Journal of Hepatocellular Carcinoma

Dovepress
open access to scientific and medical research

Open Access Full Text Article

ORIGINAL RESEARCH

Development and Validation of a Novel Prognostic Nomogram Based on Platelet and CD8$^+$T Cell Counts in Hepatocellular Carcinoma Patients with Portal Vein Tumor Thrombosis

Wanxin Shi[1,2,*], Huiwen Yan[1,*], Xiaoli Liu[1], Lihua Yu[1], Yuqing Xie[1], Yuan Wu[1], Yuling Liang[1], Zhiyun Yang[1]

[1]Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, People's Republic of China; [2]First Clinical Medical College, Beijing University of Chinese Medicine, Beijing, People's Republic of China

*These authors contributed equally to this work

Correspondence: Zhiyun Yang, Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, No. 8 Jing Shun East Street, Beijing, 100015, People's Republic of China, Tel/Fax +86-10-84322148, Email yangzhiyun2016@163.com

Purpose: Portal vein tumor thrombosis (PVTT) is one of the hallmarks of advanced Hepatocellular carcinoma (HCC). Platelet (PLT) function parameters and CD8$^+$T cells (CD8$^+$Ts) play an important role in HCC progression and metastasis. This study is committed to establishing an efficient prognosis prediction model and exploring the combined effect of PLT and CD8$^+$Ts on PVTT prognosis.

Patients and Methods: This retrospective study collected 932 HCC patients with PVTT from 2007 to 2017 and randomly divided them into a training cohort (n = 656) and a validation cohort (n = 276). We performed multivariable Cox and Elastic-net regression analysis, constructed a nomogram and used Kaplan–Meier survival curves to compare overall survival and progression-free survival rates in different substrata. Relationships between indicators involved were also analyzed.

Results: We found tumor number, size, treatment, PLT, γ-glutamyl transferase, alpha-fetoprotein, mean platelet volume, and CD8$^+$Ts were related to the 5-year OS of patients with PVTT, and established a nomogram. The area under the receiver operating characteristic curve (AUCs) for predicting the 1-year OS rates were 0.767 and 0.794 in training and validation cohorts. The calibration curve and decision curve indicated its predictive consistency and strong clinical utility. We also found those with low PLT (<100*10^9/L) and high CD8$^+$Ts (>320 cells/μL) had a better prognosis.

Conclusion: We established a well-performing prognostic model for PVTT based on platelet functional parameters and CD8$^+$Ts, and found that PT-8 formed by PLT and CD8$^+$Ts was an excellent predictor of the prognosis of PVTT.

Keywords: hepatocellular carcinoma, portal vein tumor thrombus, platelet, CD8+T cells counts, prognostic model, correlation analysis

Introduction

Hepatocellular carcinoma (HCC) is one of the major diseases that seriously endangers human life and health. According to the latest global cancer burden data released by WHO, the incidence and mortality of HCC rank sixth and fourth among malignant tumors, respectively.[1] Due to the biological characteristics of liver cancer and the anatomical characteristics of the liver, HCC easily invades intrahepatic blood vessels, especially the portal vein system. In China, the incidence rate of portal vein tumor thrombosis (PVTT) is as high as 44–62.2%. Without treatment, the median survival time of HCC patients with PVTT is only 2.7–4 months.[2] PVTT progresses rapidly and can lead to serious complications such as portal hypertension, hepatic jaundice, and refractory ascites. Therefore, the treatment strategy for PVTT has become a global medical problem.

Received: 2 December 2023
Accepted: 9 May 2024
Published: 7 June 2024

Currently, Barcelona Clinic Liver Cancer (BCLC), Child-Pugh and Model for end-stage liver disease (MELD), etc. are often used to evaluate the prognosis of HCC patients.[3] PVTT, as a marker of advanced liver cancer, is also a major factor affecting the prognosis of HCC. However, it is still unclear whether the prognostic indicators of HCC are still applicable to PVTT. Multiple studies have shown that platelets are closely related to the occurrence and development of tumors and have certain prognostic predictive value.[4–7] Decades ago, lots of scientists demonstrated that platelets could promote tumor vascular invasion and metastasis.[8,9] Platelets exercise a function as if it were a double-edged sword. Recent studies have also identified thrombocytopenia as a risk factor for recurrence and survival in patients with HCC after hepatectomy, which explains that platelet count reflects liver function to a certain extent, and thrombocytopenia implies a poor prognosis.[10] In summary, platelets play different roles in liver cancer at different stages. In addition, immune status has always been considered a prognostic factor for patients at all stages, especially for advanced liver cancer.[11] There is a correlation between PLT and immune response,[12] but there are few studies that combine them to comprehensively evaluate the prognosis of PVTT patients, which is a potential direction that has been ignored.

Here, we first explored the relationship between platelet and immune-related indicator $CD8^+$T cells counts and the prognosis of HCC patients with PVTT, and then established a model based on clinical data to screen advantaged groups, which will play a role in promoting personalized medicine. It is convenient for clinicians to predict prognosis and provides new solutions to the difficult problem of PVTT.

Materials and Methods

Patients

This retrospective study included 932 HCC patients with PVTT at Beijing Ditan Hospital of Capital Medical University from December 2007 to December 2017. The research project received approval from the ethical committee at Capital Medical University's Beijing Ditan Hospital (no. JDLKZ [2017] D [028] -02), along with a waiver of informed consent. All procedures were conducted in accordance with the 2008 Helsinki Declaration and the ethical standards outlined by the relevant national and institutional committees governing human research. The patients were divided into two groups in a ratio of 7:3, using randomization, with 656 patients in the training cohort and 276 patients in the validation cohort. The inclusion criteria were as follows: (1) age ≥18 years; (2) HCC diagnosis based on imaging or histological assessment guidelines of the Asia Pacific Society for the Study of the Liver (APSAL);[13] and (3) diagnosis of PVTT confirmed by at least one imaging test such as ultrasound, computed tomography (CT), magnetic resonance imaging (MRI), or digital subtraction angiography (DSA). The exclusion criteria were as follows: (1) patients with combined HIV and tuberculosis infection; (2) patients with serious diseases affecting important organs; (3) patients with metastatic hepatocellular carcinoma or other tumors; (4) pregnant or lactating women; and (5) patients with incomplete clinical data, relevant examinations, or test results.

Demographic and Clinical Data

Baseline data included patient's background such as age, gender, hypertension, diabetes mellitus, etiology and treatment modalities; laboratory data included routine blood markers such as white blood cells counts (WBC), hemoglobin (Hgb), platelets (PLT), mean platelet volume (MPV), liver function markers such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), ɣ-glutamyl transpeptidase (ɣ-GGT), etc., coagulation parameters such as prothrombin activity (PTA), immune cell-related parameters such as $CD4^+$Ts, $CD8^+$Ts, etc., and tumor-related parameters, such as alpha-fetoprotein (AFP), tumor size and number, and HCC comprehensive evaluation parameters, such as BCLC, Child-Pugh, Meld, APRI (AST-to-Platelet Ratio Index), FIB-4 (fibrosis-4 score). The data source was the testing report from the Laboratory Department of Beijing Ditan Hospital. We consulted relevant departments and when using flow cytometry, the following antibodies were used: anti-human BV786-conjugated anti-CD3, APC-H7-conjugated anti-CD4, BV510-conjugated anti-CD8 (BD Biosciences). Data were acquired with an FACS Canto‖ flow cytometer, and then analysis with FlowJo software (Tree Star). The observation time of patients was defined as the time from the first enrolment of patients into the study to the end of progression, death or follow-up.

Statistical Analysis

All statistical analyses were performed through R version 4.3.1, and in this study, all the data were randomly grouped in a 7:3 ratio using the "caret" package, other packages involved were rms, survival, etc. The condition when p-value of any test was less than 0.05 was considered statistically different for the study results. In order to elaborate the scientific issues more distinctly, we successively converted individual continuous variables into categorical variables during the study process. The cut-off values of categorical variables were the normal values of clinical laboratory tests. The numerical cut-offs were as follows: Platelets were 100*10^9/L, AFP was 400 ng/mL, laboratory values of which at the time of diagnosis of HCC patients with PVTT were classified as elevated when they were higher than clinical normal values, and CD8+Ts was 320 cells/μL. This means that 'Low PLT and High $CD8^+$Ts' represented PLT<100*10^9/L&$CD8^+$Ts≥320 cells/μL, 'Low PLT and Low $CD8^+$Ts/ High PLT and High $CD8^+$Ts' represented PLT<100*10^9/L&$CD8^+$Ts<320 cells/μL or PLT≥100*10^9/L&$CD8^+$Ts≥320 cells/μL, and 'High PLT and Low $CD8^+$Ts' represented PLT≥100*10^9/L&$CD8^+$Ts<320 cells/μL. In addition, we divided the nomogram into three categorical variables using quarter and three sites as cut-off values (154.3798 and 190.307). In other words, Low risk is points <154.3798, Median risk is 1.543798≤points≤190.307, and High risk is points>190.307. MELD cut-off values of 10 and 15 were determined based on the relevant literature.[14] In addition, quantitative data that conformed to normal distribution were expressed as mean ± standard deviation (SD) and compared between the two means using the *t*-test. Non-normally distributed data were expressed as median (M), interquartile range (QR) of the form (M, QR), and Mann–Whitney *U*-test was used for comparison between two groups of this type. Qualitative data were expressed as frequencies (number of individuals) and compared using the $\chi 2$ test.

