



Original Article

Comparison of the Prognostic Value of Platelet-Related Indices in Biliary Tract Cancer Undergoing Surgical Resection

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Purpose Platelet-related indices, including mean platelet volume (MPV) and plateletcrit (PCT), have been reported as new prognostic factors of overall survival (OS) in many cancers, but not yet in biliary tract cancer (BTC). We intended to assess these indices in predicting OS in BTC patients with the aim to build a new prognostic model for patients with BTC after surgical resection.

Materials and Methods Survival analysis and time receiver operating characteristic analysis were applied to screen the platelet indices. Univariate and multivariate Cox analyses were used to identify independent prognostic factors and develop a new prognostic model. Harrell's C-statistics, calibration curves, and decisive curve analysis were used to assess the model.

Results MPV and platelet distribution width (PDW)/PCT showed the best prognostic accuracy among the platelet indices. In multivariable analysis, factors predictive of poor OS were presence of nodal involvement, Non-radical surgery, poor tumor differentiation, carbohydrate antigen 19-9 > 100 U/mL, MPV > 8.1 fl, and PDW/PCT > 190. The new model was found to be superior to the TNM staging system and our new staging system showed higher discriminative power.

Conclusion MPV and PDW/PCT have high prognostic value in BTC patients, and the novel staging system based on these two indices showed good discrimination and accuracy compared with the American Joint Committee on Cancer 7th TNM staging system.

Key words Biliary tract neoplasms, Platelet, Prognosis, Mean platelet volume, Platelet distribution width/plateletcrit

Introduction

Biliary tract cancer (BTC) is a group of biliary malignancies mainly comprising intrahepatic cholangiocarcinoma (ICC), extrahepatic cholangiocarcinoma (ECC), and gallbladder cancer (GBC). ICC is estimated to account for about 0.59% of all new cancer cases in 2018, while GBC containing ECC accounts for 1.2% worldwide [1]. Despite the low incidence, the mortality of BTC is relatively high, comprising up to 2% of total cancer deaths per year [2]. BTC is acknowledged to be aggressive with a dismal prognosis; according to the RARE-CAREnet project, based on cases between 2000 and 2007 in Europe, survival for ICC was 25% at 1 year, 8% at 3 years, and 5% at 5 years, while the 5-year survival rate of GBC was less than 5% [3]. Survival for ECC was marginally better, albeit dramatically reducing from 48% at 1 year to 23% at 3 years and 17% at 5 years [4]. Increased incidence has been observed in cholangiocarcinoma and GBC [5] worldwide. Unfortunately, the only available curative treatment for BTC is surgery.

The prognosis of BTC is highly dependent on tumor stage, as the estimated 5-year survival is 50% for American Joint

Committee on Cancer (AJCC) stage I, 30% for stage II, 10% for stage III, and 0% for stage IV [6]. Both novel biomarkers and staging systems are warranted, as no prognostic biomarkers are currently used clinically, and the AJCC and Bismuth-Corlette staging system are not accurate in predicting survival [2]. Currently, nodal involvement, tumor differentiation, preoperative and postoperative serum carbohydrate antigen 19-9 (CA19-9) [7] have been confirmed as important prognostic factors in cholangiocarcinoma patients undergoing surgery. Furthermore, lymph node status and the presence of R0 curative resection are closely related to survival in GBC [8]. During the search for novel factors, inflammation-related indexes have been extensively studied as prognostic factors, but their roles in predicting overall survival (OS) for BTC are still under discussion. The neutrophil-to-lymphocyte ratio, one of the most popular inflammation-related indices, cannot maintain its role as a dependent prognostic factor of OS in BTC [9]; thus, more accurate predictors need to be identified.

It has been widely accepted that platelets contribute to tumor progression through a variety of mechanisms, including angiogenesis, assisting metastasis, and regulating immu-

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nity. Platelets can protect tumor cells from immune attack by modulating innate and adaptive immune responses [10]. The role of platelets as regulators of inflammation has been recognized, and as a result, the prognostic ability of platelet-related indices has been investigated in many cancers. Platelet count (PLT) and morphologic indices, including the mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW), have been frequently studied in a number of cancers, including pancreatic cancer [11], invasive breast cancer [12], lung cancer [13], and esophageal cell carcinoma [14]. However, the relationship between platelet-related indices and prognosis in BTC remains unknown. Therefore, we investigated the prognostic performance of platelet indices in patients with TNM stages I-III BTC after surgical resection and found two potential prognostic factors. A new prognostic model and staging system were developed based on these two factors, which may benefit patients with BTC in surgery selection and postoperative management.

Materials and Methods

1. Patients

Patients diagnosed with BTC, either ICC, ECC, or GBC, at the Peking Union Medical College Hospital (PUMCH) between December 2002 and December 2017 were included. The inclusion criteria were as follows: (1) pathologically confirmed BTC; (2) no other malignant tumors; and (3) patients who underwent resection without antitumor treatment before or during surgical intervention. The exclusion criteria were as follows: (1) patients with AJCC 7th stage IV; (2) patients with incomplete follow-up data; and (3) patients with active inflammatory diseases. A total of 527 patients were identified. Data, including patient demographics, operative outcomes, and pathologic features, were collected. Hematologic parameters, such as CA19-9, albumin (ALB), alkaline phosphatase (ALP), and γ -glutamyl transpeptidase (GGT) were collected within 5 days before surgery. OS was measured from the date of surgery to the date of death or the final follow-up. All patients were randomly split 7:3 into training (371) and validation (156) sets using `createDataPartition` from the R package 'caret', which is based on simple random sampling. To use this package, all patients were numbered with Arabic numerals and the seed was set as 2020.

