A nomogram based on combining systemic and hepatic inflammation markers for predicting microscopic bile duct tumor thrombus in hepatocellular carcinoma

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Abstract

Background

Bile duct invasion is a relatively rare event and is not well characterized in hepatocellular carcinoma (HCC). There are still very difficult to diagnose hepatocellular carcinoma (HCC) with bile duct tumor thrombus (BDTT) before surgery. Increasing evidences had revealed that inflammation had a critical role in tumorigenesis. The study aimed to develop the nomograms based on systemic and hepatic inflammation markers to predict microscopic bile duct tumor thrombus (micro-BDTT) before surgery in hepatocellular carcinoma (HCC).

Methods

A total of 418 cases with HCC who underwent hepatectomy as initial therapy between January 2012 and June 2020 were included in the study. Receiver operating characteristic (ROC) analysis was used to detect the optimal cut-off value of inflammation markers. Logistic regression was used to identify the independent risk factors of micro-BDTT. The Nomograms was constructed using the significant predictors including α-fetoprotein (AFP), alkaline phosphatase (ALP), direct bilirubin (DB), prognostic nutritional index (PNI), γ-glutamyl transferase (γ-GT)/alanine aminotransferase (ALT). The prediction accuracies of the nomograms were evaluated using the area under the receiver operating characteristic (ROC) curve.

Results

AFP, ALP, DB, PNI and γ-GT/ALT were the independent risk factors for predicting micro-BDTT (P = 0.043, P = 0.028, P = 0.012, P = 0.045 and P = 0.007, respectively), which were assembled into the nomograms. The area under the ROC curve of the nomograms combining PNI and γ-GT/ALT for predicting micro-BDTT were 0.809 (95% confidence intervals (CI): 0.747–0.871). The sensitivity and specificity value when used in predicting micro-BDTT before surgery were 0.778 (95% confidence intervals (CI): 0.656–0.899) and 0.724 (95% confidence intervals (CI): 0.678–0.769), respectively.

Conclusion

The nomogram based on combining systemic and hepatic inflammation markers is suitable for predicting micro-BDTT before surgery in hepatocellular carcinoma patients, leading to a rational therapeutic choice for HCC.

Background
Bile duct tumour thrombus (BDTT) is a relatively rare event but well-known presentation in hepatocellular carcinoma (HCC) with a reported incidence of 1.2–12.9% [1–3]. The first description of BDTT was reported in 1947, which always had the presentation with obstructive jaundice [4]. HCC with BDTT had unique clinicopathological features, such as poor differentiation, an infiltrative pattern, and a high incidence of vascular invasion [5–7]. BDTT had been classified into 2 types: macroscopic BDTT, which represents that invasion of or tumor thrombus was in the first branches of the bile duct and the common hepatic duct, and microscopic BDTT, which represents that invasion of or tumor thrombus was in the second and more peripheral branches of the bile duct [8]. Because of the rare incidence, the prognostic impact of BDTT is still controversial. However, there is a general consensus that HCC patients with BDTT has a poorer prognosis than that without BDTT [8–10]. In addition, HCC patients with BDTT had a higher propensity for early recurrence [10, 11]. At present, the current diagnosis methods for bile duct invasion before surgery was determined by ultrasonic diagnosis, computerized tomography (CT), magnetic resonance imaging (MRI), Magnetic resonance cholangiography (MRCP) or Endoscopic Retrograde Cholangiopancreatography (ERCP), preoperatively [12–14]. Due to advances in imaging an increased understanding of this entity, more and more HCC patients with macroscopic BDTT were confirmed preoperatively. However, the rate of misdiagnosis of HCC with BDTT before surgery is still very high [15]. Particularly, many HCC patients with micro-BDTT could not be accurately diagnosed preoperatively before surgery. In order to resolve this problem, some new markers including some stem cell markers and small molecule metabolite biomarkers were used for HCC with diagnosis [15, 16]. However, it is still urgently needed to find a new diagnosis method which was more accurate and less destructive.

Of note, BDTT that affects bile drainage would cause biliary obstruction and adversely affects the liver function. HCC with BDTT always had reversible hyperbilirubinemia and hypoalbuminemia, which may possibly cause by a systemic inflammatory response [17]. Therefore, it seems a reasonable approach to predict micro-BDTT with systemic and hepatic inflammation markers. It has been confirmed that the tumor growth, development, metastasis, and prognosis are associated with not only tumor characteristics but also the inflammatory response, which consists of systemic alterations and hepatic inflammation [18–21]. In the clinical setting, C-reactive protein (CRP) is an important systemic inflammation maker which had been confirmed as an independent prognostic indicator of HCC patients [22]. In addition, a series of systemic inflammation makers including neutrophil-to-lymphocyte ratio (NLR), lymphocyte-to-monocyte ratio (LMR), platelet-to-lymphocyte ratio (PLR), Prognostic Nutritional Index (PNI) and systemic immune-inflammation index (SII) had been scrutinized for the prognosis in tumor, including HCC [23, 24]. These systemic inflammation makers could also predict tumor grade and micro-vascular invasion (MVI) [25]. Based on the inflammatory biomarkers, Li et al. had developed the nomograms for predicting tumor grade and MVI with high accuracy [23]. Moreover, α-fetoprotein (AFP), γ-glutamyltransferase (γ-GT), γ-glutamyltransferase to alanine aminotransferase (γ-GT/ALT), reflect hepatic inflammation markers in the liver, which may be independent risk factors for poor prognosis of HCC patients after liver resection [25]. It is reported that γ-GT/ALT were independent risk factors for prognosis of HCC patients after liver resection [26]. At present, based on inflammation markers, many efforts on preoperative estimation of tumor
prognosis, MVI or tumor grade have been made over the past decade. However, there was not effort on preoperative estimation of micro-BDTT until now.

