 0 < No. HLE < 3, No. HLE ≥ 3, CA19-9 ≥ 37 U/mL, maximum tumour size ≥ 5 cm, multiple tumours, tumour differentiation, capsule invasion, lymph node invasion, MVI and TNM stage II/III (Supplementary Table 2).

Finally, based on the criteria described in the Methods section, the following factors were determined to be independent risk factors for OS in the discovery cohort: 0 < No. HLE < 3 (HR: 1.389, 95% CI: 1.112–1.885), No. HLE ≥ 3 (HR: 1.721, 95% CI: 1.251–2.367), CA19-9 ≥ 37 U/mL (HR: 2.051, 95% CI: 1.517–2.772), multiple tumours (HR: 1.473, 95% CI: 1.100–1.973), and lymph node invasion (HR: 1.893, 95% CI: 1.397–2.565). For RFS, the following independent risk factors were identified: 0 < No. HLE < 3 (HR: 1.354, 95% CI: 1.051–1.744), No. HLE ≥ 3 (HR: 1.520, 95% CI: 1.156–1.998), CA19-9 ≥ 37 U/mL (HR: 1.551, 95% CI: 1.218–1.976), multiple tumours (HR: 1.371, 95% CI: 1.060–1.772), and lymph node invasion (HR: 1.608, 95% CI: 1.233–2.098), MVI (HR: 1.648, 95% CI: 1.175–2.311) (Table 3). In the validation cohort, the independent risk factors related to OS and RFS were the same as those in the discovery cohort (Supplementary Table 2).

Nomogram Construction and Validation

Based on the above independent risk factors, two nomograms were developed to predict 1-, 3-, and 5-year OS and RFS in patients with ICC. The nomograms for the OS and RFS of ICC patients in the discovery cohort are shown in Figure 3A and B, respectively. The individual 1-, 3-, and 5-year OS and RFS could be easily measured by adding the specific points for each indicator.

The validation of the nomogram was performed in both the discovery and validation cohorts. As shown in Table 4, in the discovery cohort, the AUC values for 1-, 3-, and 5-year OS were 0.72, 0.75 and 0.77, respectively; the C-index (95% CI) for OS was 0.680 (0.662–0.697). The AUC values for 1-, 3-, and 5-year RFS were 0.71, 0.72 and 0.77, respectively; the C-index (95% CI) for RFS was 0.647 (0.630–0.663). In the validation group, the AUC values for 1-, 3-, and 5-year OS were 0.71, 0.73 and 0.75, respectively; the C-index (95% CI) for OS was 0.651 (0.627–0.676). The AUC values for 1-, 3-, and 5-year RFS were 0.66, 0.73 and 0.74, respectively; the C-index (95% CI) for RFS was 0.626 (0.599–0.651). Compared with the 8^{th} TNM staging system, our two nomograms showed better predictive values (Figure 4A–F and Supplementary Figure 2a–f).

Calibration curves were utilized to visualize the performances of the nomograms in both the discovery and validation cohorts. The calibration plots for 1-, 3-, and 5-year OS and RFS prediction demonstrated good coordination between the predictions of the nomograms and the observed probabilities (Figure 5A–F and Supplementary Figure 3a–f). DCA curves were used to evaluate the clinical utilization of the nomograms. We found that the two nomograms showed favourable net benefits in predicting 1-, 3-, and 5-year OS and RFS in both the training and validation cohorts (Figure 5G–L and Supplementary Figure 3g–l).

Table 3 Prognostic Factor Analysis for Overall Survival and Recurrence-Free Survival in Discovery Cohort

