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Research article

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Prognostic evaluation in gallbladder carcinoma: Introducing a composite risk model integrating nutritional and immune markers

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ABSTRACT

The importance of evaluating the nutritional status and immune condition prior to surgery has gained significant attention in predicting the prognosis of cancer patients in recent years. The objective of this study is to establish a risk model for predicting the prognosis of gallbladder carcinoma (GBC) patients. Data from GBC patients who underwent radical resection at West China Hospital of Sichuan University (China) from 2014 to 2021 were retrospectively collected. A novel risk model was created by incorporating the prognostic nutritional index and glucose-to-lymphocyte ratio, and each patient was assigned a risk score. The patients were then divided into low- and high-risk cohorts, and comparisons were made between the two groups in terms of clinicopathological features and prognosis. Propensity score matching was conducted to reduce potential bias. A total of 300 GBC patients receiving radical surgery were identified and included in this study. Patients in the high-risk group were older, had higher levels of serum carcinoembryonic antigen (CEA), cancer antigen 125 (CA125), and cancer antigen 19-9 (CA19-9), were more likely to experience postoperative complications, and had more aggressive tumor characteristics, such as poor differentiation, lymph node metastasis, and advanced tumor stage. They also had lower overall survival (OS) rates (5-year OS rate: 11.2% vs. 37.4%) and disease-free survival (DFS) rates (5-year DFS rate: 5.1% vs. 18.2%). After propensity score matching, the high-risk population still experienced poorer prognosis (5-year OS rate: 12.7% vs 20.5%; 5-year DFS rate: 3.2% vs 8.2%). The risk model combining prognostic nutritional index and glucose-to-lymphocyte ratio can serve as a standalone predictor for the prognosis and assist in optimizing the treatment approach for GBC patients.

KEYWORDS: Gallbladder carcinoma, prognostic nutritional index, glucose-to-lymphocyte ratio, risk model, curative-intent surgery, prognosis.

INTRODUCTION

Gallbladder carcinoma (GBC) stands out as the most prevalent tumor within the biliary system and holds the fifth position in terms of frequency among digestive tract tumors. However, its incidence is globally low, showing significant regional variations (1). Western countries, such as the United States, have reported lower rates of incidence at 8.5 cases per 100,000 individuals, while regions like Chile and Northern India have higher rates at 27 and 21.5 cases per 100,000, respectively (2, 3). Known risk factors for GBC include gender, age, and the presence of gallbladder stones or polyps (3). The absence of reliable screenings, coupled with the early onset of subtle symptoms and the cancer's ability to spread quickly, often results in delayed diagnosis and a poor prognosis for most GBC cases (4). Currently, the majority of GBC cases are incidental findings during surgery or postoperative analysis of cholecystectomy procedures performed for non-cancerous gallbladder conditions. The documented prevalence of incidentally discovered GBC ranges from 0.14% to 1.6% (5-7). The only treatment for GBC is surgical removal, and with advancements in surgical techniques and postoperative care, the current 5-year survival rate can range from 22% to 38% (8-10).

The prognostic indicators identified for GBC include pathologic parameters such as the AJCC eighth edition TNM staging system, tumor differentiation, and tumor necrosis (11-13). However, obtaining these parameters before surgery is often difficult since they can only be obtained from surgical resection samples. This presents a challenge in risk stratification and identifying high-risk patients who may require more aggressive treatments. Several studies have investigated the significance of preoperative inflammatory and nutritional status in predicting the prognosis of GBC patients. Inflammatory markers, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), and lymphocyte-to-monocyte ratio (LMR), have shown a correlation with prognosis (14-17). Preoperative nutritional indicators, such as the prognostic nutritional index (PNI) and controlling nutritional status

(CONUT), have also been linked to survival outcomes in tumor patients (18-20). Additionally, the preoperative glucose-to-lymphocyte ratio (GLR) has been found to be a sensitive indicator for evaluating glucose metabolism, cancer aggressiveness, and immunological status in patients with different cancers, such as hepatocellular carcinoma, gastric cancer, and T2 stage GBC (21-23). The majority of prognostic models developed for GBC thus far have relied on tumor markers or pathological parameters. For example, Chen et al. assessed the prognostic significance of systemic immune inflammation index in GBC (24). A recent study developed a predictive model to predict long-term survival in GBC based on CA19-9, peripheral organ invasion, lymph node status and tumor location (25). However, relying solely on a single factor often overlooks the tumor biology and individual patient characteristics, such as nutritional status or immune function. Moreover, the variability in case selection criteria and laboratory standards across different prognostic models limit their clinical utility (26, 27). In this study, we established an innovative risk model incorporating GLR and PNI to preoperatively stratify patients with GBC and anticipate their prognosis.

MATERIALS AND METHODS

Patient selection

We retrospectively compiled the medical data of patients diagnosed with GBC who underwent radical resection at West China Hospital of Sichuan University in China from January 2014 to December 2021. The dataset included demographic details, laboratory test results, surgical information, and reports on pathological diagnoses. To be included, patients had to meet the following criteria: (1) confirmation of GBC diagnosis according to the WHO's 2019 classification, (2) complete clinical and follow-up data (patients with sufficient survival data for a recorded survival period of > 0 months), (3) absence of diabetes, and (4) achievement of R0 resection.