We hypothesize that the combination of systemic and hepatic inflammation markers would improve the prediction of HCC with micro-BDTT before surgery. In the present study, we aimed to explore the predictive value of the combination of these hematological parameters in prediction of HCC with micro-BDTT and to develop nomograms for predicting micro-BDTT before surgery in HCC using only preoperative clinical parameters.

**Methods**

**Patient population**

HCC patients between January 2012 and June 2020 were retrospectively reviewed from Fujian Provincial Hospital, Eastern Hepatobiliary Surgery Hospital, The first affiliated Hospital of Fujian Medical University, Mengchao Hepatobiliary Hospital of Fujian Medical University, retrospectively. HCC patients were diagnosed to have pathologically confirmed BDTT through two experienced pathologists. Of these patients with BDTT, 46 (1.257%) with micro-BDTT were included in our study. The definition of micro-BDTT was in line with previously published [8]. Of these patients, 372 HCC patients without BDTT were collected for constructing nomograms for predicting micro-BDTT (Figure 1). Inclusion criteria for this study were: (1) underwent surgical resection; (2) pathological diagnosis of HCC; (3) Without distant metastasis; (4) No anticancer treatment for HCC before surgery; The exclusion criteria were: (1) patients with inflammatory disease such as hematologic disorder, human immunodeficiency virus (HIV) and infection. (2) the other serious malignant diseases. (3) patients who had previously taken anti-inflammatory medicines or received immunosuppressive therapy. (4) patients who had incomplete clinical and pathological data. (5) To make a nomogram for predicting with micro-BDTT, patients with macroscopic BDTT, which represents that invasion of or tumor thrombus was in the first branches of the bile duct and the common hepatic duct were excluded. The study was approved by the Research Ethics Committee of each institution.

**Clinicopathologic variables**

HCC Patients’ preoperative parameters such as age, gender, serum level of α-fetoprotein (AFP), ALT, Albumin, γ-GT, alkaline phosphatase (ALP), direct bilirubin (DB), total bilirubin (TB), neutrophil, lymphocyte, monocyte, platelet, maximum size of tumor, number of tumour nodules and micro-BDTT, were collected. Maximum size of tumor and number of tumor nodules were assessed by preoperative imaging studies. The NLR, PLR, LMR and PNI were calculated using the following formula: NLR = neutrophil count/lymphocyte count, PLR = platelet count/lymphocyte count, LMR = lymphocyte count/monocyte count, and PNI = 10 × serum albumin (g/dL) + 0.005 × total lymphocytes count (per mm$^3$).
**Statistical analysis**

Statistical evaluation was conducted with SPSS22.0 (IBM SPSS INC., Chicago, USA) and R3.1.2 software (Institute for Statistics and Mathematics, Vienna, Austria). Continuous data were expressed as mean±standard deviation. The receiver operator characteristics (ROC) curve analysis was used to determine the optimal cut-off values for NLR, PLR, LMR, PNI, c-GT/ALT based on the maximum of Youden's index (sensitivity + specificity – 1). These cut-off values were used to categorize the high and low groups. The univariate and multivariate logistic regression analysis were utilized to determine predictive factors for HCC with micro-BDTT and P < 0.05 was considered statistically significant. Confidence intervals (CI) were showed at the 95% confidence level. Based on the results from the multivariate logistic regression analysis, a predictive nomogram was bulided by R3.1.2 software, which underwent internally validation using the bootstrap method. AUC was calculated to evaluate the performance of the predictive model.

**Results**

**Clinicopathologic characteristics**

The baseline characteristics of the patients are shown in Table 1. A total of 418 patients with HCC were collected for our analysis, including 356 males and 62 females. The average age was 52.93 ± 11.85 years. Of all 418 HCC patients, 357 (85.40%) patients were with hepatitis B virus (HBV). 264 (63.20%) patients had an AFP level below 400 µg/L, while 154 (36.80%) patients had an AFP level greater than 400 µg/L. A majority of patients were Child-Pugh A and only 19 (4.50%) were Child-Pugh B-C. The mean values of WBC, Neutrophil, Lymphocyte, Monocyte, Albumin, Platelets, ALT, ALP, γ-GT and DB were showed in Table 1. In this study, the upper normal limits for serum ALT, ALP, γ-GT, DB values were 50, 80, 80 and 10, respectively, which were taken as the cut-off value. As the tumour characteristics, 316 (75.60%) patients had single tumour nodule, while 102 (24.40%) had multiple tumors. In addition, the mean of tumor size was 6.87 ± 4.74 cm. Histopathologically identified micro-BDTT was found in 46 patients in all of HCC patients.
Table 1
Clinicopathologic features.