Variables	Overall Survival				Recurrence-Free Survival			
	Univariate Analysis		Multivariate Analysis		Univariate Analysis		Multivariate Analysis	
	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Age, ≥65y	0.908 (0.681–1.209)	0.508			0.817 (0.636–1.049)	0.113		
Gender, male	0.908 (0.710–1.163)	0.445			0.922 (0.745–1.142)	0.458		
Body mass index	0.970 (0.923–1.021)	0.242			0.991 (0.950–1.033)	0.661		
Smoking status	1.158 (0.891–1.504)	0.273			1.079 (0.858–1.355)	0.516		
HBsAg positive	1.162 (0.889–1.519)	0.271			1.236 (0.981–1.558)	0.073	1.143 (0.841–1.554)	0.392
Hepatolithiasis	1.559 (1.154–2.107)	0.004	1.183 (0.861–1.625)	0.299	1.047 (0.789–1.389)	0.751		
Diabetes	1.196 (0.844–1.696)	0.315			1.049 (0.764–1.440)	0.768		
No. HLE = 0	Ref.		Ref.		Ref.		Ref.	
0 < No. HLE < 3	1.311 (0.951–1.806)	0.098	1.389 (1.112–1.885)	0.025	1.314 (0.944–1.756)	0.094	1.354 (1.051–1.744)	0.019
No. HLE ≥ 3	2.452 (1.813–3.317)	< 0.001	1.721 (1.251–2.367)	0.001	1.913 (1.477–2.478)	< 0.001	1.520 (1.156–1.998)	0.003
CA19-9, ≥37 U/mL	2.391 (1.800–3.177)	< 0.001	2.051 (1.517–2.772)	< 0.001	1.767 (1.404–2.223)	< 0.001	1.551 (1.218–1.976)	< 0.001
Maximum tumor size, ≥5cm	1.234 (0.958–1.588)	0.103			1.402 (1.126–1.745)	0.003	1.263 (1.000–1.594)	0.050
Multiple tumor	1.592 (1.225–2.069)	0.001	1.473 (1.100–1.973)	0.009	1.658 (1.316–2.088)	< 0.001	1.371 (1.060–1.772)	0.016
Cirrhosis	1.213 (0.916–1.607)	0.177			1.159 (0.907–1.481)	0.238		
Tumor differentiation, poor	1.954 (1.459–2.617)	< 0.001	1.185 (0.807–1.741)	0.385	1.696 (1.334–2.155)	< 0.001	1.135 (0.835–1.543)	0.418
Capsule invasion	0.964 (0.745–1.248)	0.781			1.082 (0.862–1.358)	0.497		
Perineural invasion	1.867 (1.371–2.544)	< 0.001	1.258 (0.907–1.746)	0.169	1.475 (1.112–1.955)	0.007	1.052 (0.772–1.433)	0.749
Lymph node invasion	2.492 (1.919–3.235)	< 0.001	1.893 (1.397–2.565)	< 0.001	1.994 (1.576–2.524)	< 0.001	1.608 (1.233–2.098)	< 0.001
MVI	1.526 (1.061–2.194)	0.023	1.157 (0.791–1.692)	0.452	1.790 (1.301–2.464)	< 0.001	1.648 (1.175–2.311)	0.004
TNM stage I	Ref.		Ref.		Ref.		Ref.	
TNM stage II	1.651 (0.996–2.735)	0.052	1.233 (0.935–1.625)	0.137	1.547 (1.133–2.112)	0.020	1.032 (0.732–1.455)	0.496
TNM stage III	1.444 (1.011–2.062)	0.043	1.308 (0.913–1.758)	0.092	1.696 (1.086–2.650)	0.006	1.186 (0.727–1.935)	0.858

Abbreviations: HR, hazard ratio; CI, confidence interval; HBsAg, hepatitis B surface antigen; No. HLE, number of high liver enzymes; CA-199, carbohydrate antigen 19–9; MVI, microvascular invasion.