In this study, we used univariable and multivariable Cox hazards analysis to find independent influences on HCC patients with PVTT, and subsequently combined multivariable Cox regression with Elastic-net regression to analyze the data, with the final outcome being a nomogram using R (rms package). Most of the existing linear regression methods exploit the conventional zero-one matrix as the regression targets, which greatly narrows the flexibility of the regression model, but elastic network regression avoids this shortcoming and performs well in research on multiple parameters and large parameter correlations.[15] We evaluated the degree of model differentiation by subject work characteristics (ROC) curves and area under the curve (AUC). Calibration curves were used to assess the degree of calibration between the actual and predicted probabilities of the nomogram model. Decision curves were used in this clinical study to assess the clinical utility of the nomogram model. Kaplan–Meier survival analysis was used to compare the Overall Survival (OS) and Progressive Free Survival (PFS) rates for each subpopulation in 1, 3, and 5 years, and survival comparisons were made by the Log rank test.

Results

Baseline Characteristics

We retrospectively included 932 HCC patients with PVTT between 2007 and 2017 and divided them into a training cohort (n = 656) and a validation cohort (n = 276) in a 7:3 ratio. In the training and validation cohorts, 302 (46.04%) and 139 (50.36%) patients had single tumor and 354 (53.96%) and 137 (49.64%) patients had multiple tumors, respectively (p = 0.26>0.05, Table 1). Whereas in terms of tumor size, the median tumor diameter (mm), the median quadratic range (QR) were 50.00

Table 1 Demographic Data and Clinical Characteristics of Patients of Hepatocellular Carcinoma with Portal Vein Tumor Thrombus

Variable		Overall n=932	Training Cohort, n=656(%)	Validation Cohort, n=276(%)	P value
Gender	Female	187(20.06)	131(19.97)	56(20.29)	0.98
	Male	745(79.94)	525(80.03)	220(79.71)	
Age (y)	<55	271(29.08)	198(30.18)	73(26.45)	0.29
	≥55	661(70.92)	458(69.82)	203(73.55)	
Hypertension	No	703(75.43)	494(75.30)	209(75.72)	0.96
	Yes	229(24.57)	162(24.70)	67(24.28)	
Diabetes	No	730(78.33)	515(78.51)	215(77.90)	0.91

(*Continued*)

Table 1 (Continued).

Variable		Overall n=932	Training Cohort, n=656(%)	Validation Cohort, n=276(%)	P value
	Yes	202(21.67)	141(21.49)	61(22.10)	
Etiology	HBV	719(77.15)	508(77.44)	211(76.45)	0.81
	HCV	64(6.87)	42(6.40)	22(7.97)	
	Alcohol abuse	118(12.66)	85(12.96)	33(11.96)	
	Other	31(3.33)	21(3.20)	10(3.62)	
Tumor number	Solitary	441(47.32)	302(46.04)	139(50.36)	0.26
	Multiple	491(52.68)	354(53.96)	137(49.64)	
Tumor size (mm)		50.00[27.00,62.00]	50.00[27.00,61.25]	50.00[26.75,65.25]	0.74
Treatment	Palliative	277(29.72)	180(27.44)	97(35.14)	0.06
	Minimally invasive	613(65.77)	446(67.99)	167(60.51)	
	Resection	42(4.51)	30(4.57)	12(4.35)	
Child-Pugh	A stage	374 (40.13)	265 (40.40)	109 (39.49)	0.72
	B stage	413 (44.31)	293 (44.66)	120 (43.48)	
	C stage	145 (15.56)	98 (14.94)	47 (17.03)	
BCLC	C stage	758 (81.33)	538 (82.01)	220 (79.71)	0.46
	D stage	174 (18.67)	118 (17.99)	56 (20.29)	
WBC (*10^9/L)		4.40[3.11,5.97]	4.41[3.10,5.94]	4.35[3.13,6.07]	0.77
NLR		2.83[1.79,4.26]	2.87[1.83,4.47]	2.68[1.67,3.90]	0.07
Hgb (g/L)		124.80[107.75,140.70]	124.10[107.00,140.35]	126.60[108.83,140.93]	0.31
PLT (*10^9/L)		90.00[62.15,133.77]	87.70[61.75,132.15]	95.60[63.32,139.00]	0.24
PLR		91.59[64.84,142.84]	94.38[66.47,142.84]	86.76[62.37,142.94]	0.22
BUN (mmol/L)		5.25[4.28,6.61]	5.28[4.33,6.73]	5.10[4.15,6.41]	0.13
Cr (μ mol/L)		66.00[58.00,77.00]	66.00[57.10,77.00]	66.00[58.00,77.25]	0.62
ALT (U/L)		36.60[24.60,59.95]	36.20[24.78,58.32]	37.70[24.30,61.27]	0.6
AST (U/L)		49.60[34.80,84.58]	49.50[33.70,82.93]	50.75[35.48,87.70]	0.54
TBIL (μ mol/L)		22.00[14.47,35.35]	20.95[14.38,34.15]	23.55[14.97,39.05]	0.08
ALB (g/L)		34.35[30.10,39.00]	34.50[30.28,38.92]	33.60[29.60,39.10]	0.37
γ-GGT (IU/L)		88.65[44.90,171.57]	89.15[44.90,166.43]	86.50[42.90,175.80]	0.99
ALP (U/L)		112.20[81.70,151.20]	111.65[81.77,149.43]	114.55[81.60,161.52]	0.38
TC (mmol/L)		3.63[3.09,4.07]	3.62[3.12,4.07]	3.63[3.07,4.07]	0.69
PT (s)		13.60[12.50,14.80]	13.60[12.40,14.70]	13.70[12.50,15.10]	0.44
PTA (%)		73.00[63.35,84.93]	73.30[64.00,85.00]	72.00[61.15,83.00]	0.34
AFP (ng/mL)	≤400	779 (83.58)	553 (84.30)	226 (81.88)	0.42
	>400	153 (16.42)	103 (15.70)	50 (18.12)	
CRP (mg/L)		9.80[3.20,12.50]	9.70[3.20,12.50]	9.95[3.20,12.53]	0.71
MPV (f L)		10.70[9.10,11.90]	10.60[9.07,11.90]	10.70[9.10,11.72]	0.77
PCT (%)		0.11[0.07,0.17]	0.11[0.07,0.17]	0.11[0.07,0.18]	0.59
P-LCR (%)		33.10[29.67,37.40]	33.10[29.10,37.42]	33.10[30.50,36.88]	0.51
PDW (%)		13.60[12.30,15.40]	13.60[12.20,15.60]	13.60[12.40,14.80]	0.9
T cells/Lymphocytes (%)		66.00[58.00,71.00]	66.00[58.00,70.86]	66.00[58.00,71.80]	0.98
T cells counts (cells/μL)		584.00[455.00,823.50]	588.00[455.00,829.00]	568.00[455.00,816.00]	0.85
$CD8^+$T cells counts (cells/μL)		207.00[146.00,290.00]	207.00[147.00,290.00]	207.00[142.00,288.00]	0.75
$CD4^+$T cells counts (cells/μL)		368.00[274.00,525.00]	368.00[273.75,521.50]	368.00[276.00,559.00]	0.53
$CD4^+$T/$CD8^+$T cells counts (cells/μL)		1.79[1.31,2.43]	1.79[1.31,2.39]	1.79[1.39,2.48]	0.22

Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; NAFLD, non-alcoholic fatty liver disease; BCLC, Barcelona Clinic Liver Cancer classification system; WBC, white blood cell; NLR, neutrophil-lymphocyte ratio; Hgb, hemoglobin; PLT, platelet; PLR, platelet-lymphocyte ratio; BUN, blood urea nitrogen; Cr, endogenous creatinine clearance rate; ALT, alanine aminotransferase; AST, aspartate transaminase; TBIL, total bilirubin; ALB, albumin; γ-GGT, γ-glutamyl transferase; ALP, alkaline phosphatase; TC, total cholesterol; PT, Prothrombin time; PTA, prothrombin time activity; AFP, alpha-fetoprotein; CRP, C reactive protein; MPV, mean platelet volume; PCT, plateletcrit, P-LCR, platelet -larger cell ratio; PDW, platelet distribution width.

[27.00,61.25] (training cohort) and 50.00 [26.75,65.25] (validation cohort), respectively (p = 0.74>0.05). In terms of treatment, the numbers of patients in the training cohort who underwent palliative, minimally invasive, and resection were 180 (27.44%), 446 (67.99%), and 30 (4.57%), compared to 97 (35.14%), 167 (60.51%), and 12 (4.35%) in the validation cohort, respectively (p = 0.06 > 0.05). We compared the characteristics of two cohorts and obtained a result that it did not show significant differences in demographic background, clinical indicators, and treatment, especially those indicators that would be incorporated into the nomogram model, including PLT, γ-GGT, AFP, MPV, and $CD8^+$Ts. Overall, these observations might indicate that there were no differences in baseline characteristics between the training and validation cohorts.