Follow-up assessments

All patients were regularly monitored through telephone interviews or outpatient examinations. During the initial postoperative year, follow-up assessments were conducted every three months and then every six months thereafter. The follow-up procedures included physical examinations, liver function tests, serum levels of CA19-9 and CEA, and computed tomography (CT) or magnetic resonance imaging (MRI) of the chest and abdomen. Overall survival (OS) was defined as the time from the date of radical surgery to either the date of death from any cause or the most recent follow-up date. Disease-free survival (DFS) was calculated from the date of surgery to the most recent follow-up date, unless there was a recurrence during the follow-up period. The most recent follow-up was completed in December 2023.

Data collection

Data on age, sex, BMI, preoperative lymphocyte count, preoperative blood glucose, and preoperative levels of serum CA19-9, CA125, CEA, and albumin were obtained from medical records. Observations with missing data were excluded from the analysis. GLR and PNI were calculated using the formulas GLR = preoperative blood glucose (mmol/L) / total lymphocyte count ($*10^9$ /L); PNI = albumin level (g/L) + 5 × total lymphocyte count ($*10^9$ /L). Tumor features, such as liver resection, bile duct resection, subtypes, differentiation, perineural invasion, lymph node metastasis, T stage, and postoperative complications (Clavien-Dindo grade ≥ II), were determined based on intraoperative data and postoperative pathological results. The data collection table can be found in the supplementary materials.

Construction of the risk model

Univariate and multivariate Cox-regression analysis were applied to identify the associations between GLR, PNI and survival of GBC patients to build the risk model. With the “survival” R package, the risk score of each patient was calculated using the following formula: risk score

= PNI*β1+ GLR*β2 (The R script is available in the supplementary materials). Receiver operating characteristic (ROC) curve analysis was used to determine the optimal cutoff value for the risk score. Based on this value, patients were classified into low- and high-risk populations.

Ethical statement

Approval for this study was granted by the Institutional Ethics Review Board of West China Hospital, and the requirement for informed consent was waived because the study was retrospective.

Statistical analysis

IBM SPSS 23.0 (Chicago, IL, USA), Graph-Pad Prism 8 and R statistics software (v4.2.1) were used to conduct statistical analysis. Median values and ranges were used to summarize continuous variables, while categorical variables were presented as absolute numbers and percentages. Group comparisons were made using appropriate tests such as Fisher's exact test, chi-squared test, or Mann-Whitney U test. The Kaplan– Meier method, along with log-rank tests, was utilized to estimate the probability of survival. The independent prognostic value of factors was evaluated by univariate and multivariate Cox-regression analyses. To reduce confounding bias, a propensity score matching analysis was carried out based on age, serum levels of CEA, CA125, and CA19-9, postoperative complication, tumor differentiation, node metastasis, and tumor stage. Low-risk controls were matched to high-risk cases at a 1:1 ratio using the closest matched propensity score and a caliper width of 0.02 standard deviations. A two-tailed P-value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Through database searches, we identified 401 patients with pathologically confirmed GBC. Among them, 101 patients were excluded from this study: 21 due to R1 resection, 46 due to diabetes, and 34 due to missing clinical and follow-up data. Ultimately, 300 eligible patients were included in our study. Table 1 provides the clinicopathological features of all the participants. Using the defined risk score cutoff value, we categorized these patients into low- and high-risk populations. No significant differences were observed between the two cohorts concerning the sex ratio, BMI, preoperative combined gallbladder stones, liver resection, choledochotomy, pathology subtype, presence of perineural invasion. However, high-risk patients demonstrated elevated levels of serum CEA, CA125 and CA19-9, a higher frequency of postoperative complications, and more aggressive tumor features, such as poor differentiation, the presence of node metastasis, and advanced tumor stage.

Construction and cutoff value of the risk score

Through univariate and multivariate Cox-regression analysis, we determined the prognostic significance of GLR (Multivariate cox: OS, HR: 1.811, 95%CI 1.330-3.440; DFS, HR:1.872, 95%CI 1.403-2.497) and PNI (Multivariate cox: OS, HR: 2.320, 95%CI 1.5523.368; DFS, HR: 2.225, 95%CI 1.403-2.497), as detailed in Table 2. A risk score was calculated for each GBC patient using the formula: risk score = GLR*0.012-PNI*0.07. The area under the curve (AUC) for GLR, PNI, and risk score was determined through ROC curve analysis, with the risk score having the highest AUC (0.713) compared to GLR (0.702) and PNI (0.689). Further ROC analyses were performed for T stage (AUC=0.695) and node metastasis (AUC=0.620), indicating superior predictive ability of the risk model (Figure 1). Additionally, the optimal cutoff value for the risk score was identified as 1.27.

