A prognostic nomogram in AFP-negative hepatocellular carcinoma based on LASSO Cox regression

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A prognostic nomogram in AFP-negative hepatocellular carcinoma based on LASSO Cox regression

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Abstract

Purpose

Hepatocellular carcinoma (HCC) patients with alpha-fetoprotein (AFP)-negative (<8.78 ng/mL) have special clinicopathologic characteristics and prognosis. The aim of this study was to apply a new method to establish and validate a new model for predicting the prognosis of HCC patients with AFP-negative.

Materials and Methods

A total of 410 AFP-negative patients with clinical diagnosed with HCC as a primary cohort; 148 AFP-negative HCC patients as an independent validation cohort. In primary cohort, independent factors for overall survival (OS) by LASSO Cox regression were all contained into the nomogram1; by univariate and multivariate Cox hazard analysis were all contained into the nomogram2. Nomograms performance and discriminative power were assessed with concordance index (C-index) values, area under curve (AUC), Calibration curve and decision curve analyses (DCA). The results were validated in the validation cohort.

Results

The C-index of nomogram1was 0.708 (95%CI: 0.673-0.743), which was superior to nomogram2 (0.706) and traditional modes (0.606-0.629). The AUC of nomogram1 was 0.736 (95%CI: 0.690-0.778). In the validation cohort, the nomogram1 still gave good discrimination (C-index: 0.752, 95%CI: 0.691-0.813; AUC: 0.784, 95%CI: 0.709-0.847). The calibration curve for probability of OS showed good homogeneity between prediction by nomogram1 and actual observation. DCA demonstrated that

nomogram1 was clinically useful. Moreover, patients were divided into three distinct risk groups for OS by the nomogram1: low-risk group, middle-risk group and high-risk group, respectively.

Conclusions

Novel nomogram based on LASSO Cox regression presents more accurate and useful prognostic prediction for patients with AFP-negative HCC. This model could help AFP-negative HCC facilitate a personalized prognostic evaluation.

Keywords

hepatocellular carcinoma, nomogram, prognosis, LASSO Cox regression

Introduction

HCC is the fifth most common malignancy worldwide and the third most associated with all cancer deaths [1]. As with other cancers, the prognosis for early HCC is better than late stage [2]. Since the identification of alpha-fetoprotein (AFP) in 1970s, it has been the only serologic marker that is widely used for the HCC diagnosis [3]. However, the diagnostic power of AFP has been continuously questioned and debated. For example, elevated serum AFP was only observed in 60-70% of overall HCC patients, while the proportion was merely 33-65% regarding patients harboring HCCs of <3 cm in diameter [4, 5]. HCC patients with negative serum AFP have special clinicopathologic characteristics and prognosis, they have higher tumor differentiation, earlier TNM staging, smaller tumor size, and higher survival rates [6].

Many staging systems have been employed to predict how HCC patients will respond over time, such as the BCLC, ALBI, Child-Pugh and TNM staging system. However, studies have shown that the traditional staging system is flawed to varying degrees. The BCLC staging system was reported to have the greatest potential in predicting HCC in AFP negative patients [7]. However, evidence has shown that the classification of the BCLC score is limited to the advanced stages of HCC [8]. Child-Pugh does not consider tumor-related factors, which are important for the prognosis of HCC patients [9], as the prognosis of HCC patients, tumor-related factors are crucial. ALBI model incorporates few factors, clinical indicators are easily available and easy to apply, but there are no tumor-related indicators to evaluate [10], as a model to evaluate the prognosis of HCC needs to be validated in a multicenter large sample. The TNM staging system is easy to use and is considered to be the best staging system for solid tumors, but its effect on the staging and prognosis of HCC is debatable because it only considers tumor characteristics but not liver function, which usually plays an important role in the prognosis of HCC patients[11]. Furthermore, these systems are not specifically designed to predict outcomes of HCC when patients are AFP-negative. Recently, nomograms for prediction of survival and recurrence of HCC in AFP-negative patients after radical resection have been developed in two previous studies [12, 13], respectively. However, HCC patients with transarterial chemoembolization (TACE) and radiofrequency ablation (RFA) were not included in both studies. At the same time, most of the current models are limited to Cox univariate and multivariate analysis for risk factor screening, which is unconducive to

the small sample but multi-indicators models screening.

