

Preoperative Immune Prognostic Index Can Predict the Clinical Outcomes of Patients with Gallbladder Cancer: Single-Center Experience

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Purpose: The immune prognostic index (IPI) has been used as a prognostic biomarker in various cancers. However, the prognostic value of the IPI in gallbladder cancer remains to be determined.

Patients and Methods: This study included 139 patients who were diagnosed with gallbladder cancer after surgical resection from 2003 to 2017. We used a Kaplan–Meier curve analysis to evaluate the overall survival (OS). Cox proportional hazards regression methodology was used to identify significant independent prognostic factors. Prognostic nomograms for predicting OS were established to achieve superior discriminatory abilities. The prognostic nomograms were verified according to the concordance index, calibration curves, and decision curve analyses in the training cohort and validation cohort.

Results: Of all 139 patients, 87 (62.6%) patients accepted R0 resection, 32% and 68% were stratified into the good and poor IPI group, respectively. The median OS was 55.9 (range, 5.93–182.7) months in the good IPI group and 15.47 (range, 0.29–190.37) months in the poor IPI group ($P < 0.001$). In the multivariate Cox model, the IPI was an independent predictor of OS along with the CA19-9, curative resection, and postoperative chemoradiotherapy. A nomogram based on these factors was efficient in predicting 1-, 3-, and 5-year survival probabilities. The nomogram showed higher sensitivity and specificity than the current cancer TNM staging system in the training cohort and validation cohort.

Conclusion: The IPI is an independent prognostic factor in gallbladder cancer. Our IPI-based nomogram can serve as a useful and convenient prognostic tool for gallbladder cancer.

Keywords: the immune prognostic index, gallbladder cancer, prognostic value, nomogram, overall survival

Introduction

Gallbladder cancer (GBC) is a rare type of cancer, but it is also the most common malignancy of the biliary tract, accounting for 1.2% of cancer incidence, which is more common in Chile, Eastern Europe, Northern India, and East Asia.¹ Due to the lack of typical symptoms and specific examinations, most patients with GBC present with advanced stage.^{2–4} GBC has low sensitivity to both radiotherapy and chemotherapy,⁵ and effective drugs are lacking; thus, surgical resection is the main treatment.^{6,7} Physicians are now more concerned about the prognosis of GBC after surgical resection. The currently used systemic stratification is the 8th edition of the American Joint Committee on Cancer (AJCC) staging system. However, this system cannot offer accurate and individualized prognostic information.⁸

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Therefore, identification of sensitive prognostic biomarkers for appropriate treatment is needed.

Growing evidence has recently shown that cancer-related inflammation responses have an important role in tumorigenesis, disease progression, and the patient's prognosis.^{9,10} Chronic inflammation is a consistent feature of the tumor microenvironment and a major risk factor for GBC progression.² Tumor-induced systemic inflammation leads to changes in the peripheral blood. Several studies have shown that lymphocytes, neutrophils, monocytes, and monocytes play critical roles in the tumor-induced systemic response.^{9,11} Therefore, inflammatory biomarkers have been studied as prognostic markers in various cancers. Various laboratory biomarkers, including the Glasgow prognosis score,¹² platelet-to-lymphocyte ratio, C-reactive protein, and fibrinogen level,¹³ have been proposed as prognostic biomarkers. However, in real-world clinical trials, some prognostic parameters, such as nutrition-related factors, are difficult to measure. Among them, the relative value of a combined index of the neutrophil and lymphocyte counts (neutrophil-to-lymphocyte ratio [NLR]) can be easily accessed in the clinical setting and has been shown to have association with the prognosis of several tumors including lung cancer,¹⁴ pancreatic, liver, esophageal as well as testicular diffuse large B-cell lymphoma.¹⁵ Furthermore, a derived score composed of the white cell and neutrophil counts (absolute neutrophil count divided by absolute white cell count minus absolute neutrophil count [dNLR]) has been evaluated in patients with cancer, showing its prognostic value similar to that of the NLR. The dNLR has been proven to be an independent prognostic marker in patients with colon cancer¹⁶ and metastatic melanoma.¹⁷

The baseline serum lactate dehydrogenase (LDH) level is also an independent prognostic factor for poor survival outcomes in patients with metastatic melanoma,¹⁸ non-small cell lung cancer (NSCLC),¹⁹ pancreatic carcinoma,²⁰ metastasized colorectal cancer,²¹ clear cell renal cell carcinoma (RCC),²² and gastric cancer.²³ The LDH level has also been found to be associated with progression-free survival of patients with peripheral T-cell lymphoma²⁴ and testicular diffuse large B-cell lymphoma.¹⁵

In 2019, Mezquita et al²⁵ developed the immune prognostic index (IPI), which combines the pretreatment LDH level and the dNLR to stratify patients into poor, intermediate, and good prognostic groups. Other researchers

have further examined the value of the IPI in NSCLC, RCC, and melanoma.^{24,26} However, whether the IPI has prognostic utility in GBC remains unknown.

In the study, we investigated the association of the IPI with the outcomes of GBC and attempted to establish a prognostic model based on the IPI.