Prognostic significance of risk model

In order to the investigate the prognostic significance of our risk model, we performed Coxregression analysis. Our univariate analysis revealed that age (HR 2.439, 95%CI 1.8223.266), tumor differentiation (HR 1.613, 95%CI 1.217-2.138), perineural invasion (HR 1.561, 95%CI 1.091-2.234), node metastasis (HR 2.483, 95%CI 1.857-3.322), T stage (HR 2.412, 95%CI 1.917-3.035), and risk score (HR 3.227, 95%CI 2.380-4.377) were prognostic factors for OS (Figure 2A). Subsequent multivariate analysis identified that node metastasis (HR 2.013, 95%CI 1.495-2.710), T stage (HR 2.013, 95%CI 1.495-2.710), and risk score (HR 3.293, 95%CI 2.141-5.064) were independent prognostic factors for OS (Figure 2B). In terms of DFS, univariate analysis demonstrated associations between age (HR 2.076, 95%CI 1.581-2.726), tumor differentiation (HR 1.405, 95%CI 1.075-1.838), node metastasis (HR 2.250, 95%CI 1.715-2.952), T stage (HR 1.931, 95%CI 1.565-2.382), risk score (HR 2.857, 95%CI 2.146-3.803), and DFS (Figure 2C). Multivariate analysis further highlighted node metastasis (HR 1.996, 95%CI 1.495-2.710), T stage (HR1.729, 95%CI 1.370-2.182), and the risk score (HR 3.050, 95%CI 2.014-4.621) as independent factors for DFS (Figure 2D).

Survival outcomes

We compared the survival outcomes of patients with different risk scores, specifically looking at OS and DFS. According to the Kaplan-Meier survival curves (Figure 3A, B), high-risk patients had poor OS and DFS. In the low-risk group, the 1-, 3-, and 5-year OS rates were 85.0%, 53.1%, and 37.4%, respectively. For the high-risk group, these rates were 74.5%, 15.2%, and 11.2% at 1, 3, and 5 years, respectively. Furthermore, the high-risk group had a 1-, 3-, and 5-year DFS rates of 66.3%, 29.1%, and 13.4%, respectively.

The researchers used PSM analysis to address selection bias between individuals with different risk scores. A 1:1 PSM process was employed, considering factors such as age, serum CEA, CA125, CA19-9, postoperative complications, tumor differentiation, node metastasis, and

tumor stage. As a result, the two cohorts were effectively balanced, and there were no significant differences in clinicopathological features (Table 3). However, despite this balance, patients with high-risk scores still had lower OS and DFS rates compared to those with low-risk scores (Figure 3C, D). The low-risk group had 1-, 3-, and 5-year OS rates of 82.4%, 41.3%, and 20.5%, respectively, while the high-risk group had rates of 78.5%, 70.6%, and 12.7% at 1, 3, and 5 years, respectively. Similarly, the low-risk group had 1-, 3-, and 5-year DFS rates of 71.6%, 30.6%, and 8.2%, respectively, while the high-risk group had rates of 44.7%, 12.5%, and 3.2%.

Furthermore, we examined the connection between risk score and survival in patients with GBC, separating them by T-stage and node metastasis. For T1 GBC, the OS (p=0.15) and DFS (p=0.16) were comparable between low- and high-risk cohorts (Figure 4A, B).

For T2-3 GBC, patients in low-risk group experienced significantly favorable OS and DFS (Figure 4A, B). Therefore, our results indicate that the calculated risk score effectively predicts the prognosis of patients with T2-3 GBC. Moreover, patients with higher risk scores consistently showed lower OS and DFS even when considering their lymph node status (Figure 4C, D). These findings emphasize the accuracy of our risk model in predicting outcomes for GBC patients.

DISCUSSION

Over the past few years, there has been significant focus on the significance of preoperative nutritional assessment and immune status in predicting outcomes for cancer patients. PNI, which was originally introduced by Buzby et al., is a recognized indicator of both nutritional and inflammatory conditions (28). Studies have demonstrated a link between PNI and worse prognoses in individuals with gastric, esophageal, and breast cancer (29-32). Furthermore,

multiple studies have independently verified the prognostic value of PNI in patients with biliary tract tumors (33).

The metabolic level of tumor cells is higher than normal cells, leading to a need for increased glucose consumption. 18F-fluorodeoxyglucose positron emission tomography is utilized in oncologic imaging to support this. Studies have shown that the uptake of 18F-fluorodeoxyglucose in tumors can estimate both tumor glucose metabolism and biological properties (34). An elevated blood glucose level is associated with poorer prognosis in cancer patients. In particular, elevated levels are a significant risk factor for death in gastric, lung, and liver cancer (35) and are linked to recurrence and metastasis in breast cancer (36). Cellular experiments confirm that a high-sugar environment promotes tumor cell proliferation, activation of pro-cancer signals, and inhibition of apoptosis (37, 38).