| Variable                  | All patients(n = 418) |
|---------------------------|-----------------------|
| Age                       | 52.93 ± 11.85         |
| Gender (n, %)             |                       |
| Male                      | 356, 85.17%           |
| Female                    | 62, 14.83%            |
| Neutrophil (*10⁹/L)       | 4.2745 ± 2.46045      |
| Lymphocyte (*10⁹/ L)      | 1.7433 ± 0.739        |
| Monocyte (*10⁹/ L)        | 0.4935 ± 0.2627       |
| Albumin (g/ L)            | 41.98 ± 5.88          |
| WBC (*10⁹/ L)             | 6.65 ± 2.64           |
| Platelets (*10⁹/ L)       | 190.72 ± 78.48        |
| ALP (U/L)                 | 110.20 ± 61.18        |
| γ-GT (U/L)                | 119.36 ± 130.99       |
| Dbil (umol/L)             | 7.25 ± 7.38           |
| ALT (U/L)                 | 50.07 ± 49.87         |
| NLR                       | 3.09 ± 3.45           |
| PLR                       | 122.13 ± 61.92        |
| LMR                       | 4.14 ± 2.12           |
| PNI                       | 50.63 ± 7.64          |
| HbsAg                     |                       |
| +                         | 357, 85.4%            |
| -                         | 61, 14.6%             |
| AFP(n, %)                 |                       |
| ≥ 400 µg/L                | 264, 63.20%           |
| <400 µg/L                 | 154, 36.80%           |
| Tumor size (cm)           | 6.87 ± 4.74           |
| Tumor number (n, %)       |                       |
Variable | All patients (n = 418)
--- | ---
1 | 316, 75.60%
>1 | 102, 24.40%
Child-Pugh
A | 399, 95.50%
B-C | 19, 4.50%

Hematological inflammation-based indexes and an optimal cut-off value

The mean values of NLR, PLR, LMR, PNI and γ-GT/ALT were 3.09 ± 3.45, 122.13 ± 61.92, 4.14 ± 2.12, 50.63 ± 7.64 and 2.99, respectively. According to the ROC curve, the optimal cut-off values of preoperative NLR, PLR, LMR, PNI and γ-GT/ALT was 1.409, 111.258, 3.338, 53.417 and 2.195, respectively, as shown in Table 2. These cut-off values were used to categorize the high and low groups.

Table 2
An optimal cut-off value for NLR, PLR, LMR, PNI, γ-GT/ALT was selected by the ROC curve analysis.

| Variable       | Cut-off value | AUC  | 95%CI         | Sensitivity | Specificity | p    |
|----------------|---------------|------|---------------|-------------|-------------|------|
| NLR            | 1.409         | 0.506| 0.425–0.587   | 0.9111      | 0.2198      | 0.893|
| PLR            | 111.258       | 0.548| 0.469–0.628   | 0.622       | 0.534       | 0.290|
| LMR            | 3.338         | 0.564| 0.472–0.655   | 0.71111     | 0.418       | 0.162|
| PNI            | 53.417        | 0.634| 0.559–0.710   | 0.889       | 0.3887      | 0.003|
| γ-GT/ALT       | 2.195         | 0.702| 0.628–0.776   | 0.778       | 0.595       | 0.000|

Prognostic value of systemic and hepatic inflammation markers

The results of univariate analysis for micro-BDVT were presented in Table 3. Univariate analysis showed that ALP, γ-GT, DB, ALT, AFP, γ-GT/ALT, NLR, PNI, tumor number were significantly associated with HCC with micro-BDVT. A multivariate regression performed on these significant factors showed that ALP (OR:
3.072, 95% CI: 1.131–8.347, P = 0.028), DB (OR: 2.815, 95% CI: 1.257–6.305, P = 0.012), AFP (OR: 2.060, 95% CI: 1.024–4.145, P = 0.043), PNI (OR: 0.356, 95% CI: 0.130–0.976, P = 0.045), γ-GT/ALT (OR: 3.742, 95% CI: 1.426–9.730, P < 0.007), as Table 3 showed, which were independently associated with HCC with micro-BDTT.
Table 3

Univariate logistic regression analysis of micro-BDTT presence based on preoperative data.