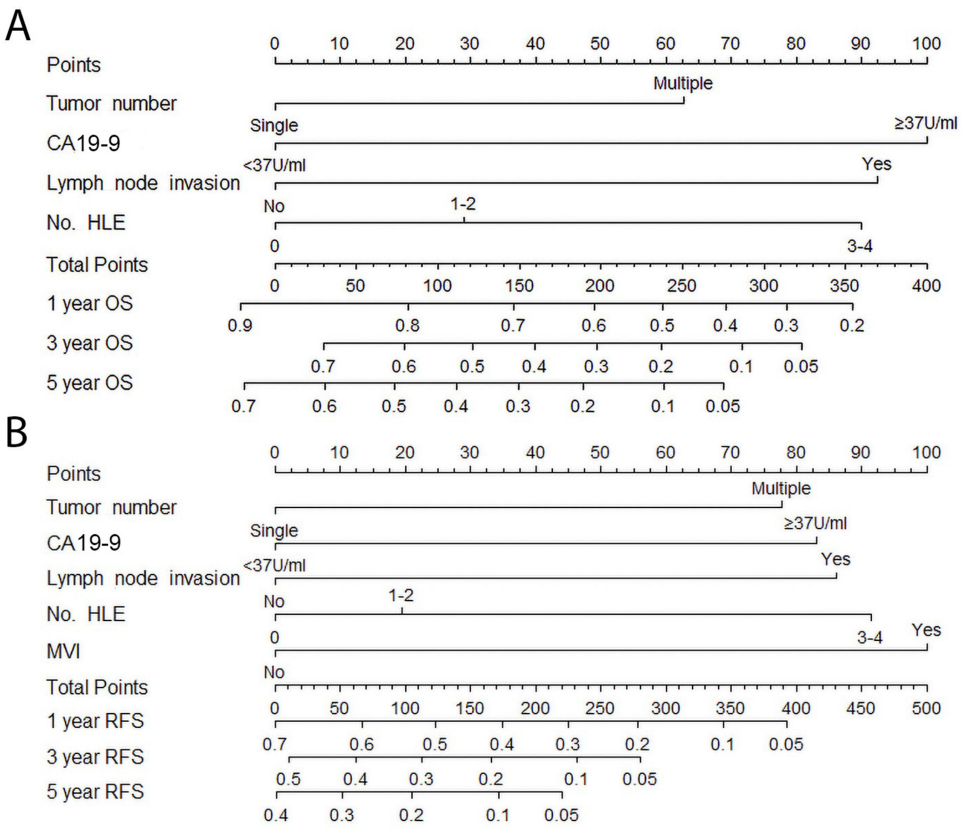

Figure 3 Nomograms predicting OS (**A**) and RFS (**B**) in ICC patients. The estimated 1-, 3- and 5-year probabilities of OS and RFS of the individual patient can be easily obtained.
Abbreviations: OS, overall survival; RFS, recurrence-free survival; ICC, intrahepatic cholangiocarcinoma.

Discussion

The high invasiveness of ICC predisposes it to multifocality, node metastasis and vascular invasions, leading to poor survival after resection, which are common obstacles faced by clinicians.[21,22] In this study, we found that each liver enzyme (ALT, AST, ALP and GGT) was associated with OS and RFS in ICC patients who underwent R0/R1 resection. In addition, according to the No. HLE, we found that patients who had more high liver enzymes had worse survival outcomes, and the NO. HLE was determined to be a significant independent risk factor. We divided the patients into two cohorts and constructed two nomograms to estimate OS and RFS in each cohort. By integrating the NO. HLE and other

Table 4 Accuracy of Nomogram and TNM Stage in Predicting Survival for Surgically Treated ICC Patients

		Models	C-Index	AUC of 1-Year	AUC of 3-Year	AUC of 5-Year
Discovery cohort	OS	Nomogram	0.680 (0.662–0.697)	0.72	0.75	0.77
	OS	8th TNM stage	0.534 (0.520–0.548)	0.55	0.53	0.51
	RFS	Nomogram	0.647 (0.630–0.663)	0.71	0.72	0.77
	RFS	8th TNM stage	0.534 (0.521–0.548)	0.55	0.51	0.56
Validation cohort	OS	Nomogram	0.651 (0.627–0.676)	0.71	0.73	0.75
	OS	8th TNM stage	0.560 (0.535–0.585)	0.55	0.64	0.64
	RFS	Nomogram	0.626 (0.599–0.651)	0.66	0.73	0.74
	RFS	8th TNM stage	0.556 (0.532–0.580)	0.58	0.63	0.72

Abbreviations: ICC, intrahepatic cholangiocarcinoma; AUC, area of under curve; OS, overall survival; RFS, recurrence-free survival.