Therefore, the aim of our study was applying a new method to establish and validate a new model that combines clinical pathological factors, biochemical indicators, for predicting the prognosis of AFP-negative HCC. In addition, a comparison between the constructed nomograms and traditional staging systems was conducted to determine whether the nomograms provided more accurate prediction in prognosis.

Materials and Methods

1.Patient selection

We performed a retrospective cohort study on 458 AFP-negative (<8.78 ng/mL) HCC patients using data from the Beijing Ditan Hospital between January 2008 and December 2016. Diagnostic criteria of HCC were consistent with previous studies [14]. The HCC diagnosis data included biopsy, radiology. First, we selected patients based on hepatic angiography, pathology in combination with ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). Next, we only included patients with complete clinical data. Our exclusion criteria included: (1) other viral infections such as human immunodeficiency virus (HIV); (2) metastatic liver cancer; (3) pregnant women; (4) incomplete data; and (5) patients with liver transplantation and survival time <15 days. The primary cohort of our study included 410 clinically diagnosed HCC patients with AFP-negative, retrospectively studied via an information system between January 2008 and December 2014. The patients were

followed for 5 years (death follow-up stopped) and first hospitalization records were kept. 148 patients with AFP-negative between January 2015 and December 2016 as an independent validation cohort. The patients were followed for 3 years (death follow-up stopped) and first hospitalization records were kept. It is recommended that all HCC patients undergo regular follow-up visits according to clinical guidelines after completion of hospital admission, usually every 3 months for the first 2 years and once a year for the next 3 to 5 years. Patients who did not come to our hospital on time for review were given treatment information and living conditions by telephone follow-up (telephone follow-up by our clinicians), the last follow-up occurred in December 2019. The outcome of our study was overall survival (OS), defined as the time from the diagnosis of HCC to the last follow-up or death. The study was approved by the ethics committee of Beijing Ditan Hospital, Capital Medical University and was conducted in accordance with the standards of the Declaration of Helsinki.

2.Laboratory Measurements

Patients are routinely examined at the first visit. Data provided include: gender, age, tumor multiplicity, tumor size, ascites, cirrhosis, etiology, portal vein tumor thrombus (PVTT), neutrophil to lymphocyte ratio (NLR), hemoglobin (HGB), platelet (PLT), creatinine (CR) , alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin (TBIL), albumin (ALB), gamma-glutamyl transpeptidase (γ-GGT), prothrombin time activity (PTA), lactate dehydrogenase (LDH), carbohydrate antigen 199 (CA199), C reactive protein (CRP), carcino-embryonic antigen (CEA),

AFP. When laboratory values at the time of HCC diagnosis were higher than clinical normal, it was classified as elevated. The tumor was staged using the BCLC staging system and liver function was scored using Child-Pugh.

3.Statistical analysis

Statistical analysis was done using SPSS 24.0 (IBM, Chicago, IL, USA), R software (version 3.6.3; http://www.Rproject.org) and MedCalc19.2.0.Categorical variables were classified based on clinical findings. All the optimal cut-off points in our study were evaluated by reference ranges and continuous variables were transformed to categorical variables. R version 3.6.3 for foreign package, survival package, rms package for Cox univariate and multivariate analysis, plotting Nomogram and calibration plots, calculating C-index; glmnet package for LASSO Cox regression; nricens package for NRI calculation; stdca package for decision curve; time ROC package for time-dependent ROC curve. MedCalc19.2.0 for low-risk group, middle-risk group and high-risk group Kaplan-Meier curve plotting.

In this study, we used the primary cohort to plot nomogram1 based on LASSO Cox regression [15], and used the primary cohort to plot nomogram2 based on multivariate COX analysis. Based on the established nomogram1 and nomogram2, the C-index and calibration curves were derived based on COX regression analysis, NRI and IDI scores were performed, and decision curves were plotted. Nomogram1 was found to be superior to nomogram2 in the primary and validation cohorts. The total score for each patient was calculated based on nomogram 1, and three groups of patients with high, medium, and low prognostic risk (based on the total score) were divided

according to interquartile values. The Kaplan-Meier curve was applied in MedCalc software, and the risk group was compared using the three-point factor, log-rank test, and the two-tailed P value < 0.05 was statistically significant.