| Variable | Micro-BDTT | 95%CI       | P     |
|----------|------------|-------------|-------|
|          | OR         |             |       |
| Age      | 0.992      | 0.967–1.018 | 0.551 |
| Gender (n, %) | 1.509      | 0.688–3.313 | 0.305 |
| WBC (*109/L) | 0.876      | 0.759–1.010 | 0.069 |
| Neutrophil (*109/ L) | 0.867      | 0.734–1.024 | 0.093 |
| Lymphocyte (*109/ L) | 0.631      | 0.386–1.031 | 0.066 |
| Monocyte (*109/ L) | 1.286      | 0.804–2.057 | 0.294 |
| Albumin (g/ L) | 0.972      | 0.926–1.020 | 0.249 |
| Platelets (*109/ L) | 0.998      | 0.994–1.002 | 0.298 |
| ALP (U/L) (n, %) | 5.870      | 2.472–14.197 | 0.000 |
| >80 (235, 56.2%) | 5.870      | 2.472–14.197 | 0.000 |
| ≤ 80 (183, 43.8%) | 5.870      | 2.472–14.197 | 0.000 |
| γ-GT (U/L) (n, %) | 3.783      | 1.894–7.558 | 0.000 |
| >80 (190, 45.5%) | 3.783      | 1.894–7.558 | 0.000 |
| ≤ 80 (228, 54.5%) | 3.783      | 1.894–7.558 | 0.000 |
| DB (umol/L) (n, %) | 4.162      | 2.065–8.393 | 0.000 |
| >10 (363, 86.8%) | 4.162      | 2.065–8.393 | 0.000 |
| ≤ 10 (55, 13.2%) | 4.162      | 2.065–8.393 | 0.000 |
| ALT (U/L) (n, %) | 1.995      | 1.058–3.762 | 0.033 |
| >50 (299, 71.5%) | 1.995      | 1.058–3.762 | 0.033 |
| ≤ 50 (119, 28.5%) | 1.995      | 1.058–3.762 | 0.033 |
| HbsAg (n, %) | 0.890      | 0.360–2.201 | 0.800 |
| + (357, 14.6%) | 0.890      | 0.360–2.201 | 0.800 |
| - (61, 85.4%) | 0.890      | 0.360–2.201 | 0.800 |
| γ-GT/ALT (n, %) | 5.146      | 2.473–10.705 | 0.000 |
| >2.195 (186, 44.5%) | 5.146      | 2.473–10.705 | 0.000 |
| Variable             | Micro-BDTT |
|----------------------|------------|
|                      | OR        | 95%CI       | P        |
| ≤ 2.195 (23, 55.5%)  | 2.888     | 1.005–8.299 | 0.049    |
| NLR (n, %) ≥ 1.409 (33, 79.4%) | 1.884     | 0.997–3.558 | 0.051    |
| ≤ 1.409 (86, 20.6%)  | 1.884     | 0.997–3.558 | 0.051    |
| PLR (n, %) >111.258 (202, 48.3%) | 1.770     | 0.900–3.481 | 0.098    |
| ≤ 111.258 (216, 51.7%) | 1.770     | 0.900–3.481 | 0.098    |
| LMR (n, %) >3.338 (249, 59.6) | 0.197     | 0.076–0.510 | 0.001    |
| ≤ 3.338 (169, 40.4)  | 0.197     | 0.076–0.510 | 0.001    |
| PNI (n, %) >53.417 (150, 35.6%) | 0.197     | 0.076–0.510 | 0.001    |
| ≤ 53.417 (268, 64.1%) | 0.197     | 0.076–0.510 | 0.001    |
| Child-Pugh (n, %) A (399, 95.50%) | 2.231     | 0.291–17.121 | 0.440    |
| B-C (19, 4.50%)    | 2.231     | 0.291–17.121 | 0.440    |
|AFP (µg/L) (n, %) ≥400 (264, 63.20%) | 1.124     | 0.454–2.782 | 0.800    |
|<400 (154, 36.80%)  | 1.124     | 0.454–2.782 | 0.800    |
| HbsAg (n, %) + (264, 63.20%) | 1.124     | 0.454–2.782 | 0.800    |
| − (154, 36.80%)   | 1.124     | 0.454–2.782 | 0.800    |
| Tumor size (cm)    | 1.017     | 0.955–1.083 | 0.592    |
| Tumor number (n, %) 1 (316, 75.60%) | 2.839     | 1.501–5.368 | 0.001    |
| >1 (102, 24.40%)   | 2.839     | 1.501–5.368 | 0.001    |
Table 4. Multivariate logistic regression analysis of micro-BDTT presence based on preoperative data.

| Variable          | Micro-BDTT |   |   |
|-------------------|------------|---|---|
|                   | OR         | 95% Cl | P  |
| ALP               | 3.072      | 1.131-8.347 | 0.028 |
| ALT               | 2.164      | 0.975-4.802  | 0.058 |
| DB                | 2.815      | 1.257-6.305  | 0.012 |
| γ-GT              | 0.672      | 0.254-1.776  | 0.432 |
| γ-GT/ALT          | 3.742      | 1.426-9.730  | 0.007 |
| NLR               | 1.390      | 0.439-4.404  | 0.576 |
| PNI               | 0.356      | 0.130-0.976  | 0.045 |
| AFP               | 2.060      | 1.024-4.145  | 0.043 |
| Tumor number (n, %) | 2.026      | 0.984-4.170  | 0.055 |

Development of a predicting nomogram

Based on the significant independent variables, a nomogram for predicting micro-BDTT probability in HCC was developed. By drawing a straight line after summing up the score assigned to each variable, we could easily obtain the total points, which be converted to predict the probability of HCC with micro-BDTT (Fig. 2A). Patients with a higher total score tended to obtain a higher probability of HCC with micro-BDTT. The accuracy of micro-BDTT nomogram model was favorable with an area under the ROC curve and the AUC of the predictive nomogram was 0.809, as shown in Fig. 3. The performance of the nomogram was validated internally with a C-index of 0.809 (95% CI 0.747–0.871). The calibration curves via internal validation showed good agreement between the predicted and actual probability of micro-BDTT (Fig. 2B). The sensitivity, specificity, positive predictive value, and negative predictive value when used in predicting micro-BDTT before surgery were 0.778, 0.724, 0.254 and 0.964, respectively (Table 5).