2. Definitions of platelet-related indices

The 10 platelet-related indices that were considered were PLT ($\times 10^9/L$), MPV (fL), PCT (%), PDW (%), platelet-to-lymphocyte ratio (PLR), red blood cell distribution width-to-platelet ratio ($\%/ \times 10^{-9}/L$), and ratios of PDW/PLT, PDW/

PCT, MPV/PLT, and MPV/PLT. PLT, MPV, PCT, PDW were checked by SYSMEX XN automatic hematological analysis (SYSMEX, Kobe, Japan). When results were doubtful, microscopic examination was still the golden standard. All values of platelet indices were collected before operation.

3. Statistical analysis

The Shapiro-Wilk normality test was used to identify non-normal continuous variables, which were presented as median (interquartile range). Categorical variables were expressed as counts and percentages, and baseline characteristics were summarized as categorical variables. Acknowledged optimal cut-off values for platelet indices, total bilirubin (TBIL) and tumor diameter were absent. Therefore, these values were determined by 'surv cutpoint' function from 'survminer' R package using the maximally selected rank statistics to provide a value of cutpoint that correspond to the most significant relation with survival outcome. The remaining cut-off values were determined based on the median values. Comparisons of the two groups were performed with the Kruskal-Wallis rank test for non-normal continuous variables and the chi-square test or Fisher exact test for categorical variables, as appropriate.

Kaplan-Meier analysis was used to perform survival tests, and the log-rank test was used for statistical comparisons between curves. Areas under time-dependent receiver operating characteristic (ROC) curves (time-AUC) curves were used to predict the efficacy of platelet indices. The association between variables and OS was assessed by univariate and multivariate Cox proportional hazards regression analyses. All statistically significant variables in the univariate analysis were included in the multivariate analysis. All Cox analyses were performed using the R package 'survival'.

A nomogram was constructed according to the multivariate Cox model. Patients with assigned prognostic scores derived from the nomogram were stratified equally into three risk groups. The predictive accuracy of the new model was evaluated by calculating Harrell's concordance index (C-index) and plotting calibration curves in training and validation cohorts. Decisive curve analysis (DCA) was also used to assess the clinical benefit. The results were compared to those of the AJCC 7th edition TNM staging system. All statistical analyses were performed using R software ver. 3.6.2 (R Foundation for Statistical Computing, Vienna, Austria). In all analyses and tests, a two-sided $p < 0.05$ was considered statistically significant.

Table 1. Baseline characteristics

Characteristic	Total	Validation datasets	Training datasets	p-value
No.	527	156	371	
Type				
GBC	122 (23.1)	37 (23.7)	85 (22.9)	0.953
ECC	296 (56.2)	86 (55.1)	210 (56.6)	
ICC	109 (20.7)	33 (21.2)	76 (20.5)	
Age (yr)				
≤ 60	233 (44.3)	64 (41.3)	169 (45.6)	0.423
> 60	293 (55.7)	91 (58.7)	202 (54.4)	
Sex				
Female	235 (44.6)	76 (48.7)	159 (42.9)	0.254
Male	292 (55.4)	80 (51.3)	212 (57.1)	
Blood type				
AB	35 (6.7)	10 (6.5)	25 (6.8)	0.752
A	114 (21.7)	38 (24.5)	76 (20.5)	
B	214 (40.8)	59 (38.1)	155 (41.9)	
O	162 (30.9)	48 (31.0)	114 (30.8)	
Fever				
No	451 (85.6)	131 (84.0)	320 (86.3)	0.586
Yes	76 (14.4)	25 (16.0)	51 (13.7)	
Emaciation				
No	259 (49.6)	71 (46.1)	188 (51.1)	0.346
Yes	263 (50.4)	83 (53.9)	180 (48.9)	
Debilitation				
No	467 (89.0)	135 (86.5)	332 (90.0)	0.320
Yes	58 (11.0)	21 (13.5)	37 (10.0)	
Drink				
No	402 (76.6)	120 (76.9)	282 (76.4)	0.991
Yes	123 (23.4)	36 (23.1)	87 (23.6)	
Fat liver				
No	453 (91.9)	135 (91.2)	318 (92.2)	0.859
Yes	40 (8.1)	13 (8.8)	27 (7.8)	
ALT (U/L)				
≤ 100	298 (56.7)	91 (58.3)	207 (55.9)	0.683
> 100	228 (43.3)	65 (41.7)	163 (44.1)	
AST (U/L)				
≤ 100	351 (69.8)	103 (69.6)	248 (69.9)	> 0.99
> 100	152 (30.2)	45 (30.4)	107 (30.1)	
GGT (U/L)				
≤ 200	232 (46.0)	70 (47.3)	162 (45.5)	0.788
> 200	272 (54.0)	78 (52.7)	194 (54.5)	
ALP (U/L)				
≤ 200	230 (45.6)	68 (45.9)	162 (45.5)	> 0.99
> 200	274 (54.4)	80 (54.1)	194 (54.5)	
ALB (g/L)				
≤ 35	112 (21.3)	34 (21.8)	78 (21.1)	0.959
> 35	413 (78.7)	122 (78.2)	291 (78.9)	
TBIL (μmol/L)^{a)}				
≤ 106	323 (61.3)	99 (63.5)	224 (60.4)	0.572
> 106	204 (38.7)	57 (36.5)	147 (39.6)	

(Continued to the next page)