Materials and Methods

Patients and Treatment

We conducted a retrospective study of a cohort of patients diagnosed with GBC by pathological examination after radical surgical resection at Peking Union Medical College (PUMC) Hospital in Beijing, China. From June 2003 to May 2017, 159 patients were reviewed. The inclusion criteria including undergoing surgery, pathologically confirmed GBC, no preoperative antitumor or anti-infection treatments, no other malignant tumors except GBC, and available clinical data at the time of the first diagnosis. The exclusion criteria were lack of a clear pathological diagnosis, history or presence of malignancy, perioperative mortality, and incomplete follow-up. Patients who underwent radical surgical protocols and had pathologically proven R0 resection are defined as curative resection.

Data Collection

Conventional clinicopathological parameters were examined within 3 days preoperatively, including complete physical examination findings, routine blood test results, biochemical test results, and levels of carbohydrate antigen 19-9 (CA19-9). A pathologist staged each patient's cancer at the time of the postoperative histopathological examination according to the 8th edition of the AJCC staging system. The incisional margins, lymph node metastasis, lymphovascular invasion, perineural invasion, and tumor size were verified based on surgical observations and pathological evaluation. Postoperative chemotherapy was obtained by follow-up. All data were obtained from the hospital's record system.

All patients were followed up via telephone, and the last follow-up was completed on 3 February 2020. The OS was calculated as the date of surgery to the date of death of any cause or censoring at the last follow-up visit. The dNLR was calculated as the absolute neutrophil count divided by the absolute white cell count minus the absolute neutrophil count.

The IPI was defined based on a dNLR of >1.4 and LDH level of >270 U/L, characterizing the patients into two groups (good, 0 factors; poor, 1–2 factors). According to the receiver operating characteristic (ROC) curve analysis, a dNLR cut-off value of 1.4 had the highest area under the curve, and the LDH cut-off value was defined according to the limit at PUMC.

Statistical Analysis

Continuous variables with a Gaussian distribution are presented as mean and standard deviation, and those without a Gaussian distribution are shown as median and interquartile range. Categorical variables are reported as number (%). The optimal cut-off values for the dNLR and CA19-9 level were calculated according to the highest area under the ROC curve.

Baseline characteristics were compared between groups using the independent-sample *t*-test and chi-square test, as appropriate. The Log-rank test and Kaplan–Meier method were used to analyze and describe survival curves. Independent prognostic factors were identified by a Cox proportional hazards regression model. Variables with a *p*-value no more than 0.05 in univariate analysis and other potential important variables were selected to enter into the multivariate model.

The patients were randomly divided into the nomogram in the training cohort and validation cohort in a proportion of 1:1. Prognostic nomograms for OS were established based on the result of the multivariate analyses. The Harrell's concordance index (C-index), calibration curves, and decision curve analysis (DCA) were further used to evaluate the predictive performance in the training cohort and validation cohort. The optimal cut-off value for our nomogram-based staging system was calculated through R package "survminer".

SPSS version 25.0 for MacOS (IBM Corporation, Armonk, NY, USA) was used for the data analyses. Nomograms were established by R version 3.5.0 software (<http://www.r-project.org/>). The extension packages included "survival," "rms," "foreign," "stdca," and "survminer". A *P* value of <0.05 indicated a significant difference.

Results

Clinicopathological Characteristics

We initially reviewed 159 patients who received surgical resection and finally 139 patients diagnosed with GBC were included in this analysis. Twenty patients were

excluded, including 4 patients lost to follow-up, 2 patients with perioperative mortality, 4 patients with other malignant tumors, and 10 patients missing key data (LDH). The patients' median age was 64 years (range, 29–85 years), and 59 (42.4%) were male. Among the study population, 67 (48.2%) patients' CA19-9 was higher than 40U/mL, 80 (57.6%) patients underwent curative surgery and 24 (17.3%) patients received postoperative chemoradiotherapy. According to the TNM staging system, 5 (3.6%) patients had stage 0 GBC, 11 (7.9%) had stage 1, 13 (9.3%) had stage 2, 44 (31.7%) had stage 3A, 40 (28.8%) had stage 3B, 9 (6.5%) had stage 4A, and 17 (12.2%) had stage 4B. Among them, the patients in stage 4 received palliative resection in order to reduce tumors and improve their life qualities. The patients' other clinicopathological and laboratory characteristics are summarized in Table 1.

At the end of follow-up, 106 patients had died. The median follow-up duration was 20.5 months (95% confidence interval [CI], 1–153 months) and the median OS was 31.2 months (95% CI, 0.27–190 months).

Clinical Features According to IPI

The IPI was calculated according to a baseline dNLR of >1.4 and LDH level of >270 U/L. Among the 139 patients, 45 (32.4%) had a good IPI and 94 (67.6%) had a poor IPI. As shown in Table 2, the NLR was higher in the poor than good IPI group (3.053 vs 1.466, respectively; $P < 0.001$). The proportions of patients with CA19-9 higher than 40U/mL (31.1% vs 56.4%, $P=0.005$), curative surgery (75.6% vs 48.9%, $P = 0.003$). As shown in Figure 1, a good IPI was associated with longer OS in the overall population and the subgroups divided according to the TNM stage. The other characteristics of the patients in each group are summarized in Table 2.

Prognostic Factors for Surgical Outcome and OS

A poor IPI is associated with poor surgical outcomes. As shown in Table 2, patients with a poor IPI tended to have a longer hospitalization (13 vs 16 days, $P=0.009$). We also found that patients with a poor IPI endured more intraoperative transfusion (2 vs 27, $P = 0.001$), as well as more intraoperative bleeding (44 vs.79, $P = 0.018$).