Table 5. Accuracy of the prediction score of the nomogram for estimating the risk of micro-BDTT presence in HCC.

| Variable                      | Value (95% CI)                  |
|-------------------------------|---------------------------------|
| Sensitivity, %                | 0.778 (0.656-0.899)             |
| Specificity, %                | 0.724 (0.679-0.769)             |
| Positive predictive value, %  | 0.254 (0.181-0.326)             |
| Negative predictive value, %  | 0.964 (0.943-0.986)             |
| Positive likelihood ratio     | 2.817 (2.245-3.533)             |
| Negative likelihood ratio     | 0.307 (0.177-0.532)             |
| Youden Index                  | 0.504                           |
| Area under ROC curve          | 0.809 (0.747-0.871)             |

Discussion

Hepatocellular carcinoma (HCC) with bile duct invasion were far more rarely than that with vascular invasion. Because of the rare incidence of BDTT, there is insufficient data to systematically analyze the prognostic implications of BDTT. However, many studies supported the hypothesis that the prognosis of HCC with BDTT can be worse than HCC without invasion [8–10]. HCC with BDTT had more advanced stage HCC with adverse histological features including higher rates of MVI and poor differentiation [5–7]. Liu et al. also reported that Patients with BDTT extending to the common bile duct usually have an unfavorable prognosis even following aggressive surgery [27]. Although Meng et al. had showed that macro-BDTT but not micro-BDTT was an independent risk factor affecting the prognosis of patients with
HCC, the number of HCC patients with micro-BDTT (only 7 patients) was too small [28]. More importantly, Kim et al. demonstrates that the prognosis of HCC patients with micro-BDTT was worser than those without BDTT [29]. The presence of micro-BDTT should therefore be considered as an adverse prognostic factor after hepatectomy.

Surgical treatment for HCC is considered as the most effective approach, including those with BDTT, and the surgical strategy in HCC with BDTT is not clearly defined in previous studies [30–32]. However, Kasai et al. had showed that in HCC patients with BDTT alone without MVI, extended hepatectomy provided a better prognosis [31]. In addition, neoadjuvant TACE reduced the surgical risk of curative liver resection and significantly prolonged median survival in HCC with BDTT [32]. Luo et al had also showed that for HCC patients with BDTT, radical hepatic resection and removal of BDTT, combined with TACE, are the best approach [33]. Peng et al. had reported that curative resection for HCC with BDTT can result in prolonging the survival [34]. These results indicated that the choice of the most appropriate therapeutic strategy is very important for the prognosis of HCC with BDTT. However, misdiagnosis of BDTT before surgery may lead to inappropriate treatments, resulting in poor survival of those patients. In our data, only 2 of 46 HCC patients with micro-BDTT were preoperative diagnosis.

Previous studies have reported that BDTT could appear even in early stage of HCC, and it could also occur in HCC patients with tumor’s diameter less than 3 cm, which indicated that the size of HCC tumor is not correlated with the occurrence of BDTT [27, 35]. In our data, we also found that the size of HCC tumor is not correlated with the occurrence of micro-BDTT. In HCC with BDTT, obstructive jaundice is a main clinical manifestation with higher preoperative bilirubin level, because of biliary tract invasion, and there were always biliary ducts dilatation. These can be used to distinguish HCC with BDTT from those without BDTT. However, the features are also observed in other biliary tract diseases, such as hepatic insufficiency, extrahepatic cholangiocarcinoma, choledochal cyst and common bile duct stone [15]. Besides, because invasion of or tumor thrombus was in the second and more peripheral branches of the bile duct, serum total bilirubin and direct bilirubin in HCC with micro-BDTT were always in the normal range, or slightly higher than normal. In addition, HCC with micro-BDTT were always ignored in dynamic contrast-enhanced CT or MRI. In our data, all patients with micro-BDTT had not clinical symptoms of jaundice. The DB in HCC with micro-BDTT were only slightly higher than that in HCC without BDTT. Therefore, HCC with micro-BDTT were hard to diagnose before surgery, since it had no visible symptoms.

With increasing knowledge, inflammation plays an essential role in tumorigenesis and progression including HCC. As more than 90% of HCC occur with hepatic injury and inflammation, the progression of HCC was inflammation-related carcinogenesis events [36]. The predictive role of systemic inflammation and hepatic inflammation markers in the prognosis of HCC received more attention in recent years. Systemic inflammatory indexes such as NLR, PLR, LMR, SII, and PNI are emerging as predictors of prognosis, tumor grade or MVI in HCC [23]. In our study, the optimal cut-off points for NLR, PLR, LMR, PNI were determined by ROC analysis, which were 1.409, 111.258, 3.338, 53.417, respectively. Herein, we found that pre-treatment NLR and PNI level were significantly associated with micro-BDTT. Through multifactor analysis, pre-treatment PNI level was also an independent predictor of HCC with micro-BDTT.
PNI is a marker combinative of albumin and lymphocyte, which has been recognized as an indicator of nutritional and immunological status. Additionally, Chan et al. showed that the PNI was independent prognostic factors for HCC patients [37], and the PNI is a simple and useful systemic inflammation marker for predicting the survival of HCC treated with sorafenib [38]. These results indicated that it is feasible for the PNI as a novel indicator of systemic inflammation. In the present study, the PNI was independent prognostic factors for HCC with micro-BDTT, which was added in the nomogram for predicting micro-BDTT before surgery as a marker of systemic inflammation. However, the specific mechanism of the observations is still unclear.