Table 1. Continued

Characteristic	Total	Validation datasets	Training datasets	p-value
CA 19-9 (U/mL)				
≤ 100	256 (51.0)	70 (48.3)	186 (52.1)	0.497
> 100	246 (49.0)	75 (51.7)	171 (47.9)	
Tumor differentiation^{b)}				
Poor	51 (10.1)	20 (13.2)	31 (8.8)	0.266
Moderate	262 (52.0)	73 (48.3)	189 (53.5)	
Well	191 (37.9)	58 (38.4)	133 (37.7)	
Radical cure				
No	200 (38.3)	64 (41.6)	136 (37.0)	0.375
Yes	322 (61.7)	90 (58.4)	232 (63.0)	
T category				
Tis	9 (1.7)	2 (1.3)	7 (1.9)	0.362
1	130 (24.7)	36 (23.1)	94 (25.3)	
2	153 (29.0)	55 (35.3)	98 (26.4)	
3	196 (37.2)	52 (33.3)	144 (38.8)	
4	39 (7.4)	11 (7.1)	28 (7.5)	
Nodal involvement				
No	364 (69.3)	113 (73.4)	251 (67.7)	0.234
Yes	161 (30.7)	41 (26.6)	120 (32.3)	
TNM stage				
0	9 (1.7)	2 (1.3)	7 (1.9)	0.737
I	179 (34.0)	57 (36.5)	122 (32.9)	
II	174 (33.0)	47 (30.1)	127 (34.2)	
III	165 (31.3)	50 (32.1)	115 (31.0)	
Tumor size (cm)^{a)}				
≤ 2.6	303 (60.2)	87 (58.8)	216 (60.8)	0.741
> 2.6	200 (39.8)	61 (41.2)	139 (39.2)	
Postoperative chemotherapy				
No	353 (76.2)	103 (73.6)	250 (77.4)	0.441
Yes	110 (23.8)	37 (26.4)	73 (22.6)	

Values are presented as number (%). ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CA 19-9, carbohydrate antigen 19-9; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GGT, γ -glutamyl transpeptidase; ICC, intrahepatic cholangiocarcinoma; TBIL, total bilirubin. ^{a)}Cut-off values of TBIL (106 μ mol/L) and tumor size (2.6 cm) were determined by 'maxstat' R package, ^{b)}Tumor differentiation was judged by pathologists. Poor includes undifferentiated or poorly differentiated, which means disordered arrangement of cancer cells, high cellular atypia and high proportion of cancer cells in division phase. Well means almost normal arrangement of cancer cells, low cellular atypia and low proportion of cancer cells in division phase. Moderate means somewhere in between.

Results

1. Baseline characteristics

A total of 527 patients met the inclusion criteria. The median follow-up was 21 months (range, 10 to 41 months), and 328 patients (62.2%) were confirmed dead. Table 1 summarizes the demographic, clinical, and pathological characteristics of the patients. Among the 527 patients, 296 (56.2%) were diagnosed with ECC, followed by GBC (n=122) and ICC (n=109). A total of 235 patients (44.6%) were female,

and 292 (55.4%) were male. According to the AJCC 7th TNM staging system, 9 (1.7%), 179 (34.0%), 174 (33.0%), and 165 (31.3%) patients were classified as TNM stage 0, I, II, and III, respectively. A total of 322 patients (61.7%) underwent radical surgery, defined as R0 resection, and 200 patients (38.3%) underwent non-radical surgery. Overall, 110 out of 463 patients received postoperative chemotherapy, most common chemo regimens were as follows: 42 patients (38.1%) received interventional therapy mainly consists of fluorouracil with pirarubicin or epirubicin, 15 patients (13.6%) received

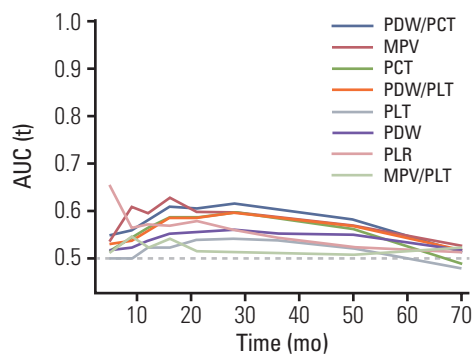


Fig. 1. Time-AUC curves of the platelet indices for overall survival prediction. AUC, area under curve; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width; PLR, platelet-to-lymphocyte ratio; PLT, platelet count; Time-AUC, areas under time-dependent ROC curves.

single-agent chemotherapy with oxaliplatin or tegafur or gemcitabine or capecitabine, 23 (20.9%) received multiagent chemotherapy with a combination of two or more agents above. The training cohort consisted of 371 patients, and the validation cohort consisted of 156 patients; these two cohorts were comparable with respect to all characteristics. Among the training cohort, 321 were included in the model construction.

2. Comparison of the prognostic efficacy of platelet indices

Of the 10 platelet indices, eight indices, including PLT, PDW, MPV, PCT, PLR, PDW/PLT, PDW/PCT, and MPV/PLT, were found to be significantly associated with OS in both Kaplan-Meier analysis (S1 Fig.) and univariate Cox analysis (S2 Table). Time-AUC curves were generated to further evaluate the prognostic accuracy of these eight platelet indices (Fig. 1). According to the curves, PDW/PCT had the highest predictive accuracy, followed by MPV. At any point of time, every subject has two outcomes, time-dependent ROC curve depicts the specificity and sensitivity of each marker in predicting survival outcome. Therefore, time-AUC curves indicate the predicting accuracy of different markers in survival outcomes. According to Fig. 1, from 10 to 60 months, time-AUC curves of PDW/PCT and MPV maintain to be on the top of all other curves representing higher predicting accuracy.

In univariate analysis of all the remaining baseline characteristics, factors associated with worse OS included nodal involvement, emaciating, non-radical surgery, ALP > 200 U/L, ALB ≤ 35 g/L, TBIL > 106 μmol/L, CA19-9 > 100 U/mL, and poorer tumor differentiation. An initial multivariable analysis revealed that the presence of nodal involvement, non-radical surgery, CA19-9 > 100 U/mL, and poorer tumor

differentiation were independent adverse prognostic factors. After integration into those four factors, MPV > 8.1 fl (hazard ratio [HR], 1.33; $p=0.045$) and PDW/PCT > 190 (HR, 0.78; $p=0.046$) remained as independent factors (Table 2). These two platelet indices were added into the final multivariable analysis instead of the initial one in order to minimize potential confounding with respect to their association with a number of baseline characteristics as described below.