In the univariate analysis, as shown in Table 3, a dNLR of >1.4 (hazard ratio [HR], 2.897; 95% CI, 1.754–4.783; $P < 0.001$), LDH level of >270 U/L (HR, 2.185; 95% CI,

Table I Baseline Characteristics of Total Patients

Characteristics	Entire Cohort (n=139)	Training Cohort (n=69)	Validation Cohort (n=70)
Age, years	64 (58–72)	63 (57–72)	64 (58–72.25)
Sex			
Male	59(42.4%)	36 (52.5%)	23 (32.9%)
Female	80(57.6%)	33 (47.8%)	47 (67.1%)
Jaundice	21(15.1%)	13 (18.8%)	8 (11.4%)
Gallstones	65(46.8%)	28 (40.5%)	37 (52.9%)
Alcohol	22 (15.8%)	13 (18.8%)	9 (12.9%)
Fatty Liver	8 (5.8%)	4 (5.8%)	4 (5.7%)
Weight loss	52 (37.4%)	28 (40.6%)	24 (34.3%)
hypertension	43 (30.9%)	18 (26.1%)	25 (35.7%)
diabetes	30 (21.6%)	13 (18.8%)	17 (24.3%)
CA19-9>40U/mL	67 (48.2%)	30 (43.5%)	37 (52.9%)
NLR	2.228 (1.649–3.703)	2.162 (1.602–3.611)	2.521 (1.715–3.738)
dNLR	1.683 (1.236–2.443)	1.590 (1.232–2.433)	1.769 (1.244–2.511)
LDH	188 (154–227)	188 (156.5–218)	185 (149.75–228)
IPI			
Good	45 (32.4%)	24 (34.8%)	21 (30.0%)
Poor	94 (67.6%)	45 (65.2%)	49 (70.0%)
R0 resection	87 (62.6%)	43 (62.3%)	44 (62.9%)
Tumor Size> 5cm	24 (17.3%)	13 (18.8%)	11 (15.7%)
Curative surgery	80 (57.6%)	41 (59.4%)	39 (55.7%)
TNM stage			
0	5 (3.6%)	1 (1.4%)	4 (5.7%)
I	11 (7.9%)	3 (4.3%)	8 (11.4%)
II	13 (9.4%)	8 (11.6%)	5 (7.1%)
IIIA	44 (31.7%)	25 (36.2%)	19 (27.1%)
IIIB	40 (28.8%)	18 (26.1%)	22 (31.4%)
IVA	9 (6.5%)	6 (8.7%)	3 (4.3%)
IVB	17 (12.2%)	8 (11.6%)	9 (12.9%)
T stage			
Tis	5 (3.6%)	1 (1.4%)	4 (5.7%)
T1	14 (10.1%)	5 (7.2%)	9 (12.9%)
T2	19 (13.7%)	10 (14.5%)	9 (12.9%)
T3	91 (65.6%)	47 (68.1%)	44 (62.9%)
T4	10 (7.2%)	6 (8.7%)	4 (5.7%)
N stage			
N0	83 (59.7%)	42 (60.9%)	41 (58.6%)
N1	46 (33.1%)	23 (33.3%)	23 (32.9%)
N2	10 (7.2%)	4 (5.8%)	6 (8.6%)
Lymph node metastasis	60 (43.2%)	28 (40.6%)	32 (45.7%)
M stage			
M0	130 (93.5%)	65 (94.2%)	65 (92.9%)
M1	9 (6.5%)	4 (5.8%)	5 (7.1%)
Distant metastasis	9 (6.5%)	4 (5.8%)	5 (7.1%)
Lymphovascular invasion	12 (8.6%)	5 (7.2%)	7 (10.0%)
Perineural invasion	9 (6.4%)	5 (7.2%)	4 (5.7%)
Hospitalization, days	15 (10–20)	14 (11–19.5)	15 (10–20)

(Continued)

Table I (Continued).

Characteristics	Entire Cohort (n=139)	Training Cohort (n=69)	Validation Cohort (n=70)
Intraoperative bleeding			
Volume≤400mL	123 (88.5%)	59 (85.5%)	64 (91.4%)
400mL<Volume<800mL	14 (10.1%)	8 (11.6%)	6 (8.6%)
Volume≥800mL	2 (1.4%)	2 (2.9%)	0 (0.0%)
Intraoperative transfusion	29 (20.9%)	17 (24.65)	12 (17.1%)
Postoperative chemoradiotherapy	24 (17.3%)	12 (17.4%)	12 (17.1%)
Postoperative complications	31 (22.3%)	16 (23.2%)	15 (21.4%)

Abbreviations: OS, overall survival; CA19-9, carbohydrate antigen 19-9; ALB, albumin; LAR, lactate dehydrogenase derived by albumin; dNLR, absolute count of neutrophils divided by the absolute white cell count minus the absolute count of neutrophils; LDH, lactate dehydrogenase.

1.211–3.945; $P = 0.009$), and the IPI (HR, 3.036; 95% CI, 1.821–5.062; $P < 0.001$) were associated with shorter OS. The univariate analysis also showed that CA19-9>40 U/mL (HR, 3.326; 95% CI, 2.073–5.051; $P < 0.001$), NLR>2 (HR, 2.433; 95% CI, 1.518–3.898; $P < 0.001$), jaundice (HR, 2.183; 95% CI, 1.322–3.607; $P = 0.002$), R0 resection (HR, 0.436; 95% CI, 0.287–0.664; $P < 0.001$), lymph node metastasis (HR, 3.096; 95% CI, 2.003–4.784; $P < 0.001$), and curative surgery (HR, 0.369; 95% CI, 0.287–0.664; $P < 0.001$) had significant differences in the Cox proportional hazards regression model. Considering that the clinical significance of postoperative chemoradiotherapy was related to prognosis, it is included in the multivariable model although the p value was close to 0.05.