Filtration for Prognostic Index

We filtrated the risk factors and independent risk factors associated with 5-year OS in the training cohort by univariable and multivariable Cox regression analysis, and the results are presented in Table 2. Univariable analysis found Etiology as Alcohol abuse, multiple tumors, tumor size, treatment as minimally invasive and Resection, WBC, PLT, ALT, AST, TBIL, γ-GGT,

Table 2 Univariable and Multivariable Cox Hazards Analysis of the Training Cohort

Variable	Univariable Analysis		Multivariable Analysis	
	HR [95% CI]	p values	HR [95% CI]	p values
Gender (Female vs Male)	1.136 [0.922, 1.399]	0.232		
Age (< 55 vs. ≥ 55)	1.001 [0.834, 1.201]	0.989		
HTN (No vs Yes)	1.138 [0.942, 1.375]	0.182		
Diabetes(No vs Yes)	1.038 [0.850, 1.266]	0.716		
Etiology				
HBV	Ref			
HCV	0.744 [0.520, 1.066]	0.107		
Alcohol abuse	1.379 [1.088, 1.747]	0.008		
Other	0.952 [0.594, 1.525]	0.837		
Tumor number (Solitary vs Multiple)	1.337 [1.131, 1.579]	0.001	1.264 [1.064, 1.502]	0.008
Tumor size	1.008 [1.005, 1.010]	<0.001	1.0031 [1.0001, 1.0061]	0.033
Treatment				
Palliative	Ref		Ref	
Minimally invasive	0.534 [0.444, 0.641]	<0.001	0.630 [0.517, 0.769]	<0.001
Resection	0.326 [0.205, 0.518]	<0.001	0.415 [0.258, 0.667]	<0.001
WBC	1.084 [1.053, 1.116]	<0.001	1.036 [0.999, 1.074]	0.06
Hgb	0.999 [0.996, 1.002]	0.644		
PLT	1.005 [1.003, 1.006]	<0.001	1.004 [1.003, 1.006]	<0.001
BUN	1.010 [0.984, 1.036]	0.461		
ALT	1.003 [1.001, 1.004]	<0.001	1.001 [0.998, 1.003]	0.565
AST	1.002 [1.001, 1.003]	<0.001	1.0004 [0.999, 1.002]	0.555
TBIL	1.0017 [1.0004, 1.003]	0.009	1.001 [0.999, 1.002]	0.345
ALB	0.994 [0.981, 1.007]	0.351		
γ-GGT	1.002 [1.002, 1.003]	<0.001	1.0012 [1.0005, 1.0021]	0.001
PTA	1.002 [0.998, 1.007]	0.316		
AFP (≤400vs. >400)	1.569 [1.257, 1.957]	<0.001	1.274 [1.012, 1.606]	0.04
MPV	1.059 [1.035, 1.084]	<0.001	1.041 [1.017, 1.067]	0.001
PCT	3.386 [1.465, 7.827]	0.004	0.690 [0.237, 2.006]	0.495
P-LCR	0.993 [0.982, 1.004]	0.204		
PDW	0.996 [0.966, 1.028]	0.821		
T cells/Lymphocytes	1.001 [0.999, 1.003]	0.346		
$CD8^+$T cells counts	0.9994 [0.9989, 0.9999]	0.023	0.9990 [0.9984, 0.9995]	<0.001
$CD4^+$T cells counts	0.9999 [0.9996, 1.0003]	0.733		

AFP, MPV, PCT, $CD8^+$Ts were the risk factors for 5-year OS in training cohort, while multivariable analysis revealed that independent risk factors included tumor number (multiple, Hazard Ratio [HR] = 1.260, 95% confidence interval [CI]: 1.064–1.502, p = 0.008), tumor size ([HR] = 1.0031, [CI]: 1.0001, 1.0061, p = 0.033), treatment (minimally invasive, [HR] = 00.630, [CI]: 0.517–0.769, p < 0.001; resection, [HR] = 0.415, [CI]: 0.258–0.667, p = 0.001), PLT ([HR] = 1.004, [CI]: 1.003–1.006, p < 0.001), γ-glutamyl transferase (γ-GGT, [HR] = 1.0012, [CI]: 1.0005, 1.0021, p = 0.001), alpha-fetoprotein (AFP > 400, [HR] = 1.274, [CI]: 1.012–1.606, p = 0.04), mean platelet volume (MPV, [HR] = 1.041, [CI]: 1.017, 1.067, p = 0.001, $CD8^+$Ts ([HR] = 0.9990, [CI]: 0.9984, 0.9995, p < 0.001). We performed Elastic-net Cox regression analysis (Figure 1a and 1b) on the clinical indicators available in Table 1, which included demographics, laboratory tests, and oncology. After setting λ = 0.09 and obtaining 10 variables, we took the intersection of the results of the elastic net analysis and the multivariable COX regression analysis. Finally, we considered tumor size, tumor number, treatment, PLT, γ-GGT, AFP, MPV, and $CD8^+$Ts to be significantly associated with 5-year prognosis of HCC patients with PVTT.

Development and Performance of Prognostic Model

Through the above screening steps, we identified 8 factors together to form the final model (Figure 1c), including tumor size, number, treatment, PLT, γ-GGT, AFP, MPV, $CD8^+$Ts. This nomogram was used to predict the OS of HCC patients

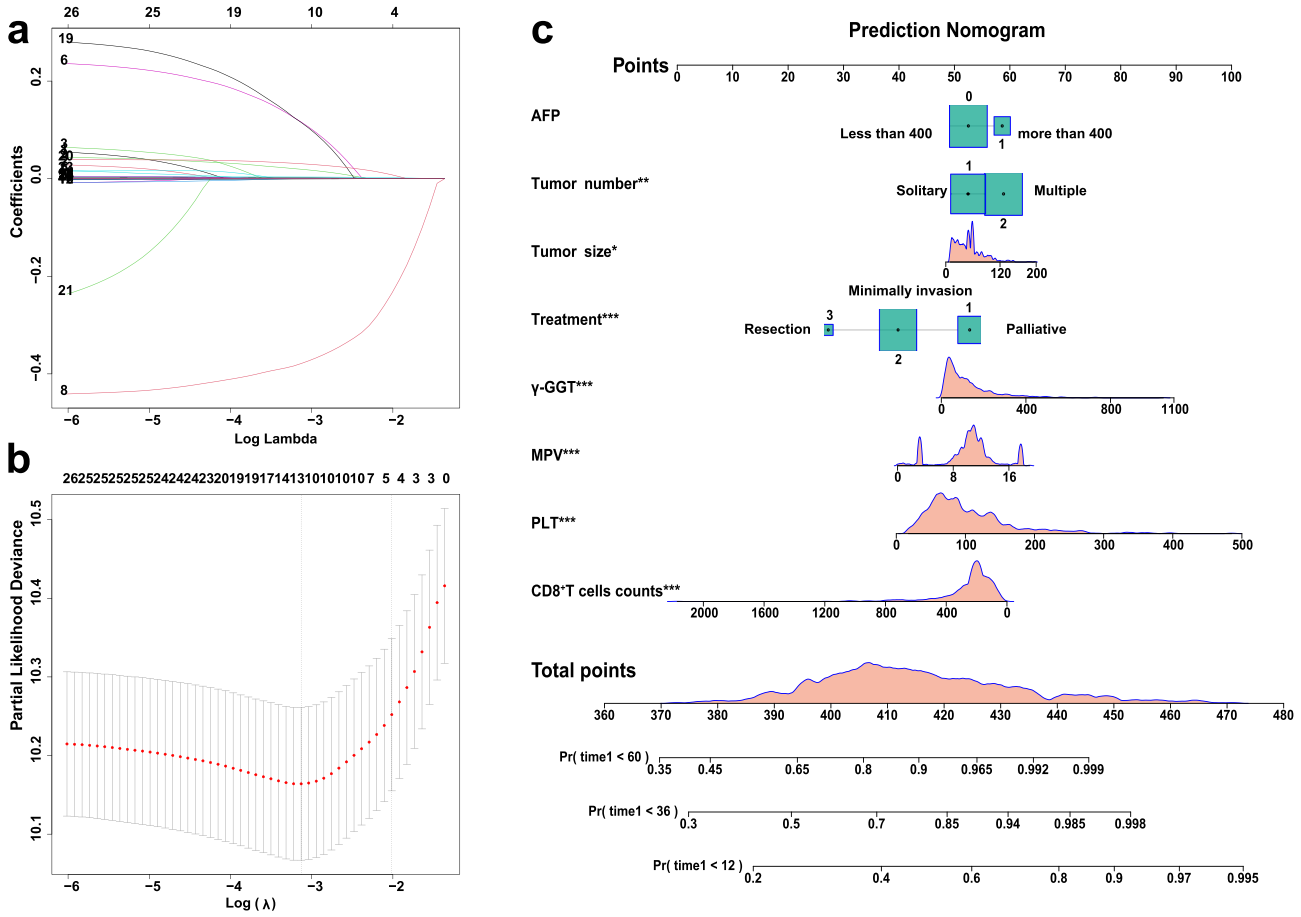

Figure 1 Visualization of elastic-net regression screening indicators and nomogram for predicting HCC patient with PVTT prognosis. (**a**) Elastic-net coefficient profiles of the 26 risk factors. (**b**) 10 risk factors selected using Elastic-net Cox regression analysis. The two dotted vertical lines were drawn at the optimal scores by minimum criteria(left) and 1-s.e. criteria(right) (At minimum criteria including: tumor number, tumor size, treatment, WBC, Hgb, PLT, BUN, ALT, AST, TBIL, ALB, γ-GGT, PTA, AFP, MPV, PCT, P-LCR, PDW, T cells/Lymphocytes, $CD8^+$T cells counts, $CD4^+$T cells counts). (**c**) Nomogram for predicting the 1, 3, 5-year-Overall Survival probability in HCC patient with PVTT. When using the nomogram, find the position of each variable on the axis and the corresponding point vertically. Then, add the points of all variables, and determine the prediction probability of OS in different 3 years on the bottom axis. The green represents categorical variables and the red represents continuous variables, and the filled portion represents the data distribution for constructing the nomogram model. The symbol "*" represents the significance of the p-value, that is, "*" indicates p=0.033, "**" indicates p=0.008, and "***" indicates ≤0.001.