The background liver inflammation has an important role on the development and prognosis of HCC patients. The clinical indicators of liver inflammation including ALT, AST, ALP and γ-GT had been confirmed to positively related with the recurrence and poor prognosis of HCC patients [39]. The ratio of γ-GT /ALT, another index reflecting liver inflammation, is a powerful prognostic factor for HCC patients. It had found that the ratio of γ-GT /ALT is a convenient prognostic marker for HCC after hepatic resection [25]. In our data, we found that DB, ALP and γ-GT /ALT were significant prognostic factors of micro-BDTT in patients with HCC. However, the exact mechanisms underlying these observations are still unclear. Besides AFP were also identified as independent predictors of HCC with micro-BDTT. In light of these findings, we suggest that HCC patients with lower pre-treatment PNI, higher DB, ALP, AFP and γ-GT /ALT levels may be potential candidates for micro-BDTT before surgery.

Herein, we successfully developed a predictive nomogram for predicting micro-BDTT before surgery in hepatocellular carcinoma based on combining systemic and hepatic inflammation markers. We generalized the sensitivity, specificity, positive predictive value, and negative predictive value in estimating the risk of micro-BDTT. Patients with a high score have a high risk of micro-BDTT. As inflammation-based indexes are routinely available and can be measured accurately, the establishment of a predictive model based on inflammation-based indexes may become a useful and inexpensive approach to predict micro-BDTT before surgery. Furthermore, it can provide guidance for choosing a more suitable therapeutic strategies for patients.

There is undoubtedly that our investigation has some limitations. First, selection bias could have been present due to HCC patients without micro-BDTT was based on data from a single center and it does not include all of HCC patients without micro-BDTT from multicenters. Second, the number of patients with micro-BDTT was fairly small. However, because of low incidence of micro-BDTT, the number of cases is difficult to increase. We had already collected HCC patients with micro-BDTT from four centers. Only 46 with micro-BDTT were included in our study. Third, since this study was a retrospective study, C-reactive protein (CRP) was not routinely measured during the study period. Nevertheless, CRP was detected in only 21 (5.02%) patients in our study. more researches are warranted concerning the relationship between the micro-BDTT and CRP. In addition, it was very necessary to improve the nomogram with more factors. Some specific markers including some stem cell markers and small molecule metabolite biomarkers may also work as good non-invasive biomarkers for predicting HCC with micro-BDTT. Fourth, further external validation is needed to confirm the reliability of our predictive model through an independent and larger
dataset. To our knowledge, this is the first study to construct a predictive nomogram including pre-treatment risk factors for predicting HCC patient with micro-BDTT, but there are still many deficiencies. Hence, further large-scale, prospective and multicentre studies are needed to confirm the results.

Conclusions

In conclusion, our study highlighted the importance of systemic inflammation and hepatic inflammation in predicting HCC with micro-BDTT. The novel inflammation-based model provided an optimal preoperative estimation of HCC with micro-BDTT, which could help choose suitable surgical methods for HCC with micro-BDTT. Nevertheless, further studies are needed to verify the effectiveness and practicability of the nomograms.

Abbreviations

HCC: Hepatocellular carcinoma, BDTT:bile duct tumor thrombus, micro-BDTT:microscopic bile duct tumor thrombus, ROC:Receiver operating characteristic, AFP:α-fetoprotein, ALP:alkaline phosphatase, TB:total bilirubin, DB:direct bilirubin, PNI:prognostic nutritional index, NLR:neutrophil-to-lymphocyte ratio, LMR:lymphocyte-to-monocyte ratio, PLR:platelet-to-lymphocyte ratio, SII:systemic immune-inflammation index, γ-GT:γ-glutamyl transferase, ALT:alanine aminotransferase, CI:confidence intervals, CT:computerized tomography, MRI:magnetic resonance imaging, MRCP:Magnetic resonance cholangiography, ERCP:Endoscopic Retrograde Cholangiopancreatography, MVI:micro-vascular invasion, HIV:human immunodeficiency virus (HIV), HBV:hepatitis B virus.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Fujian Provincial Hospital, the Shengli Clinical Medical College of Fujian Medical University. All study participants gave their written informed consent.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed in our study are available from the corresponding authors upon reasonable request.

Competing interests

The authors declare that they have no competing interests.
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Authors’ contributions

Conceived and designed the research: MLY, SQC. Data acquisition: JYW, JYW, YNB, XXH, YYZ. Data analysis: JYW, JYW, JXS, ZBZ. Drafting the manuscript: WJY, MLY. All authors read and approved the final manuscript.

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References

1. Satoh S, Ikai I, Honda G, Okabe H, Takeyama O, Yamamoto Y, Yamamoto N, limuro Y, Shimahara Y, Yamaoka Y. Clinicopathologic evaluation of hepatocellular carcinoma with bile duct thrombi. Surgery. 2000;128(5):779–83.