The multivariable model revealed that the IPI (HR, 2.478; 95% CI, 1.467–4.186; $P = 0.001$), CA19-9>40 U/mL (HR, 2.616; 95% CI, 1.658–4.128; $P < 0.001$), curative surgery (HR, 0.426; 95% CI, 0.267–0.657; $P < 0.001$) and postoperative chemoradiotherapy (HR, 0.467; 95% CI, 0.240–0.910; $P = 0.025$) were independently associated with OS.

Establishment and Validation of the Nomogram

According to the results of the Cox proportional hazards model, the nomogram for OS survival (P-risk Plus model) included IPI, CA19-9, curative surgery, and postoperative chemoradiotherapy; it was generated as shown in [Figure 2A](#). In this nomogram, each variable is imputed a weighted point, and the sum of the points can predict 1-, 3-, and 5-year survival probabilities. A higher patient grade is associated with a lower survival probability.

The results showed that the model had superior discrimination (C-index, 0.791 in the training cohort, 0.700 in

the validation cohort). Besides, the performance of the nomogram was evaluated using a calibration curve ([Figure 2B](#)). The predicted line matched the reference line well for 1-, 3-, and 5-year survival, showing good performance of the nomogram. The result showed excellent consistency between nomogram-prediction and actual observation. Similar result was obtained in the validation group ([Figure 2C](#)).

Comparison of Predictive Accuracy for OS Between Nomogram and TNM Staging System

We used DCA to compare the clinical utility between the prognostic model, TNM, and a nomogram without IPI, which is named as reference model in [Supplement Figure 1](#). Higher threshold probability represented better estimation for decision outcomes. Compared with other models, the model yielded the best net benefit across in the range of threshold probability for 1-, 3- and 5-year OS in both training set and validation set, indicating its ability for clinical decision-making is better than TNM staging. The result also showed that the model with IPI showed better net benefit than the model without IPI, which indicated that IPI was occupied a major position in the prediction of the nomogram.

Furthermore, we compared the time-dependent receiver operating characteristic (ROC) curves of the nomogram, the nomogram without IPI (Reference) as well as TNM ([Supplement Figure 2](#)). The result of time-dependent ROC curves indicated that the nomogram has better accuracy than the 8th TNM staging and the nomogram without IPI. The nomogram also showed significant relationship with longer OS in the overall population and in the subgroups

Table 2 Correlation Between IPI and Clinicopathological Characteristics

Characteristics	Good IPI (n=45)	Poor IPI (n=94)	P
Age, years	64 (59–70.50)	64 (57–73.25)	0.754
Sex			0.133
Male	30 (66.7%)	50 (53.2%)	
Female	14 (33.3%)	44 (46.8%)	
Jaundice	4 (8.9%)	17 (18.1%)	0.157
Gallstones	26 (57.8%)	39 (41.5%)	0.072
Alcohol	7 (15.6%)	15 (16.0%)	0.952
Fatty liver	5 (14.7%)	3 (4.8%)	0.124
Weight loss	12 (26.7%)	40 (42.6%)	0.070
Hypertension	17 (37.8%)	26 (27.7%)	0.227
Diabetes	9 (20.0%)	21 (22.3%)	0.754
CA19-9>40 U/mL	14 (31.1%)	53 (56.4%)	0.005*
NLR	1.466 (1.334–1.645)	3.053 (2.321–4.434)	<0.001*
dNLR	1.104 (1.014–1.232)	2.069 (1.668–2.760)	<0.001*
LDH	160 (138.50–197.50)	195 (164.75–234.25)	<0.001*
R0 resection	36 (80.0%)	51 (54.3%)	0.003*
Tumor Size> 5cm	3 (6.7%)	21 (22.3%)	0.022*
Curative surgery	34 (75.6%)	46 (48.9%)	0.003*
TNM stage			0.040*
0	3 (6.7%)	2 (2.1%)	
I	4 (8.9%)	7 (7.4%)	
II	3 (6.7%)	10 (10.6%)	
IIIA	19 (42.2%)	25 (26.6%)	
IIIB	12 (26.7%)	28 (29.8%)	
IVA	3 (6.7%)	6 (6.4%)	
IVB	1 (2.2%)	16 (17.0%)	
T stage			0.680
Tis	3 (6.7%)	2 (2.1%)	
T1	4 (8.9%)	10 (10.6%)	
T2	6 (13.3%)	13 (13.8%)	
T3	29 (64.4%)	62 (66.0%)	
T4	3 (6.7%)	7 (7.4%)	
N stage			0.012*
N0	33 (73.3%)	50 (53.2%)	
N1	12 (26.7%)	34 (36.2%)	
N2	0 (0.0%)	10 (10.6%)	
Lymph node metastasis	8 (17.8%)	52 (55.3%)	<0.001*
M stage			0.271
M0	44 (97.8%)	86 (91.5%)	
M1	1 (2.2%)	8 (8.5%)	
Distant metastasis	1 (2.2%)	8 (8.5%)	0.271
Lymphovascular invasion	1 (2.2%)	11 (11.7%)	0.103
Perineural invasion	2 (4.4%)	7 (7.4%)	0.718
Hospitalization, days	13 (9–17)	16 (11–21)	0.009*
Intraoperative bleeding			0.018*
Volume≤400mL	44 (97.8%)	79 (84.0%)	
400mL<Volume<800mL	1 (2.2%)	13 (13.8%)	
Volume≥800mL	0 (0.0%)	2 (2.1%)	

(Continued)

Table 2 (Continued).