Abbreviations: AFP, alpha-fetoprotein; PLT, platelet; γ-GGT, γ-glutamyl transferase; MPV, mean platelet volume.

with PVTT in 1, 3 and 5 years. Users of the model should bring the 8 indicators into the model, find the upward vertical point of each indicator, add the values represented by the 8 points to obtain the total score, and then draw a vertical line according to the total score at the corresponding position and intersect it with the line representing the probability of survival in 1, 3, and 5 years to obtain the survival rate in different years. The nomogram showed good predictive realism in estimating the 1, 3, and 5-year OS risk of HCC patients with PVTT. The AUC of 1 and 3-year ROC curves in the training cohort was up to 0.767 and 0.726 (Figure 2b and c), and in the validation cohort it was up to 0.794 and 0.711 (Figure 2f and g), which were significantly higher than those of the other models. The predictive efficacy of this nomogram was excellent in different outcomes, because when we used the same method to predict 1, 3, 5 years PFS, the area under the ROC curve AUC of 1, 3, 5 years in the training cohort can also be up to 0.770, 0.728, 0.741 (Figure 2a), and in the validation cohort can be up to 0.797, 0.795, 0.780 (Figure 2e). The consistency between the actual probability of events and the predicted probability of the model is an important indicator for evaluating the performance of the model, so we plotted the calibration curves and found that there was a high degree of consistency between the actual probability of events and the predicted probability of the training cohort (Figure 2d) and validation cohort (Figure 2h). DCA is used to evaluate the usability and validity of the prediction model, and this nomogram demonstrated strong clinical utility in the comparison of different time periods (Figure 2i–k) and different models (Figure 2j–l).

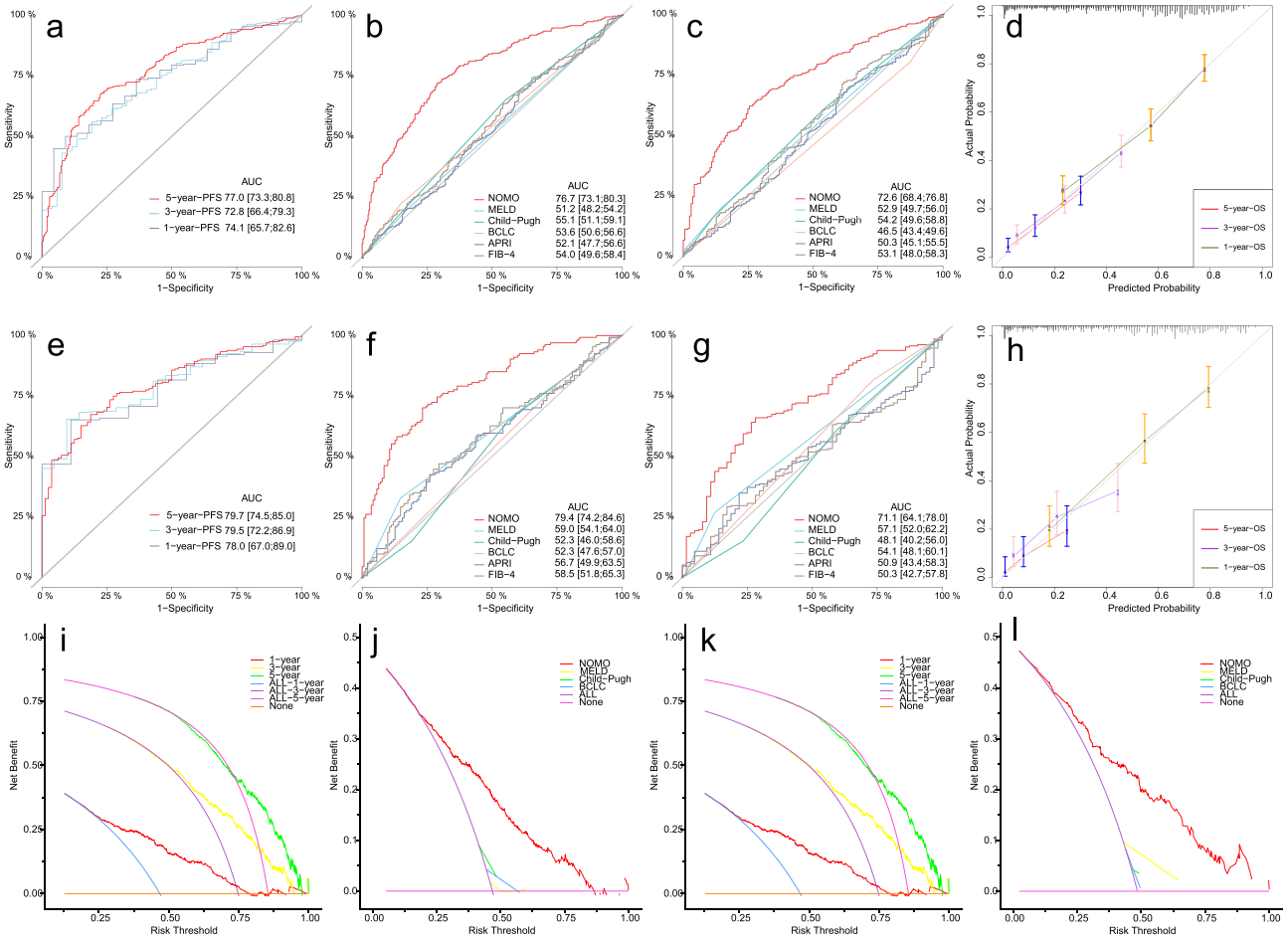

Figure 2 Performance of Nomogram model in predicting the outcome of HCC patient with PVTT. (**a**) ROC analysis of 1-year, 3-year, and 5-year PFS; (**b** and **c**) ROC analysis of 1-year, 3 years OS; (**d**) Calibration curve for predicting 1-year,3-year, and 5-year OS in the training cohort. (**e**) ROC analysis of 1-year, 3-year, and 5-year PFS; (**f** and **g**) ROC analysis of 1-year, 3 years OS; (**h**) Calibration curve for predicting 1-year,3-year, and 5-year OS in the validation cohort. (**i** and **j**) DCA for predicting 1-year,3-year, and 5-year OS and 1-year OS compared with different models in the training cohort. (**k** and **l**) DCA for predicting 1-year,3-year, and 5-year OS and 1-year OS compared with different models in the validation cohort.

Abbreviations: APRI, AST-to-Platelet Ratio Index; FIB-4, fibrosis-4 score; ROC, receiver operating characteristic; AUC, area under the curve; DCA, decision curve analysis, OS, overall survival; PFS, progression-free survival.

Risk Stratification

The nomogram model developed in this study can classify HCC patients with PVTT into low, median and high risk groups according to the two outcomes of OS and PFS in each subpopulation, whether it is the training or validation cohort, with different treatment, Child-Pugh stage, BCLC stage, large or small tumor, single or multiple tumors, AFP ≤ 400 or > 400 ng/mL, the OS and PFS of the group predicted to be at median or low risk were significantly higher than those of the high risk group ($p < 0.0001$) (Figure 3). However, in Child-Pugh Cs (Figure 3g), BCLC Ds (Figure 3i), AFP > 400 ng/mL (Figure 3o), and subgroup treated with palliative (Figure 3p), the ability of the model to stratify for low and median-risks was slightly worse than the others. Unfortunately, there was no clear advantage in the subgroup treated with resection (Figure 3r).

Prognostic Value of "PT-8", a Joint Index of PLT and $CD8^+$Ts in HCC Patients with PVTT

Analyzing the correlation between the model metrics by heatmap (Figure 4a), we found that three groups of parameters with platelets as a like item had the highest correlation and plotted scatter plots (Figure 4b–d), including PLT and $CD8^+$Ts (correlation [95% CI] = 0.249 [0.176.0.320], $p < 0.001$), PLT and γ-GGT (correlation [95% CI] = 0.248 [0.174,0.318], $p < 0.001$), PLT and tumor size (correlation [95% CI] = 0.244 [0.171,0.315], $p < 0.001$). We dichotomized platelets with a cut-off value of 100*10^9/L at and $CD8^+$Ts with a cut-off value of 320 cells/μL and formed a new triple categorical variable "PT-8". The stacked bar chart shows that this joint variable "PT-8" is closely related to the model's high, medium and low risk, in which the percentage of "Low PLT and High $CD8^+$Ts" (LP-HT) decreases with the increasing risk level, which is 19.74%, 4.72%, and 0.86%, respectively, while "High PLT and Low $CD8^+$Ts" (HP-LT) is the opposite, at 4.29%, 27.47%, and 66.09% (Figure 4e). Similarly, in the population classified as high risk by the model in HP-LT, "Low PLT and Low $CD8^+$Ts/High PLT and High $CD8^+$Ts" (LP-LT/HP-HT), and LP-HT population has a decreasing share of 52.74%, 13.51%, and 2.86%, respectively, and the opposite is true for the low-risk population, which is 3.42%, 31.05%, and 65.71%, respectively (Figure 4f). Therefore, we concluded that LP-HT was positively associated with better prognosis and HP-LT was negatively associated with better prognosis. By using the Kaplan–Meier curves analyze, we verified that the LP-HT sub-stratum had the best prognosis (OS), followed by LP-LT/HP-HT, and HP-LT had the worst prognosis (Figure 4g). By further observing the composition of PT-8 and 3-year survival of different risk groups through the Sankey diagram, we found that LP-LT/HP-HT and HP-LT had a much higher rate of death in 3 years than the LP-HT population (Figure 5a).

We usually believe that patients with poorer liver function and higher tumor load have a worse prognosis. Thus, we compared the PT-8 of patients with multiple tumors, size > 50mm, AFP > 400 ng/mL, Child-Pugh Cs, BCLC Ds with the overall population, and the results showed that the proportion of LP-HT was significantly reduced and the proportion of HP-LT was significantly increased among the above groups with poor prognosis (Figure 5b). By using the Kaplan–Meier method, we verified the survival of different Child-Pugh, BCLC, AFP, and treatments subgroups, except for the Child-Pugh Cs (Figure 5e), BCLC Ds (Figure 5g) and Resection (Figure 5l) groups, the LP-HT sub-stratum had the best prognosis (OS), followed by LP-LT/HP-HT, and HP-LT with the worst prognosis (Figure 5c, d, f, h and k, $p < 0.05$). In order to investigate the effect of PT-8 and the Nomogram on 5-year OS, based on multifactorial forest plot, this study found that APRI ([HR] = 1.0412, [CI]: 1.0022–1.0818, $p = 0.0381$), Low PLT and High $CD8^+$Ts ([HR]: 0.6970, [CI]: 0.4985–0.9744, $p = 0.0347$), Nomogram (Median risk, [HR] = 1.8816, [CI]: 1.5233–2.3241, $p < 0.0001$; High risk, ([HR] = 4.6518, [CI]: 3.5545–6.0878, $p < 0.0001$) (Figure 6), which may explain the effect of PT-8, a combined indicator of PLT and $CD8^+$Ts counts, and the Nomogram have a strong prognostic relevance for HCC patients with PVTT.