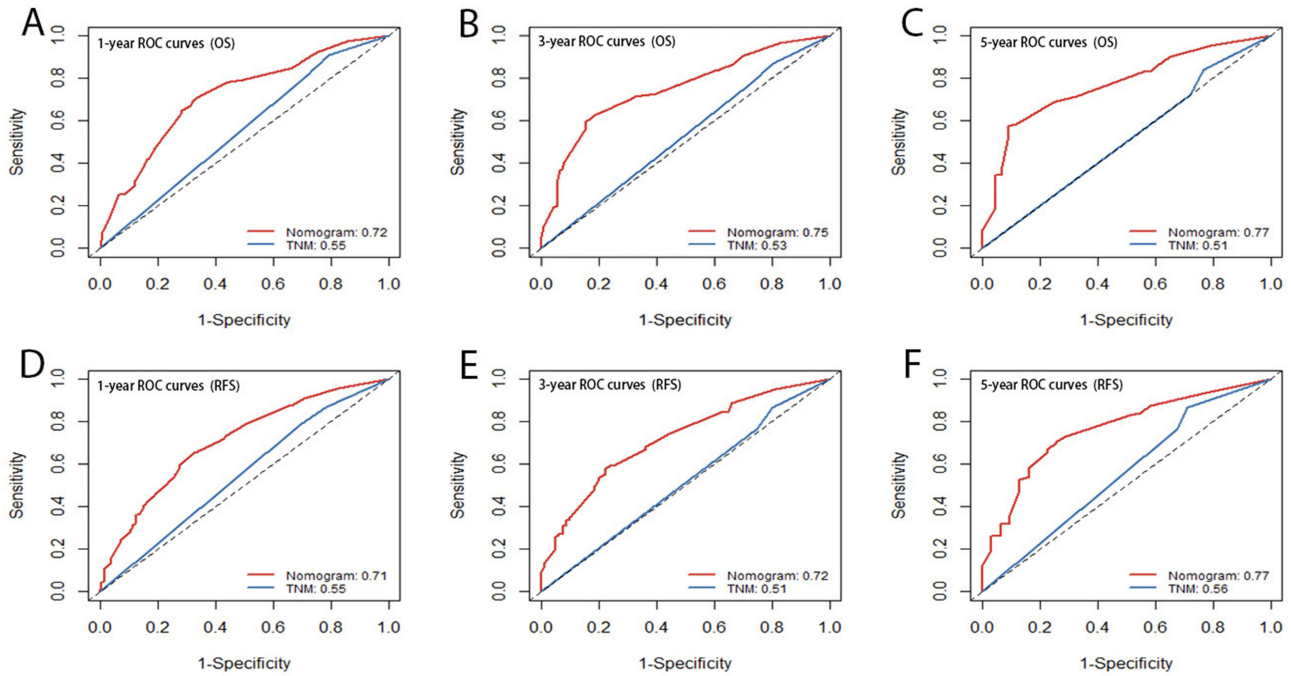

Figure 4 1-, 3- and 5-year ROC curves of OS (**A**–**C**) and RFS (**D**–**F**) for the nomogram and 8th TNM stage in prediction of prognosis in the discovery cohort.
Abbreviations: ROC, receiver operating characteristic; OS, overall survival; RFS, recurrence-free survival.

meaningful clinicopathologic characteristics, such as tumour number, CA19-9 level, lymph node invasion and MVI, our nomograms showed good predictive accuracy.

Abnormal liver enzyme levels may indicate liver damage or alterations in bile flow. In clinical practice, abnormal levels can be categorized into two categories: hepatocellular predominance, characterized by increased ALT and AST levels, and cholestatic predominance, characterized by elevated ALP and GGT levels.[23] These levels usually reflect hepatocyte integrity or cholestasis rather than liver function. Therefore, most previous studies have only investigated the role of liver function indicators in the prognosis of ICC, such as albumin and bilirubin.[13,24] However, the values of liver enzymes have been rarely reported in ICC to date; in addition, few studies have investigated the relationship between liver enzymes and survival in patients with resectable ICC. To the best of our knowledge, this is the first study to explore the effect of liver enzymes on prognosis in patients with resectable ICC.

ALT and AST are released into the blood from damaged hepatocytes after hepatocellular injury or death, and ALT and AST levels are presumed to be markers of hepatic inflammation.[18,25] ALT is an integral part of the evaluation of patients with liver disease, and the importance of ALT activity as an indicator of liver disease has recently been demonstrated in population-based studies led by Kim WR, which documented a strong association between ALT levels and subsequent mortality from liver disease.[18] In addition, patients with elevated AST levels may have higher cancer proliferation rates and more severe tissue damage.[26] In support of this hypothesis, two studies discovered that AST was a significant predictor of liver cancer.[27,28] However, a study conducted by Zhang et al showed that ALT and AST levels had a modest impact on the OS of ICC patients.[23] We explain this difference as follows: 1) recruiting a relatively small number of ICC patients and 2) recruiting patients with relatively severe ICC. When these limitations were eliminated as much as possible, the predictive values of liver enzymes in evaluating the survival outcome of ICC patients were gradually revealed in this study.