2. Moon DB, Hwang S, Wang HJ, Yun SS, Kim KS, Lee YJ, Kim KH, Park YK, Xu W, Kim BW, et al. Surgical outcomes of hepatocellular carcinoma with bile duct tumor thrombus: a Korean multicenter study. World J Surg. 2013;37(2):443–51.

3. Shiomi M, Kamiya J, Nagino M, Uesaka K, Sano T, Hayakawa N, Kanai M, Yamamoto H, Nimura Y. Hepatocellular carcinoma with biliary tumor thrombi: aggressive operative approach after appropriate preoperative management. Surgery. 2001;129(6):692–8.

4. Clark W, Schulz MD. Hepatoma, with invasion of cystic duct and metastasis to third lumbar vertebra. N Engl J Med. 1947;237(18):673–6.

5. Navadgi S, Chang CC, Bartlett A, McCall J, Pandanaboyana S. Systematic review and meta-analysis of outcomes after liver resection in patients with hepatocellular carcinoma (HCC) with and without bile duct thrombus. HPB (Oxford). 2016;18(4):312–6.

6. Zeng H, Xu LB, Wen JM, Zhang R, Zhu MS, Shi XD, Liu C. Hepatocellular carcinoma with bile duct tumor thrombus: a clinicopathological analysis of factors predictive of recurrence and outcome after surgery. Med (Baltim). 2015;94(1):e364.

7. Qiao W, Yu F, Wu L, Li B, Zhou Y. Surgical outcomes of hepatocellular carcinoma with biliary tumor thrombus: a systematic review. BMC Gastroenterol. 2016;16:11.

8. Esaki M, Shimada K, Sano T, Sakamoto Y, Kosuge T, Ojima H. Surgical results for hepatocellular carcinoma with bile duct invasion: a clinicopathologic comparison between macroscopic and microscopic tumor thrombus. J Surg Oncol. 2005;90(4):226–32.
9. Wang C, Yang Y, Sun D, Jiang Y. Prognosis of hepatocellular carcinoma patients with bile duct tumor thrombus after hepatic resection or liver transplantation in Asian populations: A meta-analysis. PLoS One. 2017;12(5):e0176827.

10. Peng SY, Wang JW, Liu YB, Cai XJ, Deng GL, Xu B, Li HJ. Surgical intervention for obstructive jaundice due to biliary tumor thrombus in hepatocellular carcinoma. World J Surg. 2004;28(1):43–6.

11. Jang YR, Lee KW, Kim H, Lee JM, Yi NJ, Suh KS. Bile duct invasion can be an independent prognostic factor in early stage hepatocellular carcinoma. Korean J Hepatobiliary Pancreat Surg. 2015;19(4):167–72.

12. Qin LX, Ma ZC, Wu ZQ, Fan J, Zhou XD, Sun HC, Ye QH, Wang L, Tang ZY. Diagnosis and surgical treatments of hepatocellular carcinoma with tumor thrombosis in bile duct: experience of 34 patients. World J Gastroenterol. 2004;10(10):1397–401.

13. Gabata T, Terayama N, Kobayashi S, Sanada J, Kadoya M, Matsui O. MR imaging of hepatocellular carcinomas with biliary tumor thrombi. Abdom Imaging. 2007;32(4):470–4.

14. Jung AY, Lee JM, Choi SH, Kim SH, Lee JY, Kim SW, Han JK, Choi BI. CT features of an intraductal polypoid mass: Differentiation between hepatocellular carcinoma with bile duct tumor invasion and intraductal papillary cholangiocarcinoma. J Comput Assist Tomogr. 2006;30(2):173–81.

15. Tan W, He J, Deng J, Yang X, Cui L, Ran R, Du G, Jiang X. Small molecule metabolite biomarkers for hepatocellular carcinoma with bile duct tumor thrombus diagnosis. Sci Rep. 2018;8(1):3309.

16. Pang YB, Zhong JH, Luo XL, Ou C, Guo Z, Xiang BD, Peng NF, Li LQ. Clinicopathological characteristics and liver stem cell marker expression in hepatocellular carcinoma involving bile duct tumor thrombi. Tumour Biol. 2016;37(5):5879–84.

17. Oshiro Y, Sasaki R, Fukunaga K, Kondo T, Oda T, Takahashi H, Ohkohchi N. Inflammation-based prognostic score is a useful predictor of postoperative outcome in patients with extrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci. 2013;20(3):389–95.

18. Hwang JE, Kim HN, Kim DE, Choi HJ, Jung SH, Shim HJ, Bae WK, Hwang EC, Cho SH, Chung IJ. Prognostic significance of a systemic inflammatory response in patients receiving first-line palliative chemotherapy for recurred or metastatic gastric cancer. BMC Cancer. 2011;11:489.

19. Khodabandehlou N, Mostafaei S, Etemadi A, Ghasemi A, Payandeh M, Hadifar S, Norooznezhad AH, Kazemnejad A, Moghoofei M. Human papilloma virus and breast cancer: the role of inflammation and viral expressed proteins. BMC Cancer. 2019;19(1):61.

20. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet. 2001;357(9255):539–45.

21. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.

22. Gan W, Yi Y, Fu Y, Huang J, Lu Z, Jing C, Fan J, Zhou J, Qiu S. Fibrinogen and C-reactive protein score is a prognostic index for patients with hepatocellular carcinoma undergoing curative resection: a prognostic nomogram study. J Cancer. 2018;9(1):148–56.