Discussion

PVTT is a disease with high incidence and mortality. According to researches, the incidence of PVTT is as high as 44–62.2%, and without treatment, the median survival time of these patients is only 2.7–4 months. So far, PVTT is a bottleneck in the treatment of HCC[16] and it is the difficulty of selecting the prediction tool with the best prognostic value[17,18] that still plagues the world.[19] This study involved two cohorts with complete records and adequate follow-up, including a training and a validation cohort. Based on the screened predictors, namely tumor-related indicators, platelet

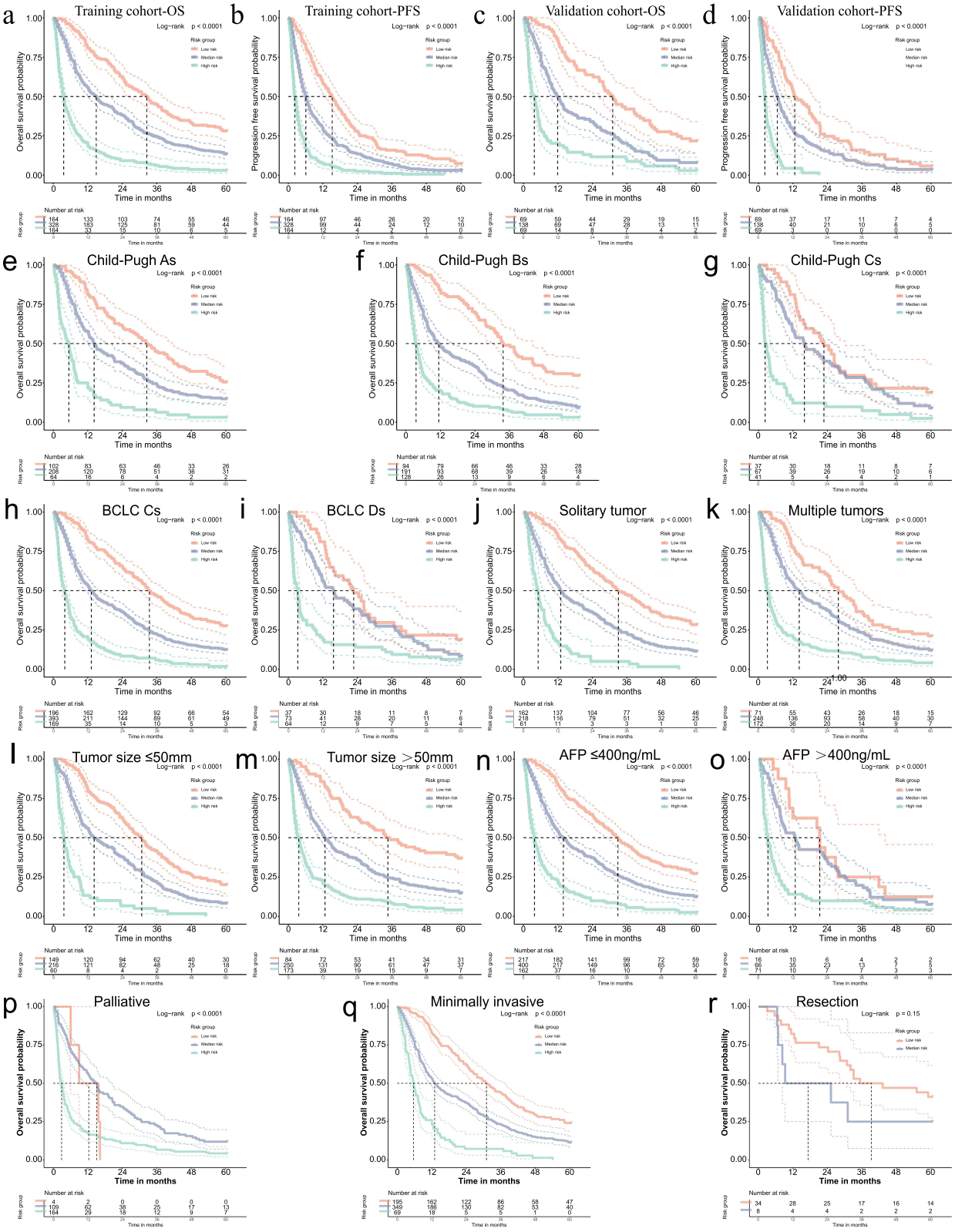

Figure 3 Risk stratification survival analysis of HCC patient with PVTT divided by nomogram model. (**a**–**d**) Kaplan–Meier survival curves of OS and PFS in training and validation cohort; (**e**–**g**) Comparison of Kaplan–Meier OS curves between the high-risk, median risk and low-risk score subgroups in subpopulations with Child–Pugh A, B, or C stage; (**h** and **i**) BCLC stage C or D stage, (**j** and **k**) solitary or multiple tumors, (**l** and **m**) tumor size ≤50mm or >50mm, (**n** and **o**) AFP≤400ng/mL or >400ng/mL, (**p**–**r**) Palliative, Minimally invasive or Resection.

Abbreviations: OS, overall survival; PFS, progression-free survival, BCLC, Barcelona Clinic Liver Cancer, AFP, alpha-fetoprotein.

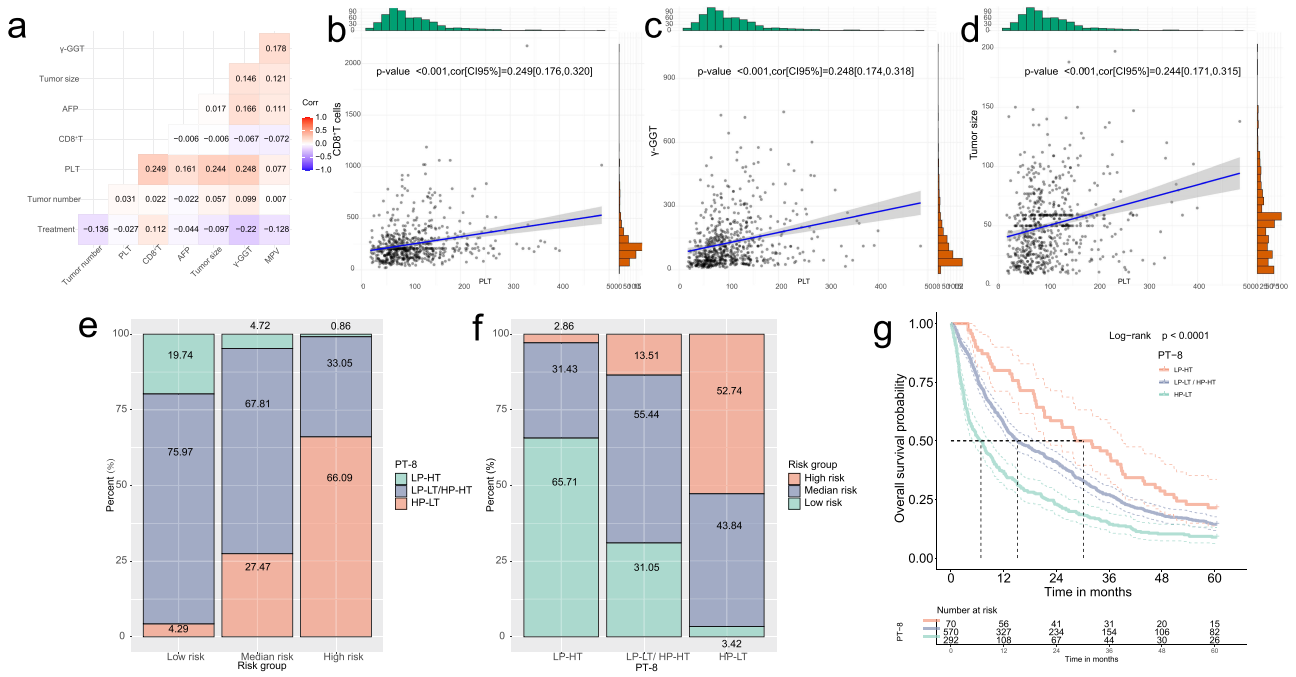

Figure 4 Correlation analysis of indicators included in nomogram model. (**a**) Heat map reflecting the correlation of indicators. The larger the absolute value of the number, the stronger the positive or negative correlation; (**b–d**) Scatter plot displaying the 3 pairs of parameters with the strongest correlation: PLT-CD8$^+$T cells counts, PLT–γ-GGT and PLT–Tumor size; (**e** and **f**) Percent Stacked Column Chart displaying the distribution of PT-8 indicators among different risk groups among all patients, as well as the proportion of PT-8 indicators among different risk groups; (**g**) Kaplan–Meier OS curves in subgroups of combination of PLT (<100*10^9/L vs. ≥100*10^9/L) and CD8$^+$T cells counts (<320 cells/μL vs. ≥ 320 cells/μL). LP-HT, Low PLT and High CD8$^+$Ts; LP-LT/ HP-HT, Low PLT and Low CD8$^+$Ts/ High PLT and High CD8$^+$Ts; HP-LT, High PLT and Low CD8$^+$Ts.

function parameters, immune-related indicators and treatment methods, we constructed a prognosis prediction model in the form of a nomogram. We confirmed the robustness of this model and its prognostic value in different subpopulations, which may provide more possibilities for the treatment direction and prognosis of HCC patients with PVTT.