Results

1.Basic characteristics

In the primary cohort, 410 patients with AFP-negative HCC met the inclusion and exclusion criteria and were included in this study. A total of 148 HCC patients with negative AFP were included in the validation cohort. The clinical characteristics of patients in the two independent cohorts are shown in Table 1. Most of the study population was male (79.8% vs 83.8%; p = 0.286), and the patients were older than 50 years (77.8% vs 83.1%; p = 0.173) in the primary and validation cohort. In addition, (78.5% vs 81.1%; p = 0.876) of the patients presented positive HBsAg. Most patients remained at BCLC stage 0-B (78.5% vs 80.4%; p = 0.632) and Child-Pugh A-B (87.1% vs 92.6%; p = 0.168), and (24.1% vs 18.2%; p = 0.212) had a tumor size ≥5cm (Table 1). In the primary cohort, 224 patients died from cancer within 5 years.

2.Biomarker Selection

All available clinical indicators, including clinicopathological features and biomarkers (Table1), were subjected to LASSO Cox regression, with a significant correlation between gender, age, tumor size, tumor number, cirrhosis, PVTT, ascites, HBV, HGB, CR, AST, ALB, LDH, γ-GGT, CA199, CRP and OS at minimum values. Further disciplinary regression was performed to take 1-s.e. criteria PVTT, ascites,

HGB, γ-GGT, CRP as independent risk factors for prognosis in HCC patients (Figure1). Inclusion of all clinical indicators in single-factor COX and multifactor COX analyses, there was a significant correlation between gender, tumor size, tumor multiplicity, cirrhosis, PVTT, ascites, HBsAg, NLR, HGB, PLT, CR, AST, TBIL, ALB, LDH, γ-GGT, PTA, CA199, CRP and OS.COX multivariate analysis was then performed to identify the factors that were distinguished in the COX univariate analysis. The results showed that gender, PVTT, CR, γ-GGT, CRP were independent risk factors for prognosis in HCC patients (Table2).

3.Development the Prediction Model

Nomogram1 and nomogram2 were constructed to predict 3 and 5-year OS based on prognostic factors determined by both instruments (Figure2). Nomogram1 and nomogram2 used consistency index (C-index), AUC and time-dependent ROC curves in the primary cohort, respectively, and the calibration curves were plotted. The C-index of nomogram1 in the primary cohort was 0.708 (95% CI: 0.673-0.743); the AUC (ROC curve) was 0.736 (95%CI: 0.690-0.778), with sensitivity (62.05%), specificity (76.34%), PPV (76.0%) and NPV (62.6%). The C-index of nomogram2 was 0.706 (95%CI: 0.673-0.739); the AUC was 0.714 (95%CI: 0.667-0.757), with sensitivity (74.55%), specificity (61.83%), PPV (70.2%) and NPV (66.9%). Continuity cut point 0.05NRI (-0.037), subtype cut point 0.5NRI (-0.037), IDI: 0.006 (p=0.29). We get similar results in the validation cohort. All suggest that nomogram1 is better than nomogram2.

4.Performance of the nomogram

In the primary cohort, the C-index (0.708) and AUC (0.736) of nomogram1 outperformed the nomogram2 and the other models (Table3). Time-dependent ROC curves suggest that nomogram 1 and nomogram 2 are similar, but significantly better than traditional modes such as Child-Pugh, BCLC, ALBI, TNM (Figure3A). Calibration plots for 3 and 5-year OS probabilities show the best agreement between the nomogram1 predictions and actual observations (Figure4A, C). In the validation cohort, the C-index (0.752) and AUC (0.784) of nomogram1 outperformed the nomogram2 and the other models (Table3). The time-dependent ROC curve suggests nomogram1 is significantly superior to nomogram2, Child-Pugh, BCLC, ALBI and TNM staging systems (Figure3B). Calibration plots for 3-year OS probabilities show the best agreement between nomogram1 predictions and actual observations (Figure4B, D).

The decision curve analysis of nomogram 1 and nomogram 2 is also meaningful (Figure 5). In the primary cohort the cue differences were small, but in the validation cohort nomogram1 and nomogram2 were better at predicting OS than either the all-patient death scenario or the no-patient death scenario if the patient threshold probability was > 20%. Moreover, the net benefit is comparable; in this range, the predicted OS of nomogram 1 is more advantageous than nomogram 2.