3. Relationships between PDW/PCT, MPV, and clinicopathological features

Patients with low PDW/PCT were characterized by emaciation, high levels of alanine aminotransferase, aspartate aminotransferase, GGT, ALP, and low levels of ALB, which suggested poor nutritional status and impaired liver function. In addition, these patients had high levels of CA19-9, TBIL, and poor TNM staging, representing quick tumor progression. High levels of TBIL may be derived from severe biliary tract obstruction (S3 Table). In addition to most of the characteristics mentioned above, patients with high MPV were prone to present nodal involvement and large tumor size, both of which indicate malignant behavior. It seemed that these two platelet indices were associated with a number of baseline characteristics, including patient-related factors and pathological features of the tumor. In our sub-analysis, we detected the relationship between these two indices with operation outcomes, including hospitalization day, biliary leakage, infection, and other complications. S4 Table shows that MPV and PDW/PCT were only significantly correlated to hospitalization days, suggesting that platelet indices may not be eligible for predicting operation outcomes.

4. Development and validation of a novel staging system

A nomogram comprising nodal involvement, radical surgery, CA19-9 level, tumor differentiation, MPV, and PDW/PCT was created based on the multivariable Cox analysis (Fig. 2) to predict OS individually. Subsequently, patients in the whole cohort were stratified into three stages almost equally, stage I ($n=153$, score < 107.5), stage II ($n=154$, score 107.5-152.0), and stage III ($n=154$, score > 107.5) according to their scores. The calibration curves for 3- and 5-year OS (S5 Fig.) illustrated good consistency of our new model in the training cohort and suboptimal in the validation cohort.

In survival analysis, patients were classified into GBC, ECC, and ICC groups according to their tumor sites to investigate the discriminative capacity of our novel staging system in each subset of BTC patients. For the convenience of comparison between our new staging system and the AJCC 7th TNM staging system, TNM stage 0 was merged into TNM stage I. In the whole cohort, the median OS for AJCC TNM stages I, II, and III was 48, 27, and 18 months, respectively

Table 2. Univariate and multivariate analyses for OS in BTC patients

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
Type				
GBC	Reference			
ECC	0.97 (0.74-1.27)	0.799		
ICC	1.12 (0.81-1.54)	0.496		
Age (yr)				
≤ 60	Reference			
> 60	1.21 (0.97-1.51)	0.087		
Sex				
Female	Reference			
Male	0.97 (0.78-1.21)	0.785		
Blood type^{a)}				
AB	Reference			
A	0.67 (0.42-1.07)	0.091		
B	0.85 (0.56-1.31)	0.467		
O	0.85 (0.55-1.32)	0.467		
Fever				
No	Reference			
Yes	1.02 (0.75-1.39)	0.902		
Emaciation				
No	Reference			
Yes	1.35 (1.08-1.68)	0.007 ^{b)}		
Debilitation				
No	Reference			
Yes	1.10 (0.78-1.55)	0.582		
Drink				
No	Reference			
Yes	0.91 (0.70-1.18)	0.491		
Fat liver				
No	Reference			
Yes	1.19 (0.80-1.77)	0.393		
ALT (U/L)				
≤ 100	Reference			
> 100	1.17 (0.94-1.45)	0.167		
AST (U/L)				
≤ 100	Reference			
> 100	1.17 (0.92-1.48)	0.198		
GGT (U/L)				
≤ 200	Reference			
> 200	1.23 (0.98-1.53)	0.073		
ALP (U/L)				
≤ 200	Reference			
> 200	1.40 (1.12-1.75)	0.003 ^{b)}		
ALB (g/L)				
≤ 35	Reference			
> 35	0.64 (0.50-0.81)	< 0.001 ^{b)}		
TBIL (μmol/L)				
≤ 106	Reference			
> 106	1.61 (1.30-2.01)	< 0.001 ^{b)}		

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Table 2. Continued

	Univariate analysis		Multivariate analysis	
	HR (95% CI)	p-value	HR (95% CI)	p-value
CA19-9 (U/mL)				
≤ 100	Reference			
> 100	2.01 (1.60-2.53)	< 0.001 ^{b)}	1.60 (1.25-2.05)	< 0.001 ^{b)}
Tumor differentiation				
Poor	Reference			
Moderate	0.68 (0.47-0.98)	0.040 ^{b)}	0.72 (0.59-0.87)	< 0.001 ^{b)}
Well	0.44 (0.30-0.65)	< 0.001 ^{b)}		
Radical cure				
No	Reference			
Yes	0.40 (0.32-0.50)	< 0.001 ^{b)}	0.47 (0.37-0.60)	< 0.001 ^{b)}
T category				
Tis	Reference			
1	5.38 (0.75-38.71)	0.095		
2	6.43 (0.90-46.14)	0.064		
3	9.03 (1.26-64.60)	0.028 ^{a)}		
4	6.07 (0.82-45.13)	0.078		
Nodal involvement				
No	Reference			
Yes	1.99 (1.58-2.50)	< 0.001 ^{b)}	1.68 (1.31-2.15)	< 0.001 ^{b)}
Tumor size (cm)				
≤ 2.6	Reference			
> 2.6	0.90 (0.72-1.13)	0.364		
Postoperative chemotherapy				
No	Reference			
Yes	0.93 (0.71-1.22)	0.624		
MPV (fl)				
≤ 8.1	Reference			
> 8.1	1.52 (1.19-1.95)	< 0.001 ^{b)}	1.33 (1.01-1.75)	0.045 ^{b)}
PDW/PCT				
≤ 190	Reference			
> 190	0.65 (0.52-0.82)	< 0.001 ^{b)}	0.78 (0.61-1.00)	0.046 ^{b)}

ALB, albumin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BTC, biliary tract cancer; CA19-9, carbohydrate antigen 19-9; CI, confidence interval; ECC, extrahepatic cholangiocarcinoma; GBC, gallbladder cancer; GGT, γ -glutamyl transpeptidase; HR, hazard ratio; ICC, intrahepatic cholangiocarcinoma; MPV, mean platelet volume; OS, overall survival; PCT, plateletcrit; PDW, platelet distribution width; TBIL, total bilirubin. ^{a)}All patients were Rh positive, ^{b)}Statistically significant ($p < 0.05$).