Lymphocytes are an essential part of the systemic inflammatory response and play a crucial role in cell-mediated anti-tumor immunity, providing valuable insight into the state of the immune system. Numerous studies have shown a strong link between immune status and the prognosis of individuals with tumors. For example, Garnelo et al. found that lower lymphocyte levels were associated with more advanced tumor stages (39). Similarly, research suggests that the local immune status of tumors can impact the prognosis of patients with BTC. This may be due to the positive effect of tumor-infiltrating lymphocytes on fighting against cancer (40). Conversely, low lymphocyte counts can lead to inadequate immune responses within the tumor microenvironment, promoting cancer progression (41). Hypoalbuminemia, a deficiency in albumin, has been linked to various dysfunctions, including abnormal activation of systemic inflammation, decreased response to drugs, and compromised immune function (42). Moreover, in individuals with advanced tumors, declining albumin levels may be attributable to factors like nutritional status, inflammation, and disease advancement, contributing to an

unfavorable prognosis (43). Furthermore, it is worth considering whether there is a connection between elevated blood sugar levels and a compromised nutritional and immune status in patients with GBC. Previous studies have shown that preoperative immunonutrition can help regulate inflammatory responses during the perioperative period. However, the specific mechanisms underlying the interaction between high blood sugar levels and nutritional and immune status remain unclear, highlighting the need for further research.

In this study, we employed preoperative hematologic parameters to develop a risk stratification model. Our risk model is linked to lymphocyte counts and incorporates both blood glucose and albumin levels to comprehensively assess the nutritional and inflammatory status of patients, which has better predictive power compared to applying GLR or PNI alone (AUC_{GLR} = 0.702, AUC_{PNI} =0.689, $AUC_{Risk\ score}$= 0.713). Based on the risk model, we divided the 300 patients into high- and low-risk population. Patients in the high-risk group exhibited more aggressive tumors, including poorer differentiation, a higher rate of node metastasis, and more advanced tumors. Furthermore, survival analysis showed a significant correlation between a higher risk score and poor long-term survival and recurrence rates.

Furthermore, to minimize any effects of selection bias and balance differences in clinical and pathological parameters between high- and low-risk populations, we utilized PSM analysis. Following PSM, GBC patients with a lower risk score still demonstrated significantly improved survival. Subgroup analysis showed that our risk model had a higher predictiveness for T2-3 GBC ($p<0.001$). Through Cox-regression analyses, we identified this indicator as an independent determinant of both OS and DFS for GBC patients. To our best knowledge, this research is the first investigation that PNI combined with GLR could provide preoperative risk stratification of patients and prognostic information for GBC patients undergoing radical surgery. These findings indicated that nutrition and immune conditions evaluated by our risk score were related to the survival outcomes of GBC individuals, which revealed the importance

of perioperative nutritional support in the management of GBC patients undergoing curative-intent surgery. Moreover, it was observed that GBC individuals with dismal outcomes could be identified preoperatively with this risk model, promising the selection of patients for aggressive treatment strategies. As such, we have confidence that this risk model can assist in making effective treatment decisions and improving prognosis for GBC. Additionally, it is worth noting that this parameter can be easily and cost-effectively obtained through preoperative examinations.

Although our study examined the prognostic significance of our risk model and uncovered its correlation with inferior survival in GBC, it is important to recognize the limitations inherent in our study. First, despite our cohort's inclusion of 300 patients, it is crucial to note that our study design was retrospective, and all patients were sourced from a single center, potentially introducing selection bias. Secon, the inclusion criteria encompassed patients who underwent curative-intent surgery with varying operative modalities and substantial variations in the extent of resection, introducing a potential impact on our results. Additionally, although we excluded GBC patients with preoperative diabetes, other factors that can elevate blood glucose levels may have influenced the accuracy of our risk model. Therefore, further high-quality studies with larger sample sizes and prospective or multicenter designs are necessary to confirm the validity of our results.

CONCLUSION

Based on our study, it can be concluded that the risk model, which combines PNI and GLR, is an independent predictor of prognosis for GBC patients who have undergone radical surgery. This easily accessible metric can accurately identify GBC patients at risk for poor outcomes prior to surgery, providing invaluable guidance for clinical treatment and improving overall prognosis.

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TABLES AND FIGURES WITH LEGENDS

Table 1. Clinical features of all included patients.

Variables	All (n=300)	High risk (n= 150, 50.0%)	Low risk (n= 150, 50.0%)	p-value
Age (years)				0.008
≤60	129 (43.0%)	57 (38.0%)	72 (48.0%)	
>60	171 (57.0%)	93 (62.0%)	78 (52.0%)	
Sex				0.724
Male	121 (40.3%)	59 (39.3%)	62 (51.7%)	
Female	179 (59.7%)	91 (60.7%)	88 (73.3%)	
BMI (kg/m^2)				0.166
≤23	144 (48.0%)	66 (44.0%)	78 (52.0%)	
>23	156 (52.0%)	84 (56.0%)	72 (48.0%)	
CEA (ng/ml)				0.01
≤5	230 (76.7%)	103 (68.7%)	127 (80.0%)	
>5	70 (23.3%)	47 (31.3%)	23 (20.0%)	
CA125 (U/ml)				0.022
≤24	193 (64.3%)	87 (58.0%)	106 (70.7%)	
>24	107 (35.7%)	63 (42.0%)	44 (29.3%)	
CA19-9 (U/ml)				0.028
≤30	149 (41.3%)	65 (38.0%)	84 (44.7%)	
>30	151 (58.7%)	85 (62.0%)	66 (55.3%)	
Gallbladder stones				0.908
Present	145 (48.3%)	72 (48.0%)	73 (48.7%)	