5.Application of the Nomogram model for risk stratification

Based on the nomogram1 we developed in this study, we subdivided the patients into low-risk, middle-risk, and high-risk groups, and the AFP-negative HCC patients showed good prognostic classification in both the primary cohort and the validation

cohort. In the primary cohort, there were 122 cases in the low-risk group, 175 cases in the middle-risk group, and 113 cases in the high-risk group. Intergroup OS was (52.111 ± 1.386) months, (40.960 ± 1.622) months, and (26.219 ± 2.138) months ($p < 0.001$) (Figure6A). In the validation cohort, there were 50 cases in the low-risk group, 70 cases in the middle-risk group, and 28 cases in the high-risk group. Intergroup OS was (35.121±0.777) months, (25.983±1.670) months, and (18.721±2.685) months ($p < 0.001$) (Figure6B).

Discussion

It is important to have an accurate prognosis of the tumor after relevant treatment. In this study, we identified 3 years and 5 years of independent risk factors for OS in AFP-negative HCC patients in the primary cohort, and established novel, effective, and verified nomograms1 to predict individuals at 3 years and 5 years OS.PVTT, ascites, HGB, γ-GGT and CRP have been included in the nomogram1. In addition, nomogram1 shows a higher discriminatory power, and the C-index of the two independent cohorts are 0.708 and 0.752, respectively. In the subsequent study, the calibration curve and decision curve analysis were used to assess the predicted precision and clinical utility of the nomogram1. Our model showed a better net benefit and higher consistency between the nomogram prediction and the actual observation.

Previous studies have demonstrated the prominent role of tumor burden and grade, liver function, degree of hepatic dysfunction, and performance status in the prognosis

of HCC. For many years, the traditional Child-Pugh rating system is the most widely used method for assessing liver function and predicting therapeutic efficacy [16]. Our study also found that the C-index (0.629) and AUC (0.672) of Child-Pugh in the primary cohort were significantly higher than ALBI, TNM, and BCLC. We also obtained the same results in the validation cohort. However, AFP-negative HCC patients have special clinicopathologic characteristics and prognosis, they have higher tumor differentiation, earlier TNM staging, smaller tumor size, and higher survival rates [6]. Most models are built without taking into account the specificity of liver biology function in AFP-negative HCC and do not fully reflect an accurate prognosis. The above studies indicate that our model is more accurate for the prognosis of AFP-negative HCC patients. We found C-index (0.708), AUC (0.736) and time-dependent ROC curves of our model was better than the other models, such as Child-Pugh, BCLC, ALBI, TNM. The calibration curve for probability of OS showed good homogeneity between prediction by our model and actual observation. DCA demonstrated that our model was clinically useful. We get similar results in the validation cohort.

A variety of nomograms have been developed to predict the prognosis of certain cancers and have been shown to be more accurate than traditional staging systems. However, most of them are limited to COX univariate and multivariate risk factor screening, which is not conducive to small sample size, multi-indicator model screening [17]. LASSO Cox regression analysis constructs a penalty function to obtain a more refined model. It compresses the regression coefficients (the sum of the

absolute values of the mandatory coefficients is less than a fixed value) and sets some regression coefficients to 0. LASSO Cox regression analysis can not only solve the problem of over-fitting, but also extract useful features effectively [15]. Meanwhile, LASSO Cox regression is more applicable in decisions with more clinical indicators. In our study, we find that the model constructed by applying the factors filtered by LASSO Cox regression has better accuracy and resolution than the model constructed by traditional Cox regression. In the primary cohort, we find the C-index (0.708) and AUC (0.736) of nomogram1 is superior to the C-index (0.706) and AUC (0.714) of nomogram2. We got better results in the validation cohort.