(Fig. 3B), with HRs of 1.0 (reference), 1.30 (1.00-1.70), and 1.76 (1.35-2.30), respectively ($p < 0.001$). The median OS for the novel staging system (Fig. 3A) was 68, 27, and 12 months, respectively, with HRs of 1.0 (reference), 2.15 (1.57-2.96), and 4.81 (3.51-6.58), respectively ($p < 0.001$). With regards to specific types of BTC, the new staging system (Fig. 3C) had a performance similar to that of the TNM staging system for GBC patients (Fig. 3D). However, for ICC patients, the new staging system (Fig. 3E) performed better than the TNM staging system (Fig. 3F). Noticeably, for ECC patients, the differences between groups classified by the new staging

system (Fig. 3G) remained significant, while the TNM staging system (Fig. 3H) failed to discriminate between patients with different OS.

The C-index for the new staging system was 0.703 in the training cohort and 0.728 in the validation cohort using the bootstrapping method (500 repetitions). The C-index for AJCC TNM stage was 0.561 in the training cohort and 0.618 in the validation cohort using the same method. Based on the results of the survival analyses and C-index, we can conclude that the new staging system has superior discrimination capacity to the AJCC TNM staging system, regardless

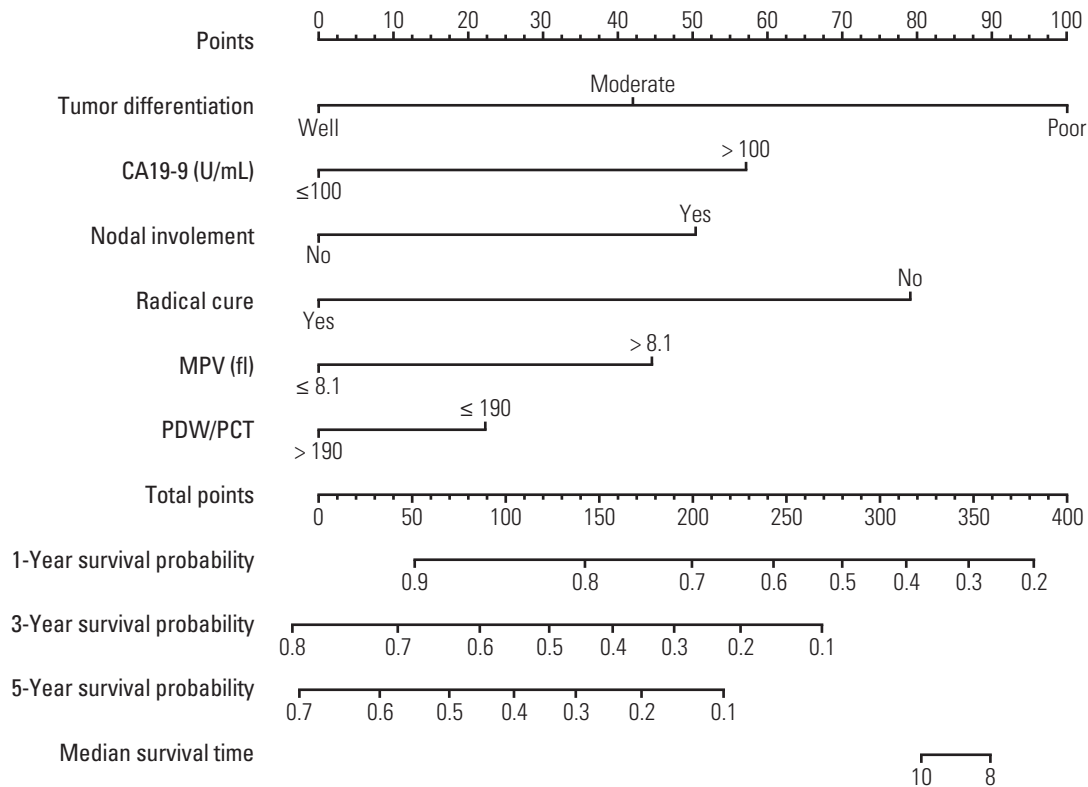


Fig. 2. Nomogram for predicting the 1-, 3-, 5-year, and median survival in patients with biliary tract cancer lines are drawn upward to determine the points for each variable. Total points represent the sum of points corresponding to each variable. Another line is drawn downward to determine the survival probability for total points. CA19-9, carbohydrate antigen 19-9; MPV, mean platelet volume; PCT, plateletcrit; PDW, platelet distribution width.

of the specific type of BTC nor the whole entity of BTC. In addition, DCA for 3- and 5-year OS (Fig. 4) indicated that the new prognostic model had more clinical benefits.

Discussion

BTC is a type of entity tumor with distinctive characteristics with respect to high heterogeneity, poor diagnosis, and quick progression without diverse effective treatment. Radical surgery is the only acknowledged curative treatment, as the role of adjuvant chemotherapy in treating BTC remains to be confirmed. Consequently, a broader and more effective prognostic model is required for the prognostication of patients with BTC undergoing surgical resection. Therefore, we constructed and internally validated a new prognostic model to predict OS and to stratify patients with BTC into different stages after surgery. The model was presented as a nomogram based on six independent prognostic factors, including two platelet indices, nodal involvement, CA19-9, radical surgery, tumor differentiation, PDW/PCT, and MPV.

By integrating tumor features, hemostatic parameters, and operation correlated factors, our novel staging system based on the model showed good discrimination and accuracy.