ALP is often used to detect obstruction and inflammation of the bile duct system. Although ALP exists in multiple tissues in the body, it could indicate the proliferation of tumour cells, such as HepG2 cells, which also show higher ALP activities in the nucleolus and changes in localization during the cell cycle.[29,30] ALP participates in tumour formation and represents both direct and indirect inflammatory reactions, and it is an independent prognostic factor for ICC

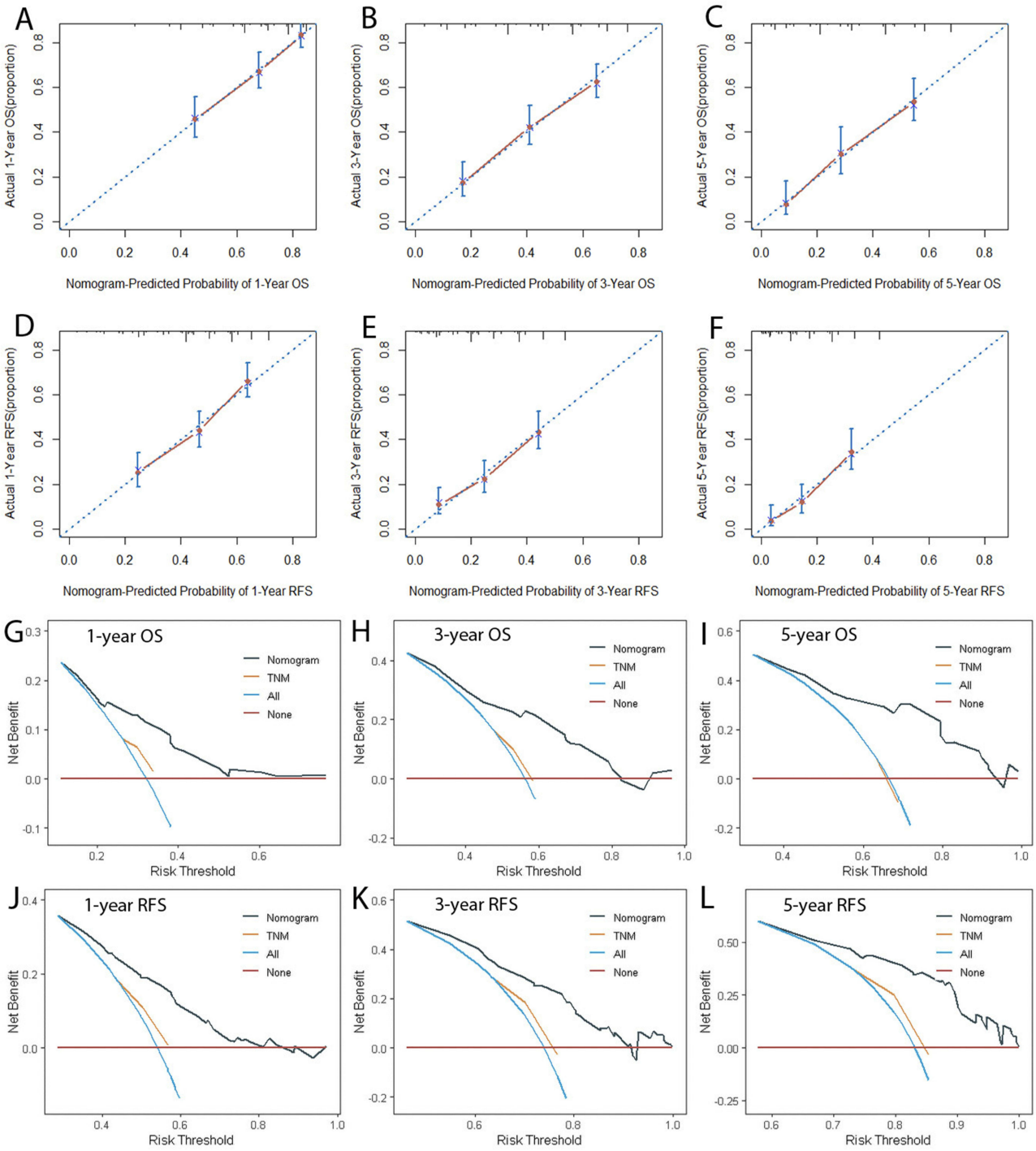

Figure 5 1-, 3- and 5-year calibration curves for probability of ICC patient OS (**A**–**C**) and RFS (**D**–**F**) nomograms construction in discovery cohort (bootstrap = 1000 repetitions) and DCA of OS (**G**–**I**) and RFS (**J**–**L**) in discovery group.