Characteristics	Good IPI (n=45)	Poor IPI (n=94)	P
Intraoperative transfusion	2 (4.4%)	27 (29.0%)	0.001*
Postoperative chemoradiotherapy	9 (20.0%)	15 (16.0%)	0.555
Postoperative complications	11 (24.4%)	20 (21.3%)	0.675

Note: Asterisks indicate statistically significant ($P < 0.05$).

Abbreviations: IPI, immune prognosis index; CA19-9, carbohydrate antigen 19-9; ALB, albumin; LAR, lactate dehydrogenase derived by albumin; dNLR, absolute count of neutrophils divided by the absolute white cell count minus the absolute count of neutrophils; LDH, lactate dehydrogenase.

divided according to the TNM stage and curative surgery (Supplement figure 3).

We also investigated whether our nomogram could distinguish different outcomes of patients with the same TNM stage of GBC. Thus, a histogram of the nomogram-predicted 1-, 3- and 5-year survival probabilities of patients with different TNM stages were developed (Figure 3). Even patients with the same TNM stage clearly had various survival probabilities. The heterogeneity of the nomogram-predicted survival probabilities in the same TNM stage group suggests that our nomogram is able to provide a more detailed classification of the prognosis in patients with GBC than is the 8th version of the AJCC TNM staging system.

Discussion

GBC is an uncommon cancer with a poor prognosis.^{27,28} Surgery is the only effective curative treatment.² Therefore, obtaining accurate prognostic information is necessary.⁶ For patients with advanced GBC, the current TNM staging system is not useful for clinicians to determine a precise treatment before the operation. Thus, a way to more precisely and specifically predict survival is required. Inflammation plays an important role in tumorigenesis.²⁹ Additionally, chronic inflammation is a major risk factor for GBC progression.²

From this perspective, inflammatory biomarkers have been proven to be associated with the prognosis of cancer. Mezquita et al demonstrated that the IPI combined with the LDH level and dNLR was significantly associated with treatment outcomes in patients with NSCLC.²⁵ Furthermore, use of the IPI has been proven effective for predicting the prognosis in patients with RCC and melanoma.^{24,26}

In this cohort of 139 patients, the level of dNLR and LDH had significant associations with OS. Using a combination of these two parameters, an IPI-based scoring system for GBC had developed. This analysis revealed that a poor IPI was significantly associated with shorter OS both in the overall population of the cohort and the subgroups divided by TNM stages. Compared with patients in the good IPI group, patients in the poor IPI group were more likely to have worse survival outcomes and poorer surgical outcomes, including longer hospitalization and more postoperative complications.

The NLR may reflect the host's systemic response to tumor-induced inflammation. Neutrophils can secrete several cytokines to help suppress the tumor-induced T-cell response which elicits tumorigenesis and metastasis.³⁰ A lower lymphocyte count, which leads to a higher NLR, indicates a decreased antitumor response.^{31,32} LDH

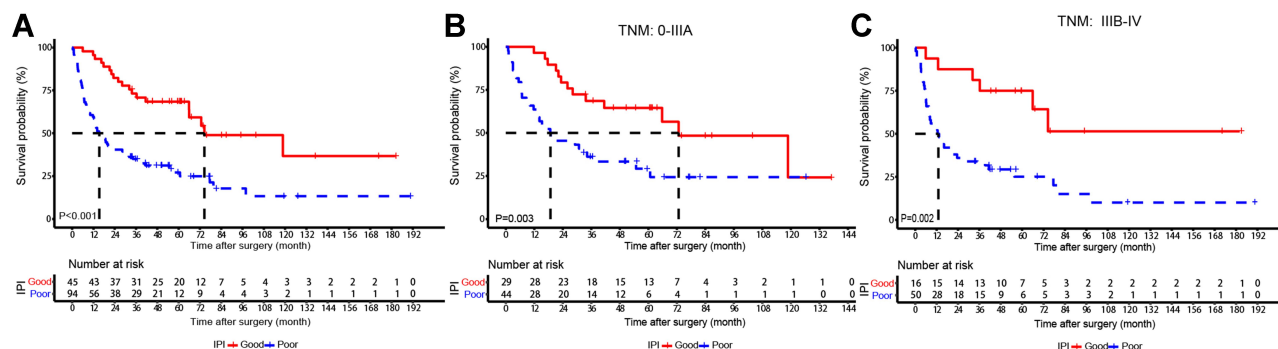


Figure 1 Kaplan-Meier (K-M) survival curves for overall survival rate stratified according to IPI. (A) Kaplan-Meier survival curves shows the overall survival rate in total gallbladder cancer patients; (B) K-M curves shows the overall survival rate in the gallbladder cancer patient graded $\leq 3A$; (C) K-M curves shows the overall survival rate in the gallbladder cancer patient graded $\geq 3B$.

Abbreviation: IPI, immune prognosis index.