Recently, an increasing number of prognostic factors for cancer have been defined, including platelet indices. The platelet indices selected in the current study were indices depicting platelet morphology including PLT, PCT, MPV, and PDW, and the ratios in between, including PDW/PCT, PDW/PLT, MPV/PLT, and MPV/PCT. PLR, an indicator of inflammation, which is drawing increasing attention, was also included. Prognostic values of most of these indices have not been investigated for BTC. Univariate Cox analysis revealed that eight out of 10 indices were associated with OS in BTC, suggesting a marked impact of platelets on tumor progression. According to time-AUC curves of these eight indices (Fig. 1), we found that MPV and PDW/PCT have the highest potential to be independent prognostic factors of BTC, which was subsequently confirmed by the final multivariable Cox analysis. MPV, representing platelet size, has been demonstrated to be an independent predictor for OS in many cancers, and a high MPV has been reported as a poor

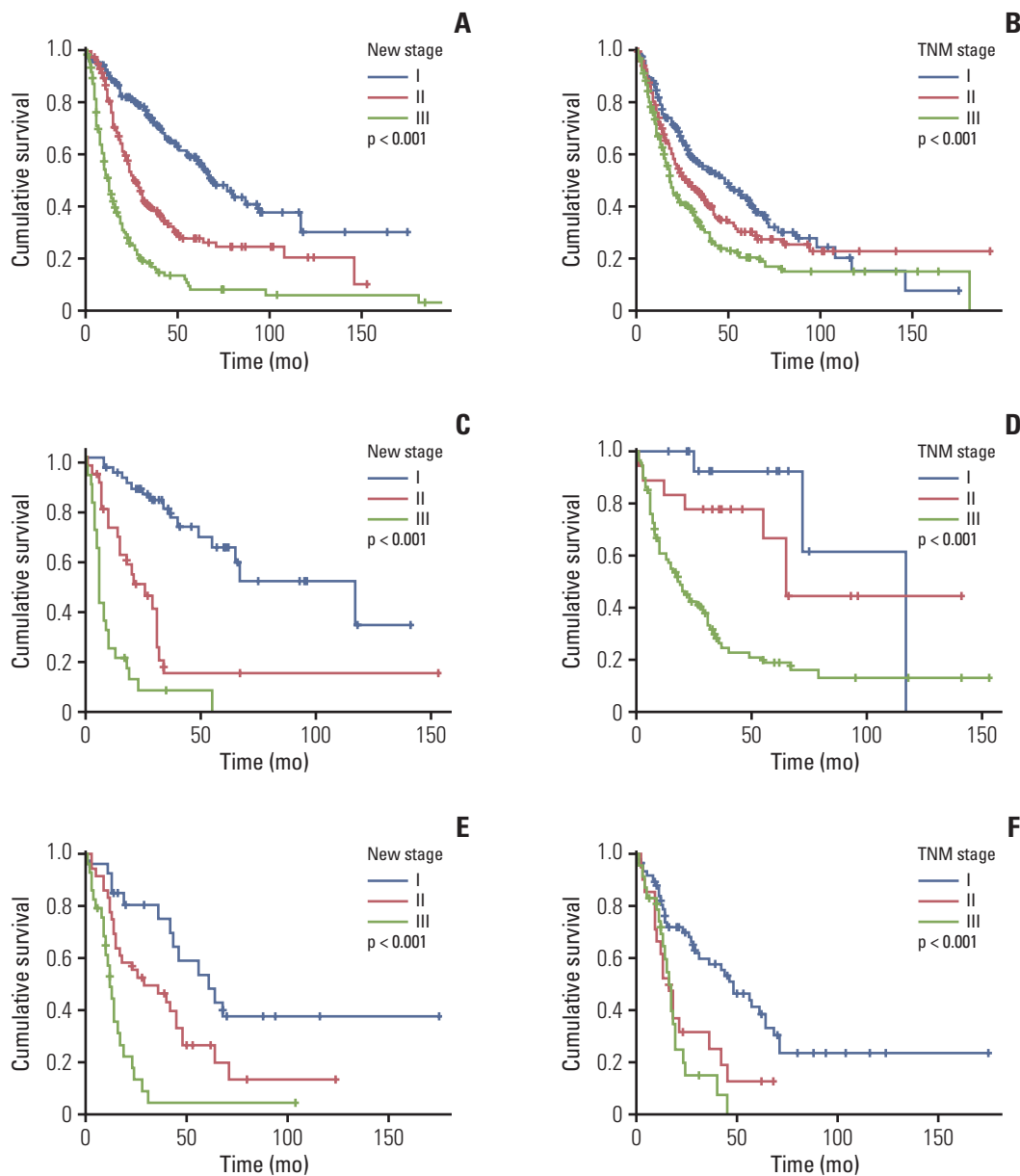


Fig. 3. Kaplan-Meier curves for OS in different subsets of patients with BTC. BTC patients (A), GBC patients (C), ICC patients (E), and ECC patients (G) were stratified by the novel staging system. BTC patients (B), GBC patients (D), ICC patients (F), and ECC patients (H) were stratified according to the AJCC TNM staging system. AJCC, American Joint Committee on Cancer; BTC, biliary tract cancer; GBC, gallbladder cancer; ECC, extrahepatic cholangiocarcinoma; ICC, intrahepatic cholangiocarcinoma; OS, overall survival. (Continued to the next page)

indicator of OS in pancreatic cancer [11], invasive breast cancer [12], lung cancer [13] and esophageal carcinoma [14]. Our findings add to the accumulating evidence that high MPV is an independent negative predictor of OS. PCT as determined by MPV and PLT, reflects the number and size of platelets simultaneously. In our study, high PLT and high MPV were both observed in patients with poor survival, resulting in a

reasonably high PCT. Furthermore, a previous study found that increased PCT levels were indicative of poor prognosis in metastatic colorectal cancer [15]. Although the relationship between PCT and OS in cancers remains to be investigated, based on the evidence that MPV and thrombocytosis are associated with poor survival in many cancers [16], we can speculate that PCT has the potential to be a good predic-