Our final nomogram included five independent risk factors, liver function parameters: ascites (C-index: 0.606) and γ-GGT (C-index: 0.596); inflammatory indicator: CRP (C-index: 0.596); and a tumor-related index: HGB (C-index: 0.604) and PVTT (C- index: 0.582). The importance of various risk factors can be shown by the Decision Tree (Figure7). CRP is synthesized in the liver and is secreted into the plasma as a pentamer, belonging to the family of pentraxins, together with serum amyloid protein [18]. CRP can predict overall survival and recurrence rates after hepatectomy in patients with HCC patients [19]. Meanwhile, CRP was independently associated with OS in non-surgical HCC patients [20].The ascites, jaundice, hepatic encephalopathy, and liver failure may induce by the formation of PVTT and thus might account for, at least in part, the difference in prognostic factors between HCC with and without PVTT [21].Previous studies indicate that increased γ-GGT is closely associated with the development of HCC because of its positive correlation with

tumor size and poor OS [22].Anemia is common in cancer patients. HGB levels have been shown to have an impact on survival both before and during anti-cancer therapy [23]. The pre-treatment anemia in HCC patients was found to be 7.0%, which is less than the 12.8% of cancer-related anemia [24]. The causes of anemia in HCC patients include nutritional deficiencies, hemolysis, blood loss, and tumor cell infiltration of the bone marrow [25]. In addition, chronic liver injury can also lead to anemia in HCC patients [26]. It has also been shown that downregulation of iron-regulated genes, including heparin, ceruloplasmin, transferrin, and transferrin receptors, disrupts the systemic iron homeostasis, leading to anemia in patients with HCC [27]. It has been demonstrated that HGB is an independent prognostic factor in patients with HCC, which is similar to the results we obtained [24]. Ascites is the hallmark of portal hypertension. Besides, ascites may also be associated with large tumor burden and vascular invasion of HCC, suggesting that the cause of ascites could be attributed to both tumoral and cirrhotic factors [28]. Studies have shown that ascites reduces long-term survival in patients with liver cancer [29]. It is similar to the results of our current study. Tumor size is one of the most important parameters of tumor burden. However, evaluation of tumor number failed to predict the prognosis of HCC patients in the present study, although other studies have demonstrated its prominent role in prognostic prediction [30]. Meanwhile, tumor markers have not been included in this study. After research and repeated comparison, our nomogram exhibited superior discrimination ability for the prediction of prognostic in AFP-negative HCC patients. Hence, our novel nomogram could be used to guide routine follow-up for patients.

PVTT, ascites, HGB, γ-GGT and CRP should be given great importance in AFP-negative HCC patients. In addition, patients given a high score by the nomogram should undergo more high-end imaging examinations, such as MRI or CT exams, and the interval time of follow up should be reduced, even if the last test results have no causes for concern.

Although our nomogram performed well, several limitations need to be addressed. Firstly, the nomogram was derived from data collected by one agency and not validated internally due to the difficulty of obtaining such data; in the validation cohort the follow-up time was shorter, and close monitoring and five-year follow-up data are still required for patients in the validation cohort. Secondly, because the present study was a retrospective study for predicting anticipated future performance, our results need to be confirmed by prospective cohort studies. Thirdly, since only three of the patients we included underwent resection, is our nomogram applicable to patients who underwent radical resection of AFP-negative patients remains uncertain.

In conclusion, we applied new methods to develop and validate nomogram to predict the OS in AFP-negative HCC patients. Nomogram1 presented in this study is statistically easier than previous model screening methods and more accurate than ALBI, TNM, Child-Pugh, BCLC, and provides a useful tool for prognosis. In order to promote the use of nomogram1 in other groups, additional validation of data from other institutions is needed.

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Authors' contributions

ZYY and XLL designed the study; DDZ collected and analyzed the data and wrote the manuscript; XLL, FNY, PW and HWY provided patients data; YYJ was responsible for the interpretation of data and revision. ZYY approved for final revision and approval.

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Availability of data and material

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

Ethics approval and consent to participate

The study was approved by the ethics committee of Beijing Ditan Hospital, Capital Medical University.

Consent for publication

Written informed consent was obtained from each patient. Information that could identify individual participants during or after data collection was not accessible.

Competing Interests

The authors have declared that no competing interest exists.