Table 3 Univariate and Multivariate Cox Proportional Hazard Analysis of Factors Associated with Overall Survival

Variates	Univariate			Multivariate		
	HR	95%CI	P	HR	95%CI	P
Age						
≤65	reference					
>65	0.906	0.584–1.404	0.657			
Sex						
Female	reference					
Male	0.965	0.631–1.475	0.869			
Jaundice						
No	reference					
Yes	2.183	1.322–3.607	0.002*			
Gallstones						
No	reference					
Yes	0.962	0.634–1.459	0.855			
Alcohol						
No	reference					
Yes	0.650	0.343–1.234	0.188			
Fatty Liver						
No	reference					
Yes	0.416	0.129–1.340	0.142			
Weight loss						
No	reference					
Yes	1.855	1.216–2.829	0.004*			
hypertension						
No	reference					
Yes	0.650	0.401–1.054	0.081			
diabetes						
No	reference					
Yes	0.858	0.516–1.426	0.555			
CA19-9						
≤40 U/mL	reference			reference		
>40 U/mL	3.326	2.073–5.051	<0.001*	2.616	1.658–4.128	<0.001*
NLR						
≤2	reference					
>2	2.433	1.518–3.898	<0.001*			
dNLR>1.4						
No	reference					
Yes	2.897	1.754–4.783	<0.001*			
LDH>270						
No	reference					
Yes	2.185	1.211–3.945	0.009*			
IPI						
Good	reference			reference		
Poor	3.036	1.821–5.062	<0.001*	2.478	1.467–4.186	0.001*

(Continued)

Table 3 (Continued).

Variates	Univariate			Multivariate		
	HR	95%CI	P	HR	95%CI	P
R0 resection No Yes	reference 0.436	reference 0.287–0.664	reference <0.001*			
Curative surgery No Yes	reference 0.369	reference 0.287–0.664	reference <0.001*	reference 0.426	reference 0.276–0.657	reference <0.001*
T stage Tis–T2 T3–T4	reference 1.887	reference 1.123–3.170	reference 0.016*			
N stage N0 N1–N2	reference 1.257	reference 0.825–1.915	reference 0.287			
M stage M0 M1	reference 2.891	reference 1.433–5.833	reference 0.003*			
Lymph node metastasis No Yes	reference 3.096	reference 2.003–4.784	reference <0.001*			
Lymphovascular invasion No Yes	reference 1.013	reference 0.468–2.195	reference 0.973			
Perineural invasion No Yes	reference 1.250	reference 0.543–2.879	reference 0.600			
hospitalization/days	1.017	1.000–1.034	0.047			
Intraoperative bleeding No Yes	reference 1.025	reference 0.661–1.590	reference 0.912			
Intraoperative transfusion No Yes	reference 1.601	reference 0.984–2.602	reference 0.058			
Tumor Size > 5cm No Yes	reference 1.169	reference 0.679–2.013	reference 0.574			
Curative No Yes	reference 0.507	reference 0.279–0.919	reference 0.025			
Postoperative complications No Yes	reference 0.881	reference 0.530–1.465	reference 0.626			

(Continued)

Table 3 (Continued).

Variates	Univariate			Multivariate		
	HR	95%CI	P	HR	95%CI	P
Postoperative chemoradiotherapy						
No	reference			reference		
Yes	0.532	0.275–1.029	0.061	0.467	0.240–0.910	0.025*

Note: Asterisks indicate statistically significant ($P < 0.05$).

Abbreviations: HR, hazard ratio; CI, confidence interval; CA19-9, carbohydrate antigen 19-9; ALB, albumin; LAR, lactate dehydrogenase derived by albumin; dNLR, absolute count of neutrophils divided by the absolute white cell count minus the absolute count of neutrophils; LDH, lactate dehydrogenase; IPI, immune prognosis index.

takes part in the last step of glycolysis by reversibly catalyzing the conversion of pyruvate to lactate.³³ Because cancer cells use glycolysis for energy production, most cancer cells rely on lactate production for their survival. Therefore, the LDH level is a classic marker revealing the metabolic activity of tumor in patients with cancer. Many analyses have revealed that the NLR and LDH level are significantly associated with OS in patients with melanoma,³⁴ biliary cancer,³⁵ and testicular diffuse large B-cell lymphoma.¹⁵ Because the absolute lymphocyte count is not routinely documented in clinical trials despite measurement of the differential white blood cell count, the dNLR is calculated using the white blood cell count and absolute neutrophil count and has become an alternative to the NLR. A study conducted by Proctor et al³⁶ showed that the prognostic value of the dNLR was similar to that of the NLR in different solid cancers.

The similar combination of metabolic and inflammation including GLR (glucose to lymphocyte ratio). It is proved to be one of the independent factors affecting the OS of GBS patients after surgical resection,³⁷ which represent the aggressive biologic behavior of gallbladder cancer. However, the research of IPI mainly focused on all the GBS patients with radical resection, the investigation of GLR was mainly focused on the GBS patients with diabetes. It is currently difficult to determine which indicators' combination is better because of the different population. Further research is needed to explore the contrast between GLR and IPI.

The previously published dNLR cut-off value by Proctor³⁶ is 2.0, which provides less prognostic value than the cut-off value of 1.4 in our cohort. The same situation occurred in the research conducted by Gyeong-Won.³⁸ The discrepancy between these cut-off values might be due to the differences in tumor types and our

limited population. The cut-off values of IPI require further validation in larger and independent cohorts.