Absent	155 (51.7%)	78 (52.0%)	77 (51.3%)	
Liver resection				0.465
Yes	198 (66.0%)	102 (68.0%)	96 (64.0%)	
No	102 (34.0%)	48 (32.0%)	54 (36.0%)	
Bile duct resection				0.133
Yes	145 (48.3%)	79 (52.7%)	66 (44.0%)	
No	155 (51.7%)	71 (47.3%)	84 (56.0%)	
Postoperative complication				0.012
Present	77 (25.6%)	48 (32.0%)	29 (19.3%)	
Absent	223 (74.3%)	102 (68.0%)	121 (80.7%)	
Pathology				0.197
Adenocarcinoma	267 (89.0%)	130 (86.7%)	137 (91.3%)	
Others	33 (11.0%)	20 (13.3%)	13 (8.7%)	
Differentiation				0.049
Poor	139 (46.3%)	78 (52.0%)	61 (40.7%)	
Moderate/Well	161 (53.7%)	72 (48.0%)	89 (59.3%)	
Perineural invasion present	49 (16.3%)	27 (18.0%)	22 (14.7%)	0.435
Absent	101 (83.7%)	123 (82.0%)	128 (85.3%)	
Node metastasis				0.003
Yes	117 (39.0%)	71 (47.4%)	46 (30.7%)	
No	183 (61.0%)	79 (52.6%)	104 (69.3%)	
pT (8th AJCC)				0.031

T1/T2	228 (76.0%)	106 (70.7%)	122 (81.3%)	
T3	72 (24.0%)	44 (29.3%)	28 (18.7%)	

AJCC: American Joint Committee on Cancer, BMI: body mass index, CA125: carbohydrate antigen 125, CA19-9: carbohydrate antigen 19-9, CEA: carcinoembryonic 427 antigen.

Table 2. Univariate and multivariate analyses of overall survival and disease-free survival.

Variables	**Univariate analysis**			**Multivariate analysis**		
	HR	**95%CI**	**p-value**	**HR**	**95%CI**	**p-value**
Overall survival						
Age (<60 vs ≥60)	2.439	1.822-3.266	<0.001	/	/	0.182
Sex (male vs female)	/	/	0.713	/	/	/
BMI (≤23 vs >23)	/	/	0.476	/	/	/
CEA (≤5 vs >5)	/	/	0.872	/	/	/
CA125 (>24 vs ≤24)	/	/	0.61	/	/	/
CA19-9(>30 vs ≤30)	/	/	0.723	/	/	/
GLR	1.506	1.136-1.996	0.004	1.811	1.330-3.440	<0.001
PNI	2.639	1.962-3.549	<0.001	2.320	1.552-3.368	<0.001
Gallbladder stones	/	/	0.728	/	/	/
Liver resection	/	/	0.184	/	/	/
Bile duct resection	/	/	0.12	/	/	/
Postoperative complication	/	/	0.415	/	/	/
Pathology (Adenocarcinoma vs other)	/	/	0.549	/	/	/

Differentiation (Poor vs moderate/well)	1.613	1.217-2.138	0.001	1.359	1.011-1.827	0.420
Perineural invasion (positive vs negative)	1.561	1.091-2.234	0.015	/	/	0.606
Node metastasis (Positive vs negative)	2.483	1.857-3.322	<0.001	1.778	1.320-2.397	<0.001
pT (8th AJCC) (T1/T2 vs T3)	2.412	1.917-3.035	<0.001	1.816	1.423-2.317	<0.001
Disease-free survival						
Age (<60 vs ≥60)	2.076	1.581-2.726	<0.001	/	/	0.356
Sex (male vs female)	/	/	0.702	/	/	/
BMI (≤23 vs >23)	/	/	0.994	/	/	/
CEA (≤5 vs >5)	/	/	0.592	/	/	/
CA125 (>24 vs ≤24)	/	/	0.846	/	/	/
CA19-9(>30 vs ≤30)	/	/	0.92	/	/	/
GLR	1.663	1.273-2.173	<0.001	1.872	1.403-2.497	<0.001
PNI	2.314	1.755-3.052	<0.001	2.225	1.528-3.241	<0.001
Gallbladder stones	/	/	0.808	/	/	/
Liver resection	/	/	0.325	/	/	/
Bile duct resection	/	/	0.18	/	/	/
Postoperative complication	/	/	0.12	/	/	
Pathology (Adenocarcinoma vs other)	/	/	0.606	/	/	
Differentiation	1.405	1.075-1.838	0.013	/	/	0.234

(Poor vs moderate/well)						
Perineural invasion	/	/	0.06	/	/	/
(positive vs negative)						
Node metastasis	2.25	1.715-2.952	<0.001	1.824	1.380-2.412	<0.001
(Positive vs negative)						
pT (8th AJCC)	1.931	1.265-2.382	<0.001	1.486	1.19-1.857	<0.001
(T1/T2 vs T3)						

AJCC: American Joint Committee on Cancer, BMI: body mass index, CA125: carbohydrate antigen 125, CA19-9: carbohydrate antigen 19-9, CEA: carcinoembryonic antigen, GLR: glucose-to-lymphocyte ratio, PNI: prognostic nutritional index.