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Figures

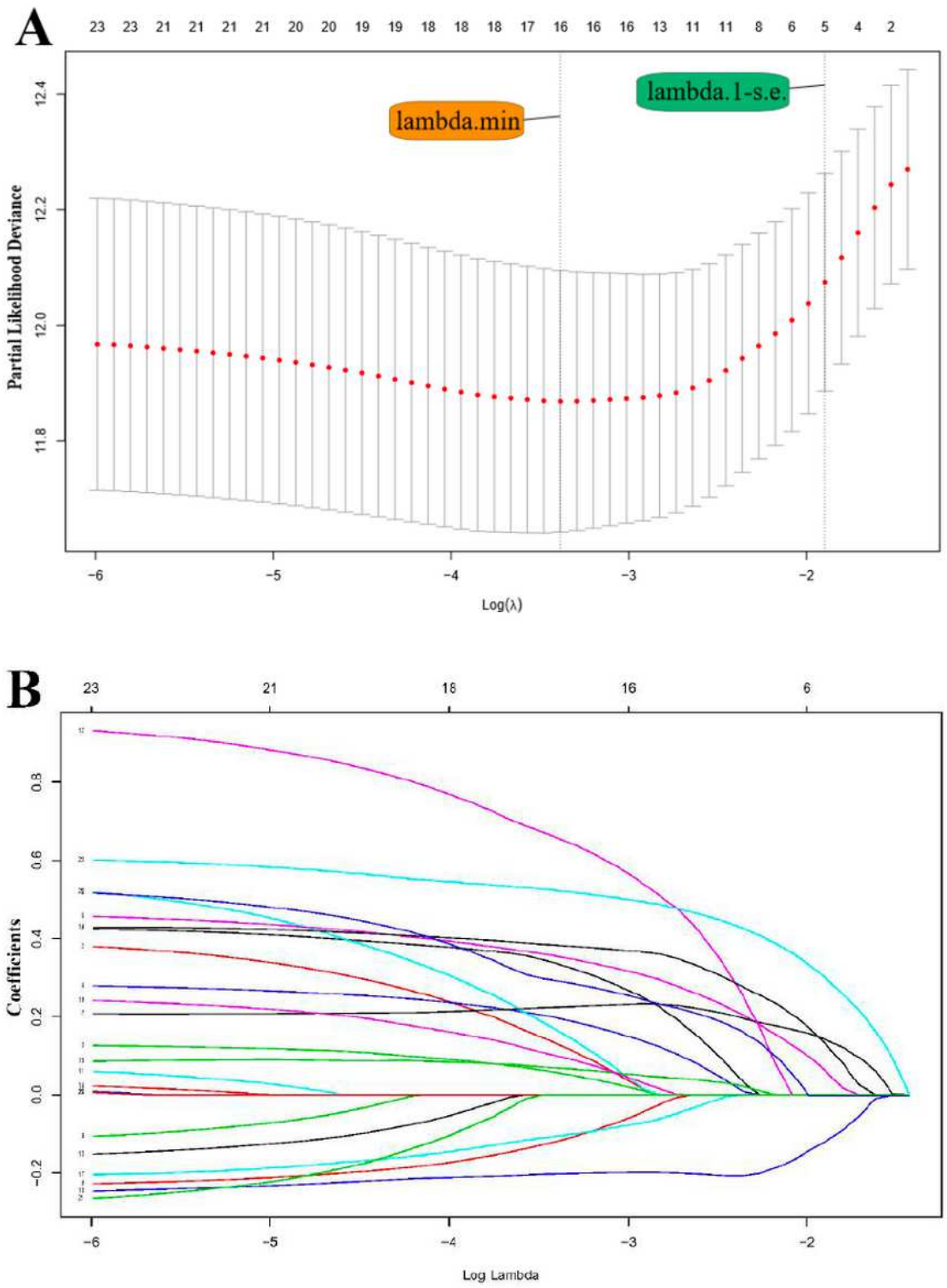

Figure 1

Five risk factors selected using LASSO Cox regression analysis. A. The two dotted vertical lines were drawn at the optimal scores by minimum criteria and 1 s.e. criteria(At minimum criteria including Gender, Age, Tumor size, Tumor number, Cirrhos is, PVTT, Ascites, HBV, HGB, CR, AST, ALB, LDH, γ GGT, CA199

and CRP; At 1 s.e. criteria including PVTT, Ascites, HGB, γ GGT and CRP). B. LASSO coefficient profiles of the 23 risk factors.