In terms of grouping, Mezquita et al divided the IPI into three groups: good, intermediate, and poor. This analysis found that the main difference of IPI in OS was limited in the poor IPI group, and there was no significant difference in OS between the good and intermediate IPI groups. This was also found in patients with RCC in a study by Daniel et al.³⁹ Considering the smaller population size of our cohort, we merged the good and intermediate IPI groups into one group.

For more accurate prediction of survival of patients with GBC, a prognostic nomogram using was constructed, using the factors selected according to the results of the Cox proportional hazards model: IPI, CA19-9, curative surgery, and postoperative chemoradiotherapy. The nomogram predicts the 1-, 3-, and 5-year survival probabilities. More assessments were performed using DCA, concordance index and ROC curves, which showed that our predictive model had satisfactory predictive ability, clinical utility, and improvement over the current staging system. The comparison of DCA, C-index, and ROC between the nomogram with and without IPI showed that IPI is an important factor in predicting the prognosis of GBS patients. We found that according to the nomogram, the survival of patients in the same TNM staging group can still be differed. The result indicates that the nomogram provides more precise prognostic information.

Besides, just as like other tumors, GBC results from the accumulation of multiple genetic alterations. Extensive research showed that a number of genetic alterations, such as TP53, KRAS, NRAS, and IDH1, played an important role in progression of GBC. The molecular characteristics of GBC have obvious heterogeneity and there were significant differences in survival between different molecular subgroups based on molecular characteristics.

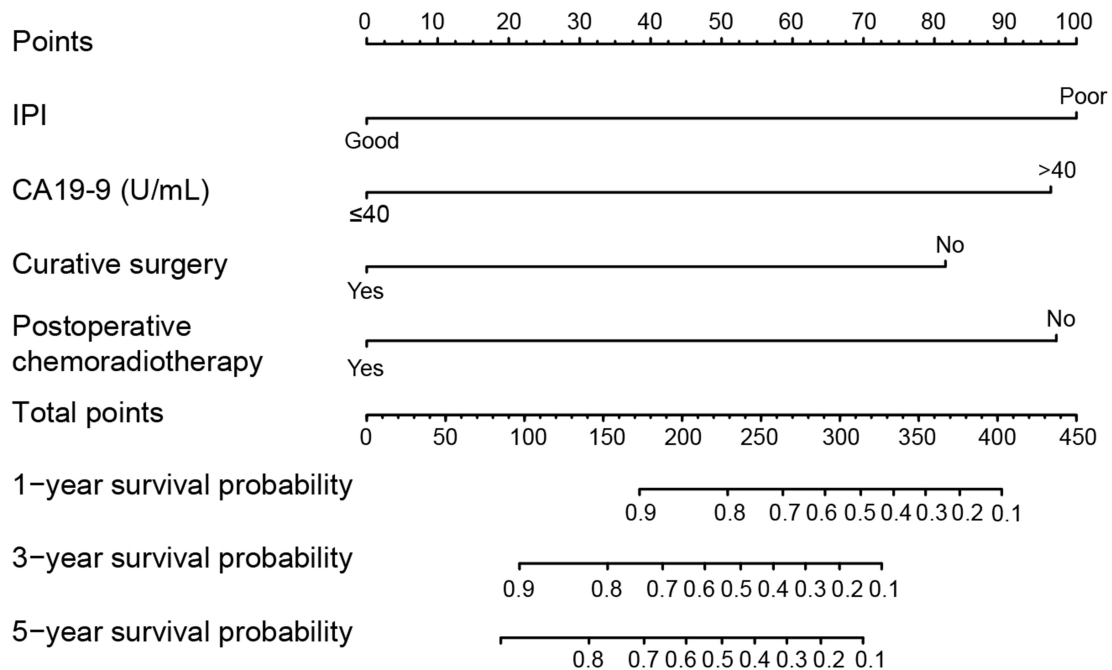
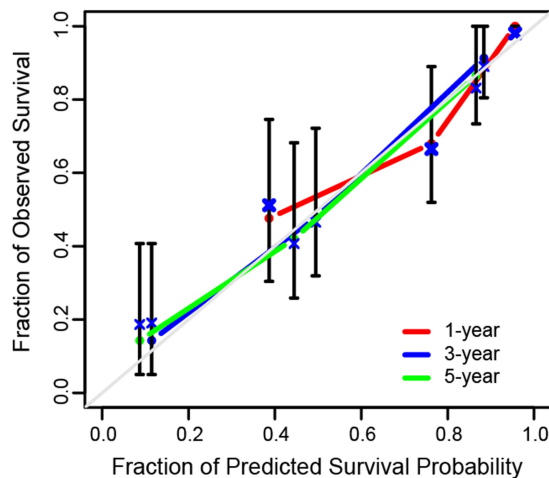
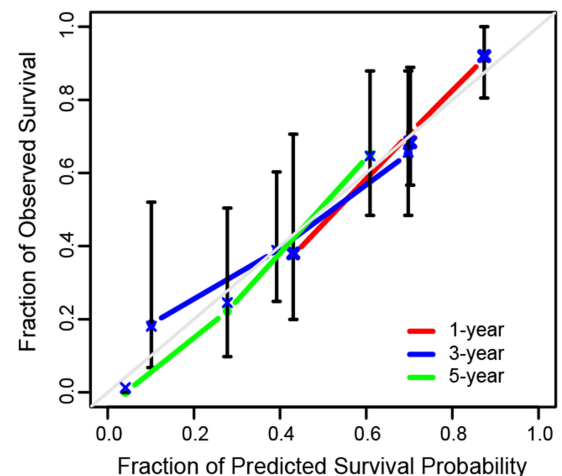
A**B****C**

Figure 2 Nomogram and calibration curves. **(A)** Nomogram based on IPI, CA19-9, curative surgery, and postoperative chemoradiotherapy for predicting overall survival. Calibration curves of the nomogram for 1-year, 3-year, and 5-year OS in training set **(B)** and validation set **(C)**. The x-axis represents nomogram predicted probability of OS, and the y-axis is the actually observed survival probability.