Table 3. Clinical features of patients with different risk after propensity score matching.

Variables	Low risk (n= 103)	High risk (n= 103)	p-value
Age			0.780
≤60	48 (32.0%)	46 (44.7%)	
>60	55 (68.0%)	57 (55.3%)	
Sex			0.203
Male	38 (36.2%)	46 (44.7%)	
Female	65 (63.1%)	57 (55.3%)	
BMI (Kg/m^2)			0.676
≤23	50 (48.5%)	53 (51.5%)	
>23	53 (51.5%)	50 (48.5%)	
CEA (ng/ml)			0.503
≤5	82 (79.6%)	78 (75.7%)	
>5	21 (20.4%)	25 (24.3%)	
CA125 (U/ml)			0.236
≤24	73 (70.9%)	65 (63.1%)	
>24	30 (29.1%)	38 (36.9%)	
CA19-9 (U/ml)			0.889
≤30	52 (50.5%)	53 (51.5%)	
>30	51 (49.5%)	50 (48.5%)	
Gallbladder stones			0.329
Present	56 (54.4%)	49 (47.6%)	
Absent	47 (45.6%)	54 (52.4%)	
Liver resection			0.769
Yes	67 (65.0%)	69 (67.0%)	
No	36 (35.0%)	34 (33.0%)	
Bile duct resection			0.329
Yes	46 (44.7%)	53 (51.5%)	

No	57 (55.3%)	50 (48.5%)	
Postoperative complication			0.870
Present	25 (24.3%)	24 (23.3%)	
Absent	78 (75.7%)	79 (76.7%)	
Pathology			0.250
Adenocarcinoma	95 (92.2%)	90 (87.4%)	
Others	8 (7.8%)	13 (13.6%)	
Differentiation			0.889
Poor	50 (48.5%)	52 (50.5%)	
Moderate/Well	53 (51.5%)	51 (49.5%)	
Perineural invasion			0.856
Present	19 (18.4%)	18 (17.5%)	
Absent	84 (81.6%)	85 (82.5%)	
Node metastasis			0.666
Yes	40 (38.8%)	37 (35.9%)	
No	63 (61.2%)	66 (64.1%)	
pT (8th AJCC)			0.330
T1/T2	75 (72.8%)	81 (78.6%)	
T3	28 (27.2%)	22 (21.4%)	

AJCC: American Joint Committee on Cancer, BMI: body mass index, CA125: 435 carbohydrate antigen 125, CA19-9: carbohydrate antigen 19-9, CEA: carcinoembryonic
antigen.

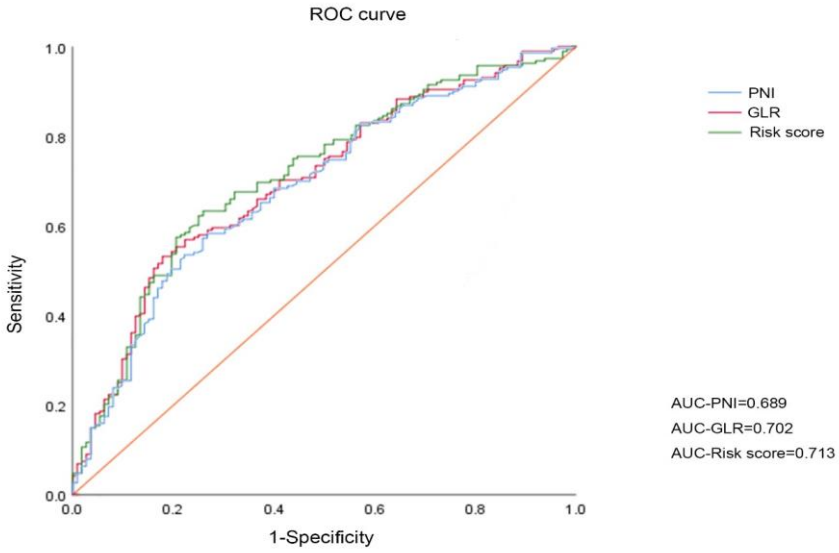

Figure 1. Analysis of the ROC curve for predicting overall survival with the risk score, PNI and GLR.