This prediction model contains 8 features. Tumor size and number,[20,21] and AFP are widely accepted as important prognostic markers for HCC.[22–24] The independent prognostic value of γ-GGT has also been demonstrated in previous studies.[25,26] Due to its independent influence on prognosis, treatment modalities are often used to construct prognostic tools for HCC patients.[27] In multiple cancer-related studies, the impact of MPV on prognosis is not uniform. According to a meta-analysis, among 12 types of cancer (excluding liver cancer), the MPV of patients with gastric cancer, breast cancer, endometrial cancer, thyroid cancer, and lung cancer was significantly increased, while the MPV of patients with renal cell carcinoma and gallbladder cancer was decreased. In addition, for patients with colon cancer and lung cancer, an increase in MPV implies a poor prognosis.[28] In studies related to HCC, the ratio of mean platelet volume to platelet is found significantly elevated in HCC patients,[29] low-level MPV might be one of the risk indicators for liver cancer recurrence after liver transplantation,[30] and for patients with liver cirrhosis and those who cannot undergo resection, patients with advanced HCC, those with thrombocytopenia and higher MPV have longer survival times.[31]

HCC patients with PVTT are often accompanied by abnormal platelet counts.[32,33] Recently, the opinion that high-platelet is an independent risk factor for OS and PFS in HBV-HCC patients with BCLC C-Ds is articulated by members of our group.[34] In addition, platelet count may affect the prognosis of patients with PVTT. Among patients with PVTT after liver resection, the patient with thrombocytopenia had a better prognosis (median OS, 16.6 vs. 8.6; median RFS, 3.7 vs. 1.7, $p < 0.05$).[35] For HBV-HCC patients with PVTT, high platelet count (platelet>130*10^9/L) is a risk factor for OS ([HR]=1.329 [CI]: 1.091–1.617, $p = 0.005$).[36] Similarly, among patients with PVTT who underwent TACE, survivors had a significantly higher rate of thrombocytopenia than those who died (89.0*10^9/L vs. 119.0*10^9/L, $p = 0.046$).[37] In addition, patients with PVTT who did not develop distant tumor metastases were more likely to exhibit thrombocytopenia than those who did.[38] In the reverse direction, a major study of the Lancet concluded that antiplatelet therapy could inhibit tumor metastasis and improve patient prognosis, but the survival rate of the low-concentration drug group was

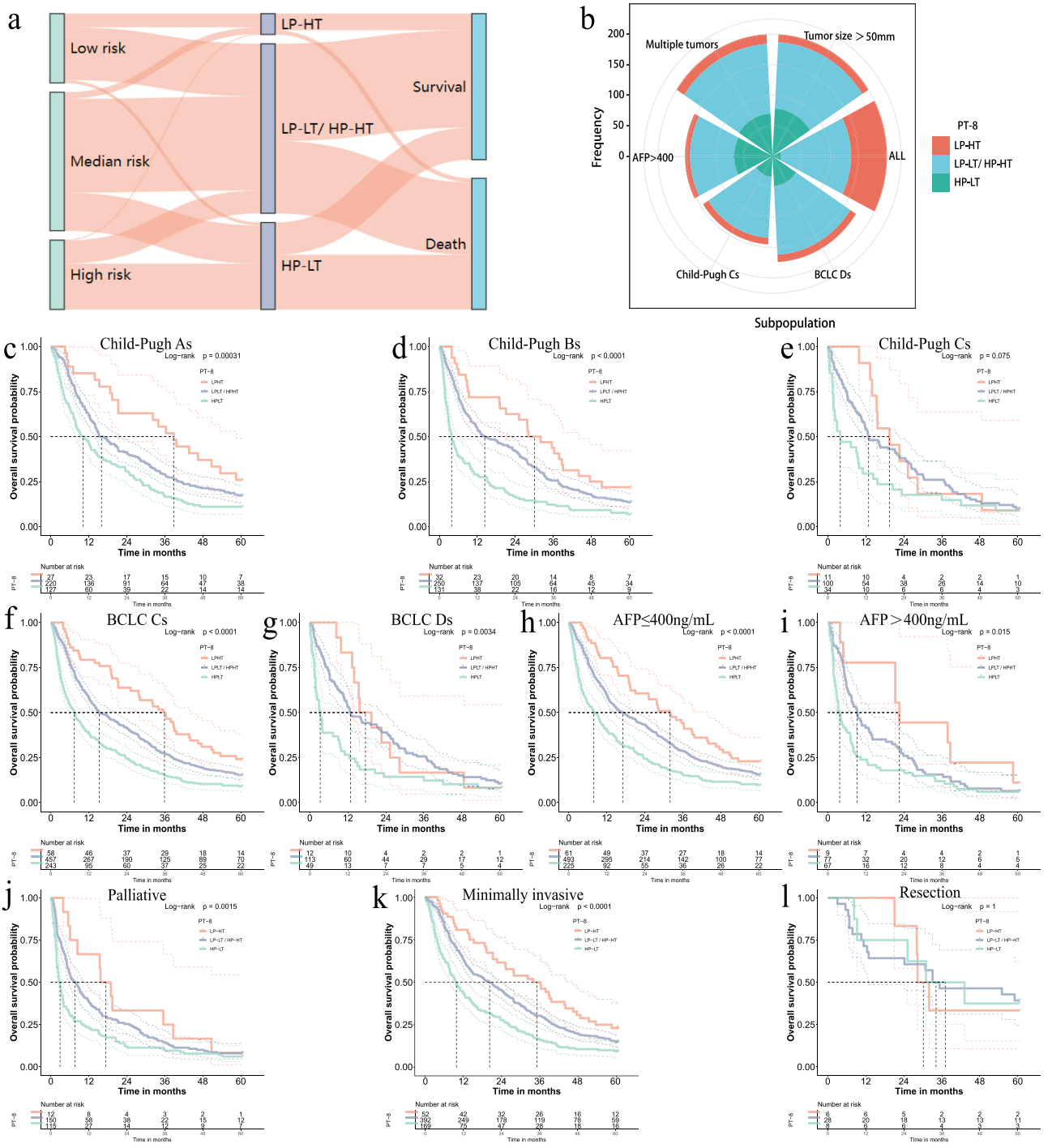

Figure 5 Comparison of survival distribution by the risk score and PLT-CD8$^+$T cells counts in different subpopulations. (**a**) Sankey diagram displaying the relationship between different risk subgroups, PLT-CD8$^+$T cells counts subgroups and 3-year outcome; (**b**) The rose plot displaying the distribution of PT-8 indicators in all patients and subgroups with tumor size>50mm, multiple tumors, Child Pugh C stage, BCLC D stage, and AFP>400ng/mL. (**c**–**e**) Comparison of Kaplan–Meier OS curves between patients with different PT-8 metrics at Child-Pugh A, B, or C stage, (**f** and **g**) BCLC C or D stage, (**h** and **i**) AFP ≤400ng/mL or >400ng/mL, and (**j**–**l**) Palliative, Minimally invasive or Resection. LP-HT, Low PLT and High CD8$^+$Ts; LP-LT/ HP-HT, Low PLT and Low CD8$^+$Ts/ High PLT and High CD8$^+$Ts; HP-LT, High PLT and Low CD8$^+$Ts.

significantly higher than that of the high-concentration group.[39] These support our findings that thrombocytopenia may be an important prognostic factor in patients with PVTT. The study also found that platelets were significantly correlated with CD8$^+$Ts, γ-GGT and tumor size, which suggested that platelets could aggravate liver inflammation and tumor infiltration.[40]

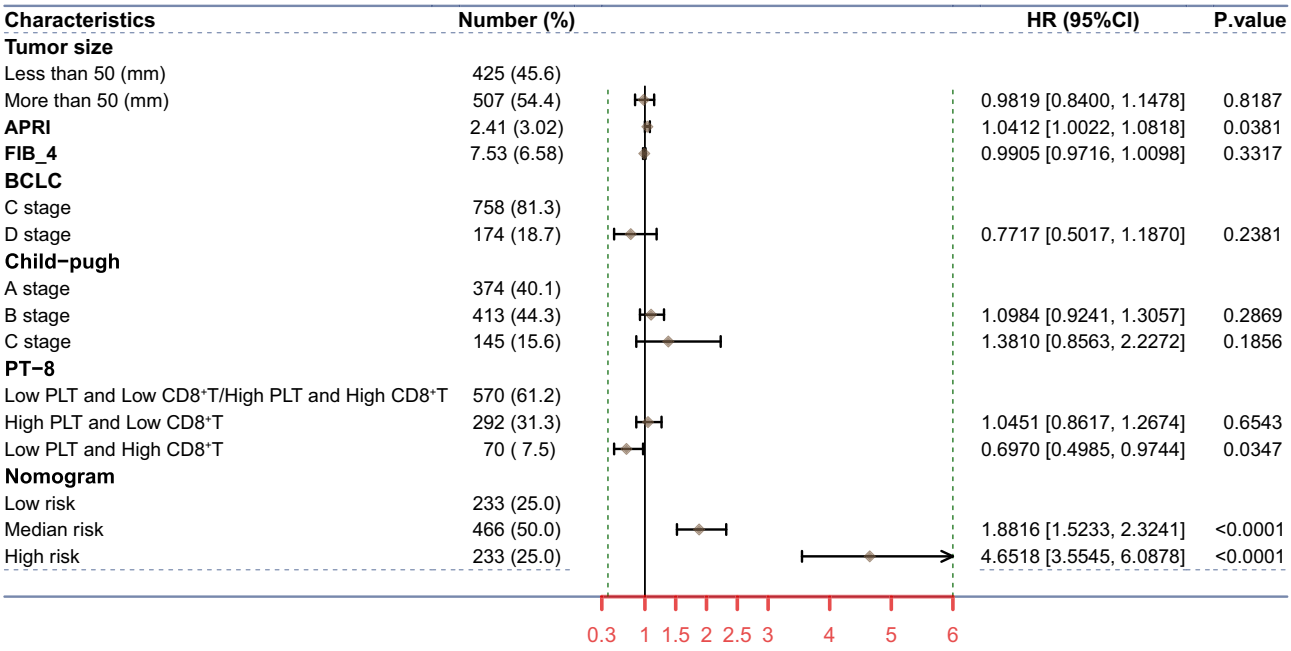

Figure 6 Forest map comparing mortality risk in overall survival. PT-8, indicators of binding of different levels of platelets and $CD8^+$T cells counts.
Abbreviations: HR, hazard ratios; CI, confidence interval, BCLC, Barcelona Clinic Liver Cancer, Child-Pugh; APRI, AST-to-Platelet Ratio Index; FIB-4, fibrosis-4 score.