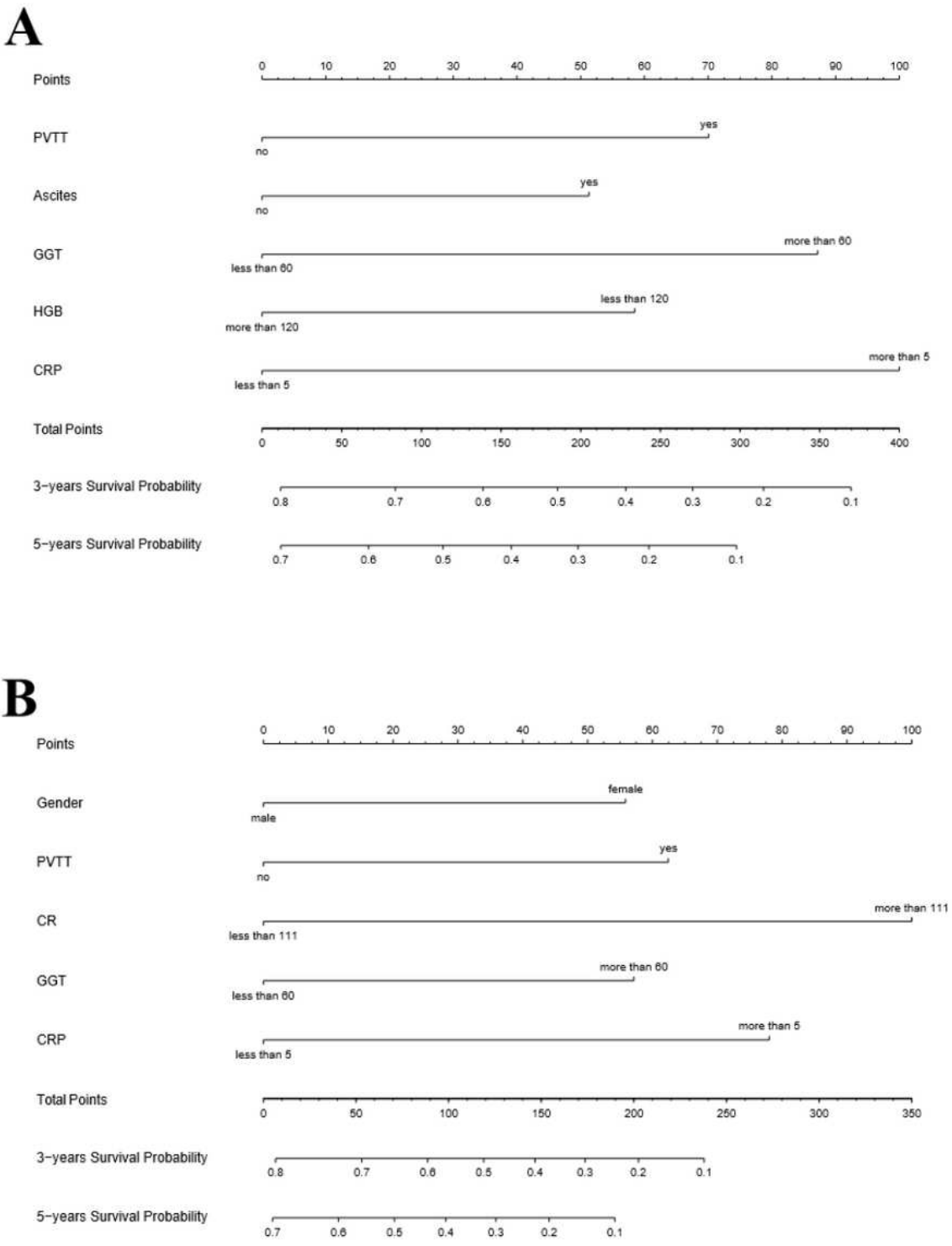

Figure 2

A. Nomogram1 including PVTT, Ascites, γ GGT, HGB and CRP, for three and five years overall survival (OS) in patients with AFP negative HCC. B. Nomogram2 including Gender, PVTT, CR, γ GGT and CRP, for three and five years overall survival (OS) i n patients with AFP negative HCC. The nomogram1 and nomogram2

are valued to obtain the probability of three and five years survival by adding up the points identified on the points scale for each variable.