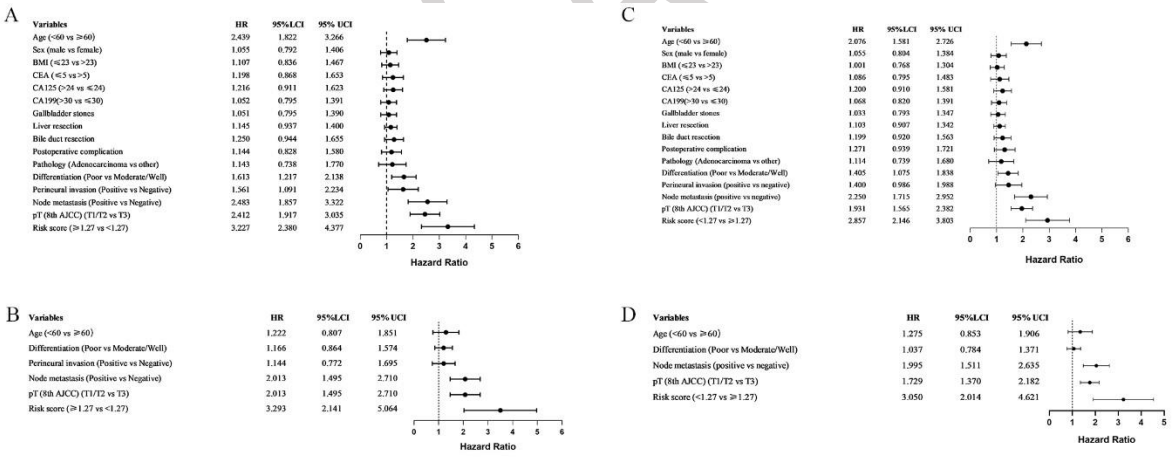

Figure 2. Cox-regression analysis for overall survival and disease-free survival. (A) univariate analysis for overall survival; (B) multivariate analysis for overall survival; (C) univariate analysis for disease-free survival; (D) multivariate analysis for disease-free Survival.