Abbreviations: ICC, intrahepatic cholangiocarcinoma; OS, overall survival; RFS, recurrence-free survival; DCA, decision curve analysis.

patients.[13,31] GGT has been proven to be related to the prognosis of renal cell carcinoma, endometrial carcinoma and oesophageal squamous cell carcinoma.[15,32,33] In a study containing 107 ICC patients who underwent resection, Zhang et al demonstrated that elevated serum GGT concentration was associated with an increased risk of postoperative death and tumour recurrence in patients with HBV-associated ICC.[34] As an oxidative stress marker, GGT overexpression in

cells has been reported to be involved in tumour formation, cell proliferation[34] and inflammatory processes in the extracellular microenvironment.[23] The inseparable relationships of the inflammatory microenvironment with tumours, including ICC, have been widely accepted. Our research further confirmed this viewpoint on the basis of previous achievements.

In the present study, we determined the cut-off values of ALT, AST, ALP and GGT according to previous studies. Then, we explored the prognostic value of these liver enzymes in ICC patients treated by curative liver resection and found that patients with high preoperative ALT, AST, ALP and GGT levels might have unfavourable OS and RFS, as shown in Figure 2. Thereafter, we performed survival analysis in three subgroups that were classified by the NO. HLE. We found that the greater the NO. HLE, the worse the prognosis patients might have, and vice versa. Moreover, in the multivariate analysis of Table 3, the No. HLE, accompanied by several clinicopathological factors, was found to be a significant independent prognostic factor of OS and RFS. The results strongly highlight the importance of liver enzymes in predicting the prognosis of surgically resected ICC patients. Interestingly, as shown in Table 2, we found that CA19-9 ≥ 37 U/mL, perineural invasion and lymph node invasion were significantly correlated with high ALT, AST, ALP and GGT levels; hepatolithiasis was significantly correlated with high ALT, ALP and GGT levels; and maximum tumour size was significantly correlated with high ALP and GGT levels, which confirmed that elevated liver enzymes had a close relationship with the inflammatory microenvironment and tumour formation, progression and recurrence. To the best of our knowledge, few studies have explored the prognostic value of these four liver enzymes in surgically treated ICC patients. Zhang et al's study was slightly different from ours. They found that ALP and AST were independent risk factors for prognosis in ICC patients; however, ALT and AST were not.[23] To reduce the selection bias, only patients who underwent surgical resection were included in our study. We found that ALT and AST also had significant impacts on the survival of ICC patients. Our findings were not contradictory to the results of previous studies but rather elucidated the predictive value of ALT and AST and ALP and GGT, which are characterized by hepatocellular injury and cholestasis, respectively.

However, the current study has several limitations. First, there is no convincing basic medical research to support the concrete effects of ALT, AST, ALP and GGT on tumour formation and progression. Second, selection bias, withdrawal bias and other clinical biases were inevitable due to the retrospective nature of the study. Third, liver enzymes were measured on only one occasion; thus, we could not take individual variability into account. Fourth, we did not conduct external verification. Finally, in this study, we only discussed the impact of liver enzymes on the long-term prognosis of resectable ICC. However, further investigation is needed for other types of cholangiocarcinoma or patients receiving other treatments. Nevertheless, our study was the first to explore the prognostic value of ALT, AST, ALP and GGT levels in ICC patients treated by curative liver resection, which could be effective in clinical practice. In the future, multicentric clinical studies with greater sample sizes are urgently needed to confirm our conclusions and promote the clinical application of serum liver enzymes.

Conclusion

In conclusion, our present study provided important evidence that elevated liver enzymes, such as ALT, AST, ALP and GGT, indicate poor OS and RFS in surgically treated ICC patients, and the more No. HLE the patient has, the worse the prognosis. No. HLE, CA19-9 ≥ 37 U/mL, multiple tumours and lymph node invasion were independent risk factors for OS, and No. HLE, CA19-9 ≥ 37 U/mL, multiple tumours, lymph node invasion and MVI were independent risk factors for RFS. Based on the above independent risk factors, we constructed and validated two nomograms for predicting 1-, 3- and 5-year OS and RFS and confirmed the precise calibration and excellent discrimination power of our nomograms. Our nomograms could be useful for making clinical decisions.

Data Sharing Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Consent to Participate

According to the regulations of the ethics committees at our institution, patient consent is not mandatory for retrospective studies involving the review of medical records. This is because the study does not have a direct impact on patient privacy or health, and the data used were anonymized to ensure the confidentiality of patients' personal information.

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Disclosure

The authors have no relevant financial or non-financial interests to disclose.

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