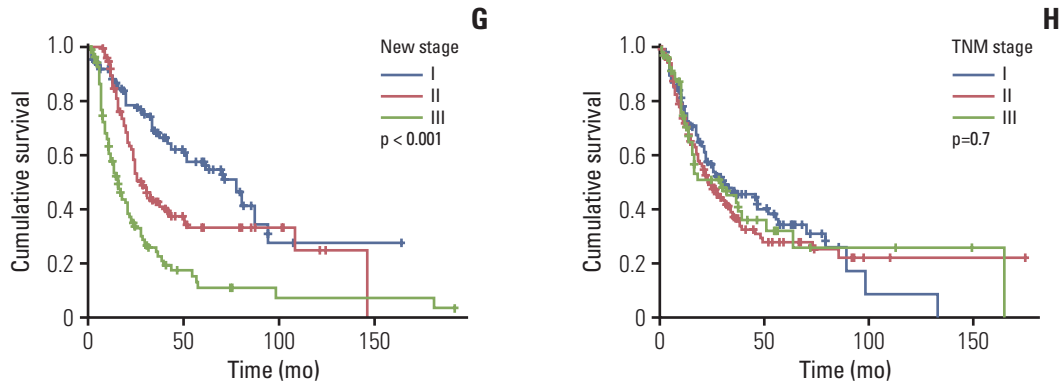


Fig. 3. (Continued from the previous page)

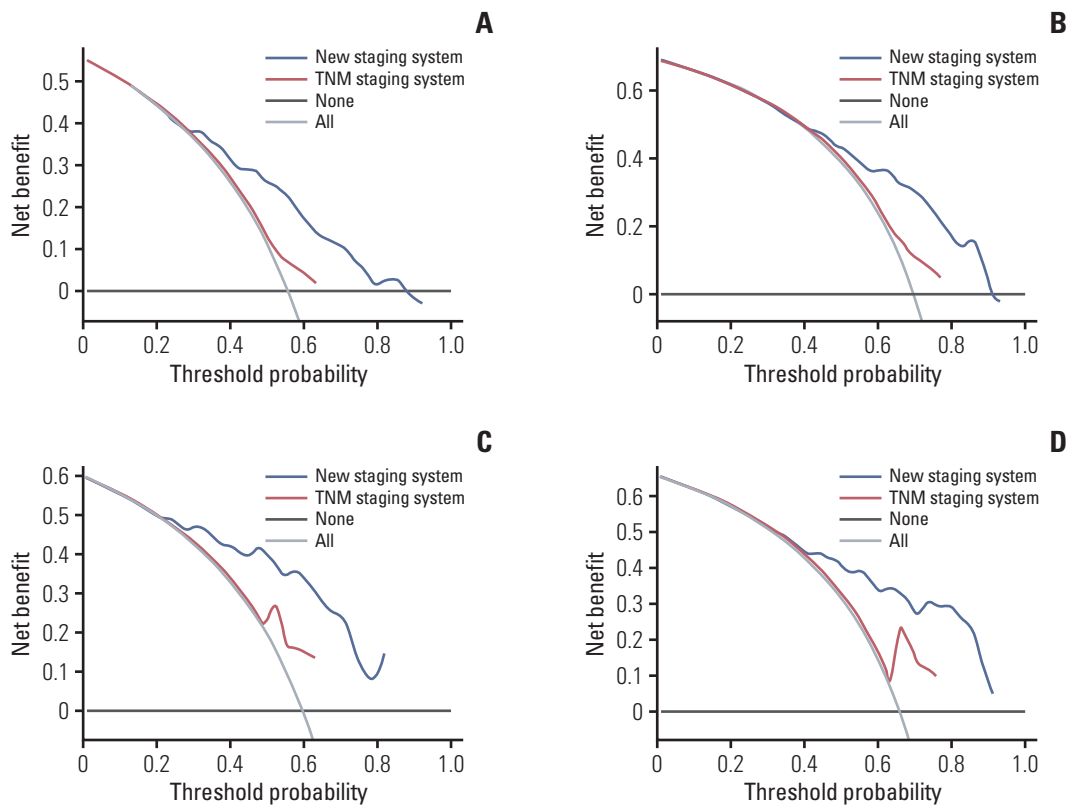


Fig. 4. Decisive curve analysis of the nomogram. (A) 3-Year survival of the training cohort. (B) 5-Year survival of the training cohort. (C) 3-Year survival of the validation cohort. (D) 5-Year survival of the validating cohort. The horizontal black line denotes no survival, while the gray curve represents all survival.

tor of tumor progression. The role of PDW in predicting OS is controversial. Low PDW was found to be correlated with poor OS in non-small cell lung cancer [17], colorectal cancer [18], and gastric cancer [19]. The mechanism underlying high PDW with poor tumor stage remains to be elucidated. An emerging notion is that the interplay between PDW and MPV should be considered when evaluating their prognos-

tic performance. Overall, the correlation between PDW and tumor progression remains to be investigated, and whether the underlying mechanisms are unique for heterogeneous tumors is unknown. In our study, by combining the PDW and PCT, PDW/PCT showed better performance in terms of predicting prognosis than PDW or PCT alone, while the prognostic performance of PDW/PCT has not yet been

revealed in cancers.