Abbreviations: IPI, immune prognosis index; CA19-9, carbohydrate antigen 19-9; OS, overall survival.

Therefore, the molecular characteristics should be considered as factors added to the prognostic model of GBC in the future. Besides, Whether IPI is associated with some of these genetic changes is unknown.

Our study has several limitations. First, retrospective data were used from a rather small population at a single institution; thus, bias cannot be avoided. Second, the

validation of this study is an internal validation, further external validation is needed. Third, the TNM staging of the patients may have heterogeneity, which needs to be excluded through more precise and strict research. Finally, other factors that may influence survival, such as jaundice and lymph node metastasis, were not considered in this prognosis model.

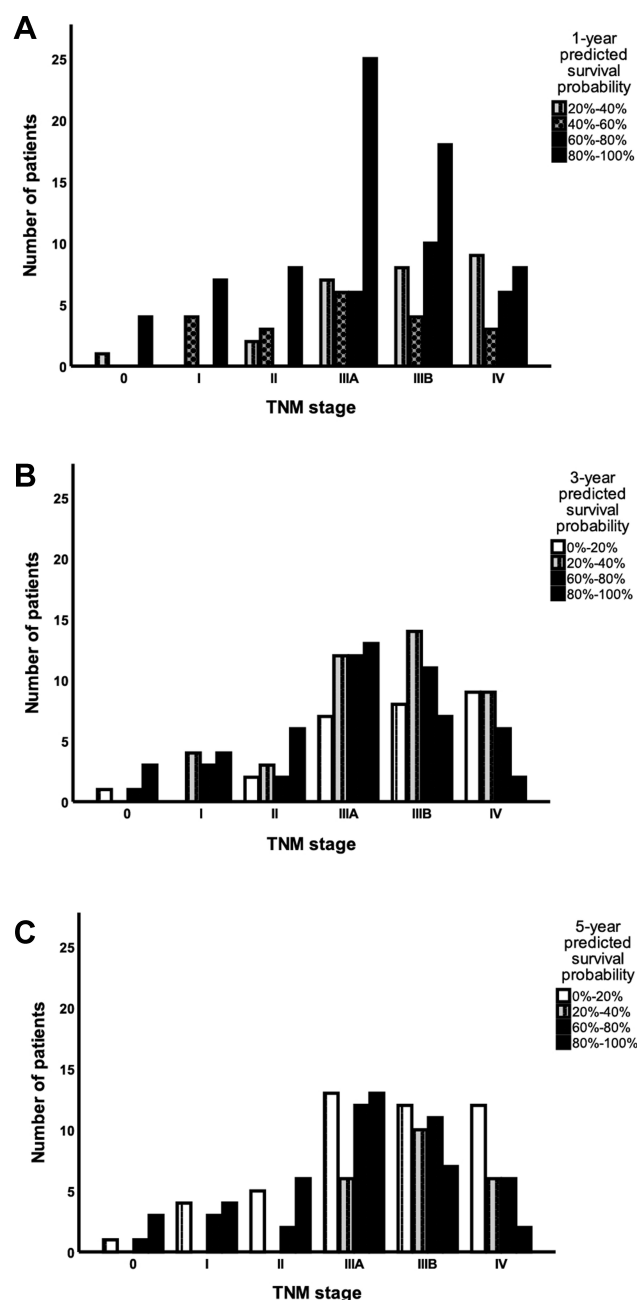


Figure 3 1-year (A), 3-year (B), and 5-year (C) survival probability predicted by nomogram in patients divided in TNM stage. The x-axis represents patients in different TNM stages, and the y-axis is the number of patients within the corresponding interval of nomogram-predicted survival probability.

Conclusions

In conclusion, this study is the first to demonstrate that the IPI is a prognostic indicator for patients with GBC. Our data showed that the IPI was an independent predictor of OS in patients with GBC, and a suitable prognostic nomogram was established. If these results are validated, this IPI-based nomogram can be reliably used for prognostic prediction in patients with GBC. Further validation

through a large-population, multicenter, prospective study is needed.

Ethics and Consent

Ethical approval was obtained from the ethics committees of the PUMC Hospital of the Chinese Academy of Medical Sciences. The study was accompanied with the ethical criteria of the Declaration of Helsinki. Informed consent was not required because of the retrospective nature of the study. All patient data accessed complied with relevant data protection and privacy regulations. The study was registered in Research Registry and the unique Identifying number is 5561. The Hyperlink to this specific registration is: <https://www.researchregistry.com/browse-the-registry#home/registrationdetails/5eac1b9fd0b7cd0015832876/>.

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Disclosure

The authors report no conflicts of interest in this work.

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