Mechanistically, higher level of platelets releases more proteins, nucleotides, and bioactive lipids, which can promote the extravasation of liver cancer cells from the primary focus. Platelets promote liver cancer invasion and colonization through adhesion, aggregation and coagulation, leading to tumor progression. Based on research on the relationship between platelets and inflammatory response, we believe that inflammatory response plays a decisive role in prognosis in advanced tumors.[41] Peritonitis and sepsis are the main causes of death in patients with cirrhosis, and thrombocytopenia inhibits the biological behavior of inflammatory response. Improve the prognosis of PVTT patients.[42]

The formation of PVTT is based on the vascular invasion of cancer cells and hemodynamic changes,[43,44] which are closely related to the release, adhesion and immune regulation of platelets. Fortunately, among the included indicators, platelet counts and $CD8^+$Ts showed the strongest correlation. At the same time, we made a breakthrough discovery that the new indicator PT-8 established based on these two indicators showed different effects on the subgroup of patients with PVTT has a significant impact on the prognosis, that is, the LP-HT group has a better prognosis, the LP-LT/HP-HT group is second, and the HP-LT group has the worst prognosis. This is consistent with the findings of a study that examined the mechanism of action of $CD8^+$Ts.[45] Based on PT-8, we stratified patients into five subgroups: Child-Pugh Cs, BCLC Ds, AFP > 400ng/mL, multiple tumors, and tumor size > 50mm, and found that compared with the overall population, their proportion of LP-HT decreased significantly, and the proportion of HP-LT increased significantly, and in extensive studies, these 5 subgroups all showed poor prognosis. In addition, we observed that the survival difference between LP-HT and LP-LT/HP-HT in patients with Child-Pugh Cs, BCLC Ds or who have undergone resection was not obvious, which may indicate that for these three subgroups of patients, it is no longer possible to obtain prognostic prediction suggestions with the help of PLT and $CD8^+$Ts, and the predictive value of HP-LT is not affected by factors such as liver function and tumor burden.

In recent years, the phenomenon that platelets play an immune regulatory role as non-immune cells has gradually attracted the attention of scholars.[46,47] It has been demonstrated that platelets and their parent cells, the megakaryocytes (MK), can also uptake, process and present both foreign and self-antigens to $CD8^+$Ts conferring on them the ability to directly alter adaptive immune responses.[48] In addition, platelets inhibit the number and function of antigen-specific $CD8^+$T cells by increasing MHC-1.[49] Immune checkpoints have been a hot topic in cancer-related research and a study found that platelets regulated the expression and diffusion of PD-1 and PD-L1 and the production of IFN-γ and TNF-α through direct or indirect contact with T cells, thus affecting the function of immune cells including $CD8^+$Ts.[50] At the same time, after the balance between $CD8^+$Ts and blood cells is disrupted, CXCL4 produced by platelets can induce

monocytes to differentiate into bone marrow-derived suppressor cells (MDSCs), thereby inhibiting $CD8^+$Ts function.[45] These can well explain the results of our study: the prognosis of the LP-HT subgroup is significantly better than that of the other subgroups.

This study still has certain limitations. First, the sample size included in this study was not large enough, and the sample size of certain subgroups during the research process was small, which may affect the research results. Second, some studies believe that antiplatelet therapy has no significant relationship with bleeding events in patients with liver cancer and the main influencing factors of bleeding events are Child-Pugh and tumor stage. However, some studies have found that in antiplatelet therapy, the survival rate of the low-concentration group was lower than that of the high-concentration group. Therefore, based on the clinical fact that gastrointestinal bleeding is one of the causes of death in patients with advanced liver cancer, this study should further analyze thrombocytopenic patients with low and lower platelet levels. Third, compared with other major research countries or regions around the world, liver cancer in my country is mainly derived from hepatitis B and exhibits different tumor characteristics. Therefore, there are differences in screening HCC patients with PVTT and pursuing appropriate treatment strategies. Aspects still require further exploration.

Conclusion

In summary, based on two imaging indicators (tumor size, tumor number), five laboratory indicators (PLT, MPV, γ-GGT, AFP and $CD8^+$Ts) and treatment methods, this study constructed a nomogram for predicting prognosis of HCC patients with PVTT. It not only has robust performance but also has the function of distinguishing among high, medium and low-risk patients. The new parameter, PT-8, discovered by platelets and $CD8^+$Ts is considered to be significantly related to the prognosis of PVTT. PVTT patients with thrombocytopenia and high $CD8^+$Ts have a better prognosis, which provides a simple and reliable basis for clinical decision-making.

Data Sharing Statement

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

Ethics Statement

The studies involving human participants were reviewed and approved by The Ethics Committee of Beijing Ditan Hospital (no. JDLKZ [2017] D [028] −02), Capital Medical University. This study is a retrospective research. The ethics committee approved it to be used in this study while ensuring patient privacy, not interfering with the patient's treatment methods, and not bringing risks to the patient's physiology. Retrospective researches are unable to obtain written informed consent. The main reasons are as follows: (1) we only collect the clinical laboratory data from patients, and the risks are manageable; (2) some patients have lost contact or died and are unable to track their informed consent signatures. This study was based on the approval of the ethics committee and the oral informed consent of the patients or their families who survived and could be contacted during the regular follow-up period. All procedures were conducted in accordance with the 2008 Helsinki Declaration and the ethical standards outlined by the relevant national and institutional committees governing human research.

Acknowledgments

We thank YTL (Yuting Liu, Center of Integrative Medicine, Beijing Ditan Hospital, Capital Medical University, Beijing, China) and MRH (Mengru Hua, First Clinical Medical College, Beijing University of Chinese Medicine, Beijing, China) for patient information collection.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This work was supported by the National Natural Science Foundation of China (No. 82274479), the Special Fund of Capital Health Research and Development (No. 2020-2-2173), High-level Public Health Technical Personnel Construction Project (Subject leaders-02-16), Beijing Hospitals Authority Clinical Medicine Development of Special Funding Support (No. ZYLX202127), and Dengfeng Talent Support Program of Beijing Municipal Administration of Hospitals (No. DFL20191803).

Disclosure

The authors report no conflicts of interest in this work.