An increasing body of evidence has highlighted the role of platelets in various stages of tumorigenesis, including tumor growth, invasion, metastasis, and chemotherapy resistance. The interaction between platelets and tumor cells is mutual, and recent studies have demonstrated that cancer can influence platelet's, physiology, and activation, even in the RNA profile; thus, providing potential markers for cancer prognosis [20]. Mechanisms of cancer-stimulated thrombocytosis have been elucidated, and primary tumors can influence megakaryopoiesis and thrombopoiesis by modulating a variety of cytokines directly or indirectly. Granulocyte colony-stimulating factor and macrophage colony-stimulating factor secreted by tumor cells can stimulate megakaryopoiesis [20], while elevated interleukin-6 released from cancer cells can increase the ploidy of megakaryocytic nuclei, resulting in an elevated platelet volume [21]. The ploidy of megakaryocytes has been observed to correlate with the MPV [22]. Moreover, platelets with a high MPV have been confirmed to be a potential indicator of platelet activation [23]. Larger platelets contain higher absolute amounts of proteins and more receptors on the surface, which enables them to interact with tumor cells [22]. For example, during the process of platelet activation, granules containing ADP released by platelets [20] interact with P2Y₁₂ receptors on platelets, triggering epithelial-mesenchymal transition, followed by invasion and metastasis [24]. Platelets with high MPV can also release more growth and prothrombotic factors, promoting angiogenesis and tumor progression [21]. Furthermore, activated platelets can crosslink, forming clots, and protecting circulative tumor cells against shear stress or immune attack [24]. In addition, increased platelet turnover is often observed in cancer patients, possibly arising from increased platelet consumption or sequestration. When platelet turnover increases, new platelets are produced with a larger size [22]. Whether larger platelets are more prothrombotic remains unclear. Evidence clarifying the role of small platelets in cancer-associated thrombosis is limited. Different mechanisms for platelets with different sizes interfering with cancer cells may exist, considering the diversity of cancer and the extensive functions of platelets. Lower PDW represents lower heterogeneity of platelets, which can be interpreted by a higher proportion of large new platelets under the stimulation of cancer cells. In contrast, platelets with a high MPV have more potential to interfere with cancer cells, assisting tumor progression in many stages. Based on these evidences and our findings, we propose that high MPV and low PDW/PCT are indicators of poor prognosis in BTC.

After integrating MPV and PDW/PCT into the multivariable Cox model, we developed a new prognostic model and a novel staging system. The OS of patients belonging to differ-

ent stages were significantly different, either limited to specific types of BTC or the entire BTC cohort. Meanwhile, the AJCC 7th TNM staging system presented modest prognostic power in patients with ECC (Fig. 3H), and has been shown to have poor prognostic performance in several subsets of patients with BTC [25]. The C-index of our novel prognostic model was 0.703 in the training cohort and 0.728 in the validation cohort. Compared to the AJCC TNM stage, with a C-index of 0.561 and 0.618, our prognostic model has superior discrimination to the AJCC TNM stage. DCA also implied increased clinical benefits with the utility of our new staging system. Based on these results, we can conclude that our new prognostic model outperformed the AJCC TNM staging system in many aspects. An obvious advantage of our new prognostic model is the universal application to patients with BTC without the need to adjust the prognostic standard for any specific type. The AJCC TNM staging system is applicable for all subclasses of BTC, whereas the staging standards are not uniform with respect to different types of BTC. For example, T stage in GBC represents the infiltration depth of the tumor, while in ECC, T stage depends on the extent of invasion. In contrast, our model performed well in predicting the survival of patients with all types of BTC, which allows for greater convenience and wider application in comparison to the TNM staging system. Prognostic models have been proposed for GBC [26] and ICC [27], respectively, while relatively few models have been developed for BTC independent of a tumor site. Schweitzer et al. [28] proposed a prognostic score for patients with BTC at the time of diagnosis before any intervention. Bridgewater et al. [29] developed a model to predict OS in advanced BTC with limited predictive prognostic accuracy. A suitable method for survival prediction of patients with BTC undergoing resection has not yet been determined. Our novel staging system had extraordinary discriminative capability in predicting the OS of patients with BTC independent of the tumor site. The nomogram cannot only inform patients after surgery about their prognosis but also help to identify candidates for aggressive surgery among patients with BTC beforehand. With respect to the involvement of platelet indices, our new prognostic model can be applied to stratify patients in clinical trials intended to explore the efficacy of anti-platelet therapy, since post-diagnosis aspirin use has been reported to be associated with prolonged BTC survival [30].

One thing needs to be explained is the relatively high proportion of non-radical surgery (38.3%). This could be attributed to several reasons. Symptoms of biliary tumors were of insidious onset and most patients were found to be in advanced stage during the first medical visit. In our research, 31.3% patients were classified as TNM stage III with diverse severity of perinephric tissue invasion which increased

the difficulty of surgery. Another situation was that preoperative assessment underestimate the range of tumor invasion where only radical surgery had the chance to remove all lesions. However, due to patient intolerance or family disagreement, non-radical surgery was performed instead of radical surgery. Another reason was that surgeries were performed between December 2002 and December 2017, intraoperative frozen-section was not routinely performed until recent years. From 2002 to 2012, 111 patients out of 213 (52.1%) received radical surgery while from 2013 to 2017, 168 patients out of 245 (68.6%) received radical surgery. The development of new technology elevated the probability of radical surgery.

Our study has several limitations. First, our data need to be completed, as detailed features of tumors, including invasion or local extension, are absent. However, the prognostic ability of perineural invasion and papillary tumors are inconsistent in BTC due to correlation with other factors [6], the absence of these data probably had a slight influence on the outcome. Second, our study was a single-center retrospective study with inevitable selection bias; multi-center and prospective studies are needed before generalization to a broader population. Third, the lack of external validation may decrease the credibility of the model, although we performed internal validation and obtained a high C-index value in the validation cohort.

To the best of our knowledge, our study is the first to systematically evaluate platelet indices as prognostic factors for BTC. We found that MPV and PDW/PCT are both independent prognostic factors for OS in patients with BTC. Subsequently, a new prognostic model and novel staging system

were proposed for patients with BTC undergoing surgical resection, whose prognostic ability outperformed TNM staging in many aspects.

Electronic Supplementary Material

Supplementary materials are available at Cancer Research and Treatment website (<https://www.e-crt.org>).

Ethical Statement

This study was approved by the Medical Ethics Committee of PUMCH (S-K1110), and written informed consent was obtained from all patients. The study conformed to the ethical guidelines of the 1975 Declaration of Helsinki.

Author Contributions

Conceived and designed the analysis: Sun L, Yang H, Mao Y.

Collected the data: Chen Y, Hu W, Ji X, Xu H, Du S, Zhao H, Lu X, Sang X, Zhong S.

Contributed data or analysis tools: Sun L, Chen Y.

Performed the analysis: Wei Y.

Wrote the paper: Wei Y.

Conflicts of Interest

Conflict of interest relevant to this article was not reported.

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