23. Li P, Huang W, Wang F, Ke YF, Gao L, Shi KQ, Zhou MT, Chen BC. Nomograms based on inflammatory biomarkers for predicting tumor grade and micro-vascular invasion in stage I/II hepatocellular
24. Sun Y, Huang Z, Chi P. An inflammation index-based prediction of treatment response to neoadjuvant chemoradiotherapy for rectal mucinous adenocarcinoma. Int J Clin Oncol. 2020;25(7):1299–307.

25. Zhang T, Liu Z, Zhao X, Mao Z, Bai L. A novel prognostic score model based on combining systemic and hepatic inflammation markers in the prognosis of HBV-associated hepatocellular carcinoma patients. Artif Cells Nanomed Biotechnol. 2019;47(1):2246–55.

26. Ju MJ, Qiu SJ, Fan J, Zhou J, Gao Q, Cai MY, Li YW, Tang ZY. Preoperative serum gamma-glutamyl transferase to alanine aminotransferase ratio is a convenient prognostic marker for Child-Pugh A hepatocellular carcinoma after operation. J Gastroenterol. 2009;44(6):635–42.

27. Liu QY, Lai DM, Liu C, Zhang L, Zhang WD, Li HG, Gao M. A special recurrent pattern in small hepatocellular carcinoma after treatment: bile duct tumor thrombus formation. World J Gastroenterol. 2011;17(43):4817–24.

28. Meng KW, Dong M, Zhang WG, Huang GX. Clinical characteristics and surgical prognosis of hepatocellular carcinoma with bile duct invasion. Gastroenterol Res Pract. 2014;2014:604971.

29. Kim JM, Kwon CH, Joh JW, Sinn DH, Park JB, Lee JH, Kim SJ, Paik SW, Park CK, Yoo BC. Incidental microscopic bile duct tumor thrombi in hepatocellular carcinoma after curative heptectomy: a matched study. Med (Baltim). 2015;94(6):e450.

30. An J, Lee KS, Kim KM, Park DH, Lee SS, Lee D, Shim JH, LimYS, Lee HC, Chung YH, et al. Clinical features and outcomes of patients with hepatocellular carcinoma complicated with bile duct invasion. Clin Mol Hepatol. 2017;23(2):160–9.

31. Kasai Y, Hatano E, Seo S, Taura K, Yasuchika K, Uemoto S. Hepatocellular carcinoma with bile duct tumor thrombus: surgical outcomes and the prognostic impact of concomitant major vascular invasion. World J Surg. 2015;39(6):1485–93.

32. Shen Y, Li P, Cui K, Wang Z, Yu F, Tian H, Li S. Neoadjuvant Transcatheter Arterial Chemoembolization for Biliary Tumor Thrombosis: A Retrospective Study. Int J Technol Assess Health Care. 2016;32(4):212–7.

33. Xiangji L, Weifeng T, Bin Y, Chen L, Xiaojie Q, Baihe Z, Feng S, Mengchao W. Surgery of hepatocellular carcinoma complicated with cancer thrombi in bile duct: efficacy for criteria for different therapy modalities. Langenbecks Arch Surg. 2009;394(6):1033–9.

34. Peng BG, Liang LJ, Li SQ, Zhou F, Hua YP, Luo SM. Surgical treatment of hepatocellular carcinoma with bile duct tumor thrombus. World J Gastroenterol. 2005;11(25):3966–9.

35. Liu QY, Huang SQ, Chen JY, Li HG, Gao M, Liu C, Liang BL. Small hepatocellular carcinoma with bile duct tumor thrombus: CT and MRI findings. Abdom Imaging. 2010;35(5):537–42.

36. Bishayee A. The role of inflammation and liver cancer. Adv Exp Med Biol. 2014;816:401–35.

37. Chan AW, Chan SL, Wong GL, Wong VW, Chong CC, Lai PB, Chan HL, To KF. Prognostic Nutritional Index (PNI) Predicts Tumor Recurrence of Very Early/Early Stage Hepatocellular Carcinoma After Surgical Resection. Ann Surg Oncol. 2015;22(13):4138–48.
38. Caputo F, Dadduzio V, Tovoli F, Bertolini G, Cabibbo G, Cerma K, Vivaldi C, Faloppi L, Rizzato MD, Piscaglia F, et al. The role of PNI to predict survival in advanced hepatocellular carcinoma treated with Sorafenib. PLoS One. 2020;15(5):e0232449.

39. Cheung YS, Chan HL, Wong J, Lee KF, Poon TC, Wong N, Lai PB. Elevated perioperative transaminase level predicts intrahepatic recurrence in hepatitis B-related hepatocellular carcinoma after curative hepatectomy. Asian J Surg. 2008;31(2):41–9.

**Figures**

![Flow diagram of the study selection process.](image)

**Figure 1**

Flow diagram of the study selection process.
Figure 2

Nomogram to estimate HCC with micro-BDTT presence preoperatively. (A) Nomogram for predicting HCC with micro-BDTT. (B) Calibration plot of the nomogram for predicting the risk of micro-BDTT in HCC.
Figure 3

The accuracy of the nomogram for predicting micro-BDTT in HCC using ROC curve.