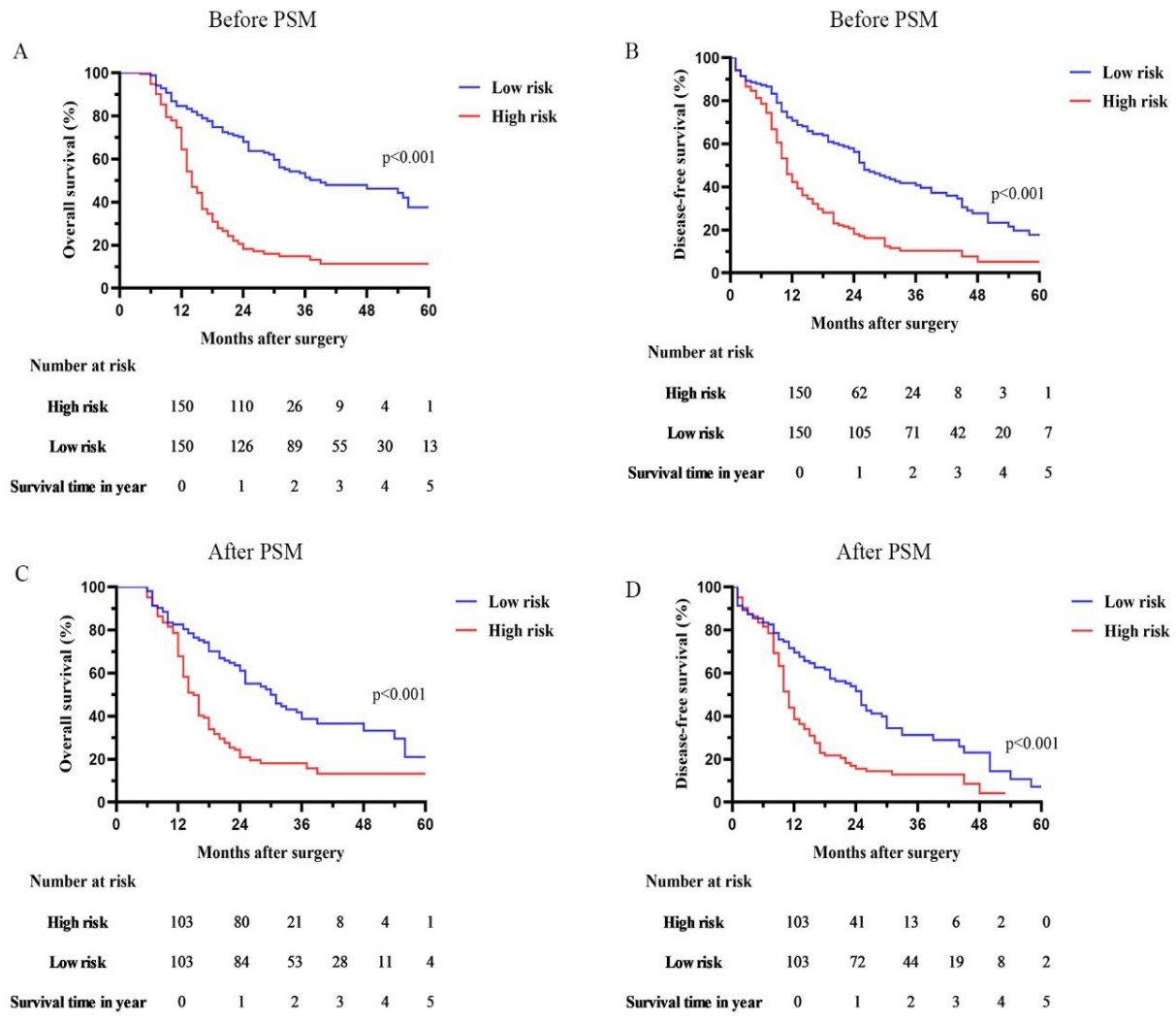

Figure 3. Comparison of survival outcomes between low- and high-risk cohort. (A) overall survival before PSM; (B) disease-free survival before PSM; (C) overall survival after PSM; (D) disease-free survival after PSM.

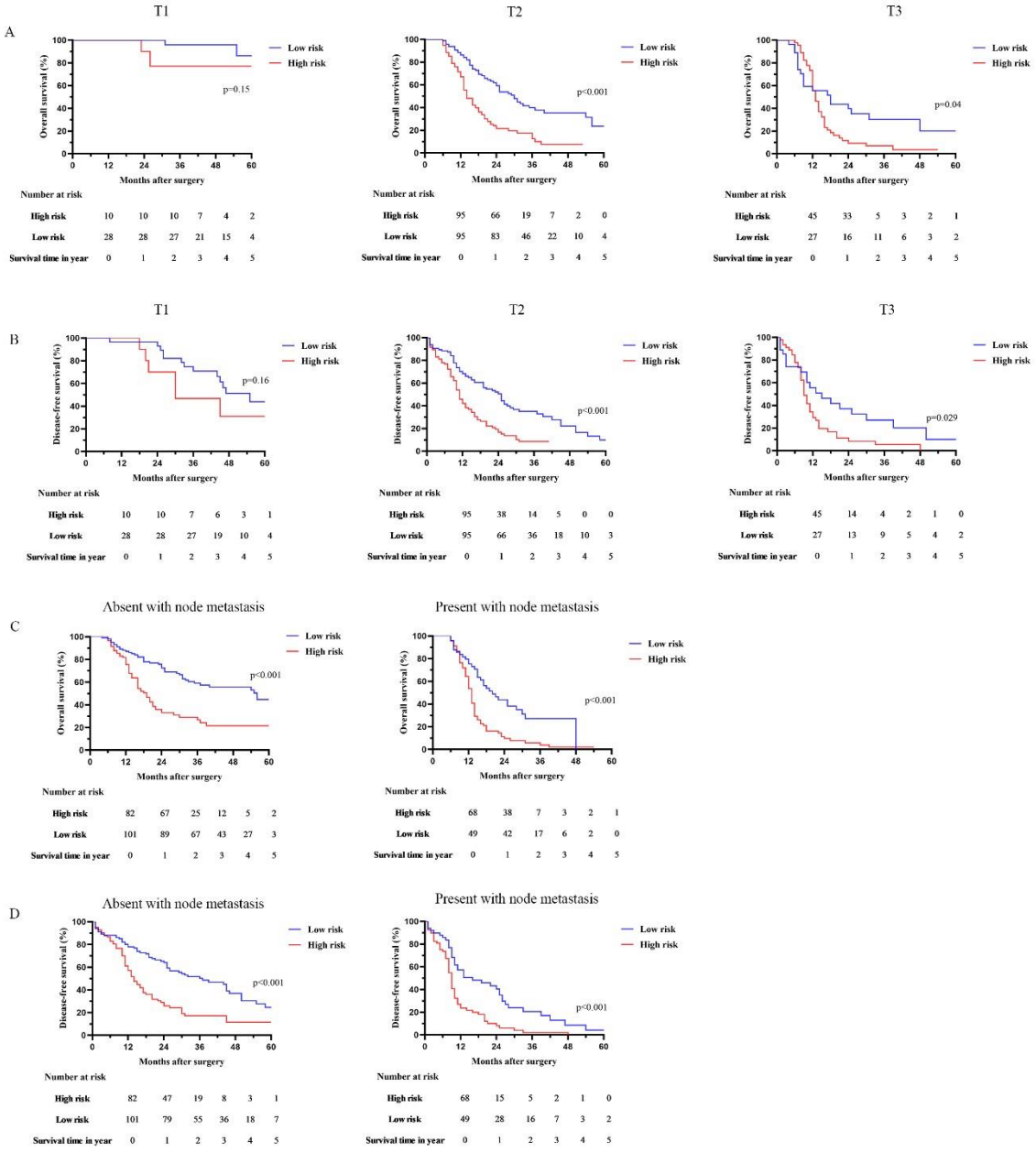

Figure 4. Associations of risk score with the survival outcome of GBC patients stratified based on the T stage. (A) Overall survival; (B) disease-free survival, and node metastasis. (C) overall survival; (D) disease-free survival.