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2021;71(3):209–249. doi:10.3322/caac.21660
2. Sun J, Guo R, Bi X, et al. Guidelines for diagnosis and treatment of hepatocellular carcinoma with portal vein tumor thrombus in china (2021 edition). *Liver Cancer*. 2022;11(4):315–328. doi:10.1159/000523997
3. Reig M, Forner A, Rimola J, et al. BCLC strategy for prognosis prediction and treatment recommendation: the 2022 update. *J Hepatol*. 2022;76(3):681–693. doi:10.1016/j.jhep.2021.11.018
4. Bekendam RH, Ravid K. Mechanisms of platelet activation in cancer-associated thrombosis: a focus on myeloproliferative neoplasms. *Front Cell Dev Biol*. 2023;11:1207395. doi:10.3389/fcell.2023.1207395
5. Zhang X, Yu S, Li X, et al. Research progress on the interaction between oxidative stress and platelets: another avenue for cancer? *Pharmacol Res*. 2023;191:106777. doi:10.1016/j.phrs.2023.106777
6. Patmore LA, Van Eekhout KMA, Buti M, et al. Hepatocellular carcinoma risk in sub-Saharan African and Afro-Surinamese individuals with chronic hepatitis B living in Europe. *J Hepatol*. 2023;S0168–S8278(23):05219.
7. Morris K, Schnoor B, Papa AL. Platelet cancer cell interplay as a new therapeutic target. *Biochim Biophys Acta BBA*. 2022;1877(5):188770. doi:10.1016/j.bbcan.2022.188770
8. Zanetto A, Campello E, Pelizzaro F, et al. Haemostatic alterations in patients with cirrhosis and hepatocellular carcinoma: laboratory evidence and clinical implications. *Liver Int*. 2022;42(6):1229–1240. doi:10.1111/liv.15183
9. Ramadori P, Klag T, Malek NP, Heikenwalder M. Platelets in chronic liver disease, from bench to bedside. *JHEP Rep*. 2019;1(6):448–459. doi:10.1016/j.jhepr.2019.10.001
10. Toubert C, Guiu B, Al Taweel B, et al. Prolonged survival after recurrence in HCC resected patients using repeated curative therapies: never give up! *Cancers*. 2022;15(1):232. doi:10.3390/cancers15010232
11. Sangro B, Sarobe P, Hervás-Stubbs S, Melero I. Advances in immunotherapy for hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*. 2021;18(8):525–543. doi:10.1038/s41575-021-00438-0
12. Ferdous F, Scott T. The immunological capacity of thrombocytes. *Int J Mol Sci*. 2023;24(16):12950. doi:10.3390/ijms241612950
13. Omata M, Cheng AL, Kokudo N, et al. Asia–Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. *Hepatol Int*. 2017;11(4):317–370. doi:10.1007/s12072-017-9799-9
14. Kim WR, Mannalithara A, Heimbach JK, et al. MELD 3.0: the model for end-stage liver disease updated for the modern era. *Gastroenterology*. 2021;161(6):1887–1895.e4. doi:10.1053/j.gastro.2021.08.050
15. Tay JK, Narasimhan B, Hastie T. Elastic net regularization paths for all generalized linear models. *J Stat Softw*. 2023;106(1). doi:10.18637/jss.v106.i01
16. Zhou XH, Li JR, Zheng TH, et al. Portal vein tumor thrombosis in hepatocellular carcinoma: molecular mechanism and therapy. *Clin Exp Metastasis*. 2023;40(1):5–32. doi:10.1007/s10585-022-10188-1
17. Cheng S, Hu G, Jin Z, Wang Z, Xue H. CT-based radiomics nomogram for prediction of survival after transarterial chemoembolization with drug-eluting beads in patients with hepatocellular carcinoma and portal vein tumor thrombus. *Eur Radiol*. 2023;33(12):8715–8726. doi:10.1007/s00330-023-09830-7
18. Xu L, Wang P, Li L, et al. circPSD3 is a promising inhibitor of uPA system to inhibit vascular invasion and metastasis in hepatocellular carcinoma. *Mol Cancer*. 2023;22(1):174. doi:10.1186/s12943-023-01882-z
19. Xu Y, Zhang Z, Xu D, Yang X, Zhou L, Zhu Y. Identification and integrative analysis of ACLY and related gene panels associated with immune microenvironment reveal prognostic significance in hepatocellular carcinoma. *Cancer Cell Int*. 2021;21(1):409. doi:10.1186/s12935-021-02108-2
20. Jiang H, Qin Y, Wei H, et al. Prognostic MRI features to predict postresection survivals for very early to intermediate stage hepatocellular carcinoma. *Eur Radiol*. 2023;1:4.
21. Pelizzaro F, Trevisani F, Simeon V, et al. Predictors of non-transplantable recurrence in hepatocellular carcinoma patients treated with frontline liver resection. *Liver Int*. 2023;43(12):2762–2775. doi:10.1111/liv.15719
22. Liu X, Hou Y, Wang X, et al. Machine learning-based development and validation of a scoring system for progression-free survival in liver cancer. *Hepatol Int*. 2020;14(4):567–576. doi:10.1007/s12072-020-10046-w
23. Norman JS, Li PJ, Kotwani P, Shui AM, Yao F, Mehta N. AFP-L3 and DCP strongly predict early hepatocellular carcinoma recurrence after liver transplantation. *J Hepatol*. 2023;79(6):1469–1477. doi:10.1016/j.jhep.2023.08.020
24. Kotwani P, Chan W, Yao F, Mehta N. DCP and AFP-L3 are complementary to AFP in predicting high-risk explant features: results of a prospective study. *Clin Gastroenterol Hepatol*. 2022;20(3):701–703.e2. doi:10.1016/j.cgh.2021.01.043
25. Liu X, Wang X, Yu L, et al. A novel prognostic score based on artificial intelligence in hepatocellular carcinoma: a long-term follow-up analysis. *Front Oncol*. 2022;12:817853. doi:10.3389/fonc.2022.817853

26. Xu W, Wang Y, Yang Z, Li J, Li R, Liu F. New Insights into a classification-based microvascular invasion prediction model in hepatocellular carcinoma: a multicenter study. *Front Oncol*. 2022;12:796311. doi:10.3389/fonc.2022.796311
27. Foerster F, Gairing SJ, Müller L, Galle PR. NAFLD-driven HCC: safety and efficacy of current and emerging treatment options. *J Hepatol*. 2022;76(2):446–457. doi:10.1016/j.jhep.2021.09.007
28. Detopoulou P, Panoutsopoulos GI, Mantoglou M, et al. Relation of Mean Platelet Volume (MPV) with cancer: a systematic review with a focus on disease outcome on twelve types of cancer. *Curr Oncol*. 2023;30(3):3391–3420. doi:10.3390/curroncol30030258
29. Yin J, Niu Y, Qian L, Zhang X, Liu Z, Wang R. Mean platelet volume predicts survival in patients with hepatocellular carcinoma and type 2 diabetes. *Diabet Res Clin Pract*. 2019;151:120–127. doi:10.1016/j.diabres.2019.04.012
30. Zhang AB, Zhang ZH, Zhang J, et al. Lower mean platelet volume is a risk indicator of hepatocellular carcinoma recurrence following liver transplantation. *Hepatobiliary Pancreat Dis Int*. 2019;18(3):223–227. doi:10.1016/j.hbpd.2019.04.009
31. Scheiner B, Kirstein M, Popp S, et al. Association of platelet count and mean platelet volume with overall survival in patients with cirrhosis and unresectable hepatocellular carcinoma. *Liver Cancer*. 2019;8(3):203–217. doi:10.1159/000489833
32. Gallo P, Terracciani F, Di Pasquale G, Esposito M, Picardi A, Vespasiani-Gentilucci U. Thrombocytopenia in chronic liver disease: physiopathology and new therapeutic strategies before invasive procedures. *World J Gastroenterol*. 2022;28(30):4061–4074. doi:10.3748/wjg.v28.i30.4061
33. Lim J, Kim HI, Kim E, et al. Variceal bleeding is aggravated by portal venous invasion of hepatocellular carcinoma: a matched nested case-control study. *BMC Cancer*. 2021;21(1):11. doi:10.1186/s12885-020-07708-1
34. Yu L, Liu X, Wang X, et al. Impact of gender as a prognostic factor in HBV-related hepatocellular carcinoma: the survival strength of female patients in BCLC stage 0-B. *J Cancer*. 2019;10(18):4237–4244. doi:10.7150/jca.33430
35. Cheng Y, Wang K, Zhang X, et al. Thrombocytopenia: a prognostic factor for hepatocellular carcinoma patients with portal vein tumor thrombus after hepatectomy. *J Gastroenterol Hepatol*. 2019;34(7):1214–1221. doi:10.1111/jgh.14537
36. Li M, Dang Z, Ma S, et al. A novel prognostic scoring system to predict portal vein tumor thrombosis in patients with hepatitis B virus-associated hepatocellular carcinoma. *Am J Transl Res*. 2023;15(7):4600–4609.
37. Chen KL, Gao J. Factors influencing the short-term and long-term survival of hepatocellular carcinoma patients with portal vein tumor thrombosis who underwent chemoembolization. *World J Gastroenterol*. 2021;27(13):1330–1340. doi:10.3748/wjg.v27.i13.1330
38. Li M, Zhao Y, Liu X, Zhang S, Jiang Y, Yang Z. Early risk warning system for distant metastasis of hepatitis B virus-associated hepatocellular carcinoma with portal vein tumor thrombus. *Oncol Lett*. 2020;19(4):3249–3257. doi:10.3892/ol.2020.11423
39. Rothwell PM, Wilson M, Price JF, Belch JF, Meade TW, Mehta Z. Effect of daily aspirin on risk of cancer metastasis: a study of incident cancers during randomised controlled trials. *Lancet*. 2012;379(9826):1591–1601. doi:10.1016/S0140-6736(12)60209-8
40. Wenpei G, Yuan L, Liangbo L, et al. Predictive value of preoperative inflammatory indexes for postoperative early recurrence of hepatitis B-related hepatocellular carcinoma. *Front Oncol*. 2023;13:1142168. doi:10.3389/fonc.2023.1142168
41. Peng W, Li C, Zhang X, Wen T, Chen Z. The impact of thrombocytopenia on prognosis of HBV-related small hepatocellular carcinoma: a propensity score matching analysis. *World J Surg Oncol*. 2021;19(1):46. doi:10.1186/s12957-021-02160-2
42. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999–2016: observational study. *BMJ*. 2018;362:k2817. doi:10.1136/bmj.k2817
43. Palumbo JS. Crosstalk between hemostasis and immunity in cancer pathogenesis. *Thromb Res*. 2022;213:S3–S7. doi:10.1016/j.thromres.2021.12.013
44. Zhang M, Ding Q, Bian C, Su J, Xin Y, Jiang X. Progress on the molecular mechanism of portal vein tumor thrombosis formation in hepatocellular carcinoma. *Exp Cell Res*. 2023;426(1):113563. doi:10.1016/j.yexcr.2023.113563
45. Joseph R, Soundararajan R, Vasaikar S, et al. CD8+ T cells inhibit metastasis and CXCL4 regulates its function. *Br J Cancer*. 2021;125(2):176–189. doi:10.1038/s41416-021-01338-5
46. Scherlinger M, Richez C, Tsokos GC, Boilard E, Blanco P. The role of platelets in immune-mediated inflammatory diseases. *Nat Rev Immunol*. 2023;23(8):495–510. doi:10.1038/s41577-023-00834-4
47. Koupenova M, Livada AC, Morrell CN. Platelet and megakaryocyte roles in innate and adaptive immunity. *Circ Res*. 2022;130(2):288–308. doi:10.1161/CIRCRESAHA.121.319821
48. Maouia A, Rebetz J, Kapur R, Semple JW. The immune nature of platelets revisited. *Transfus Med Rev*. 2020;34(4):209–220. doi:10.1016/j.tmrv.2020.09.005
49. Guo L, Shen S, Rowley JW, et al. Platelet MHC class I mediates CD8+ T-cell suppression during sepsis. *Blood*. 2021;138(5):401–416. doi:10.1182/blood.2020008958
50. Polasky C, Wendt F, Pries R, Wollenberg B. Platelet induced functional alteration of CD4+ and CD8+ T cells in HNSCC. *Int J Mol Sci*. 2020;21(20):7507. doi:10.3390/ijms21207507