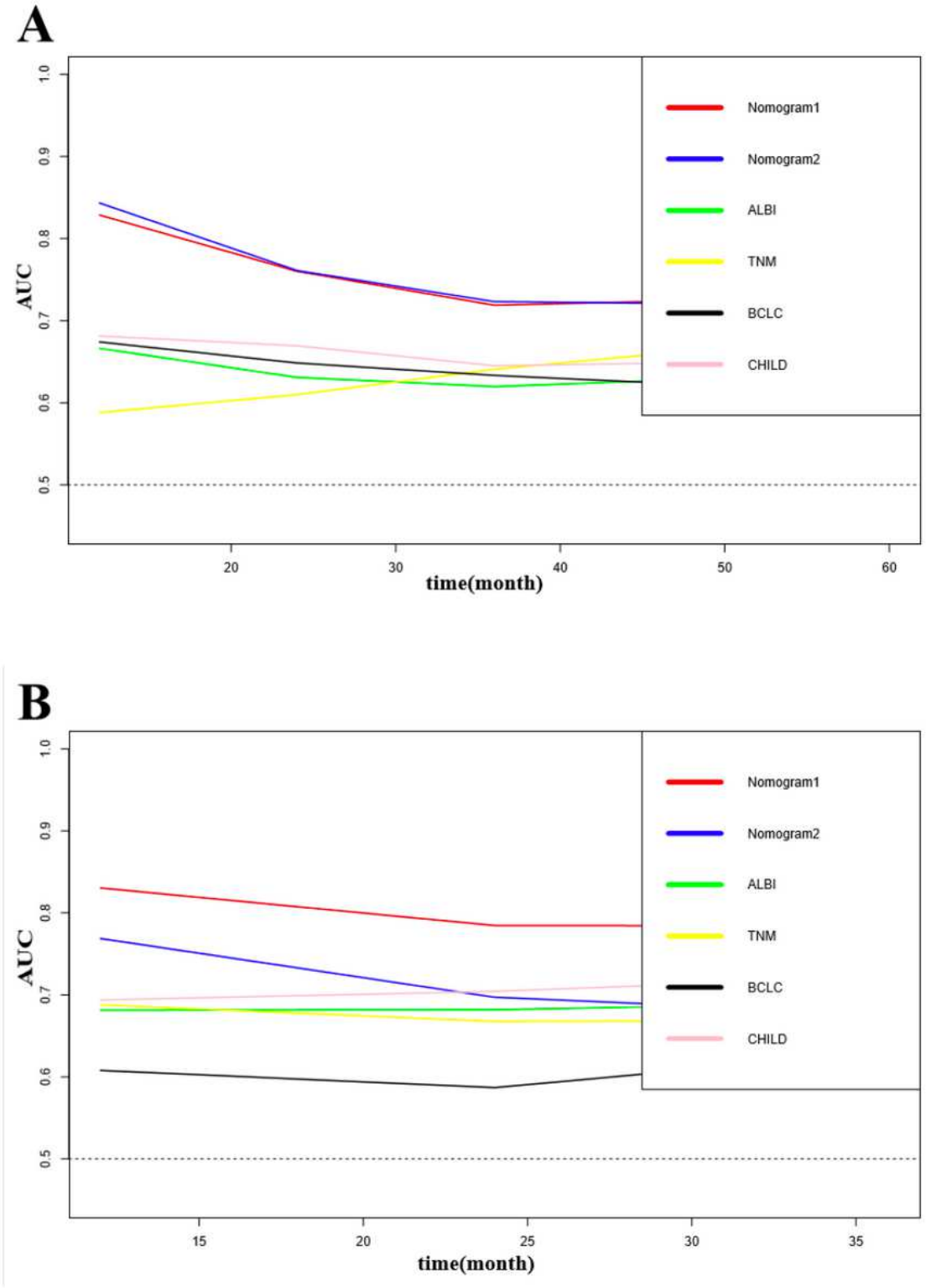

Figure 3

Time ROC curve of the six models in the primary and validation cohort. Red line: Nomogram1.Blue line: Nomogram1.Green line: ALBI. Yellow line: TNM. Black line: BCLC. Pink line: CHILD. A. Time ROC curve of the six models in the primary. B. Time R OC curve of the six models in the validation cohort.

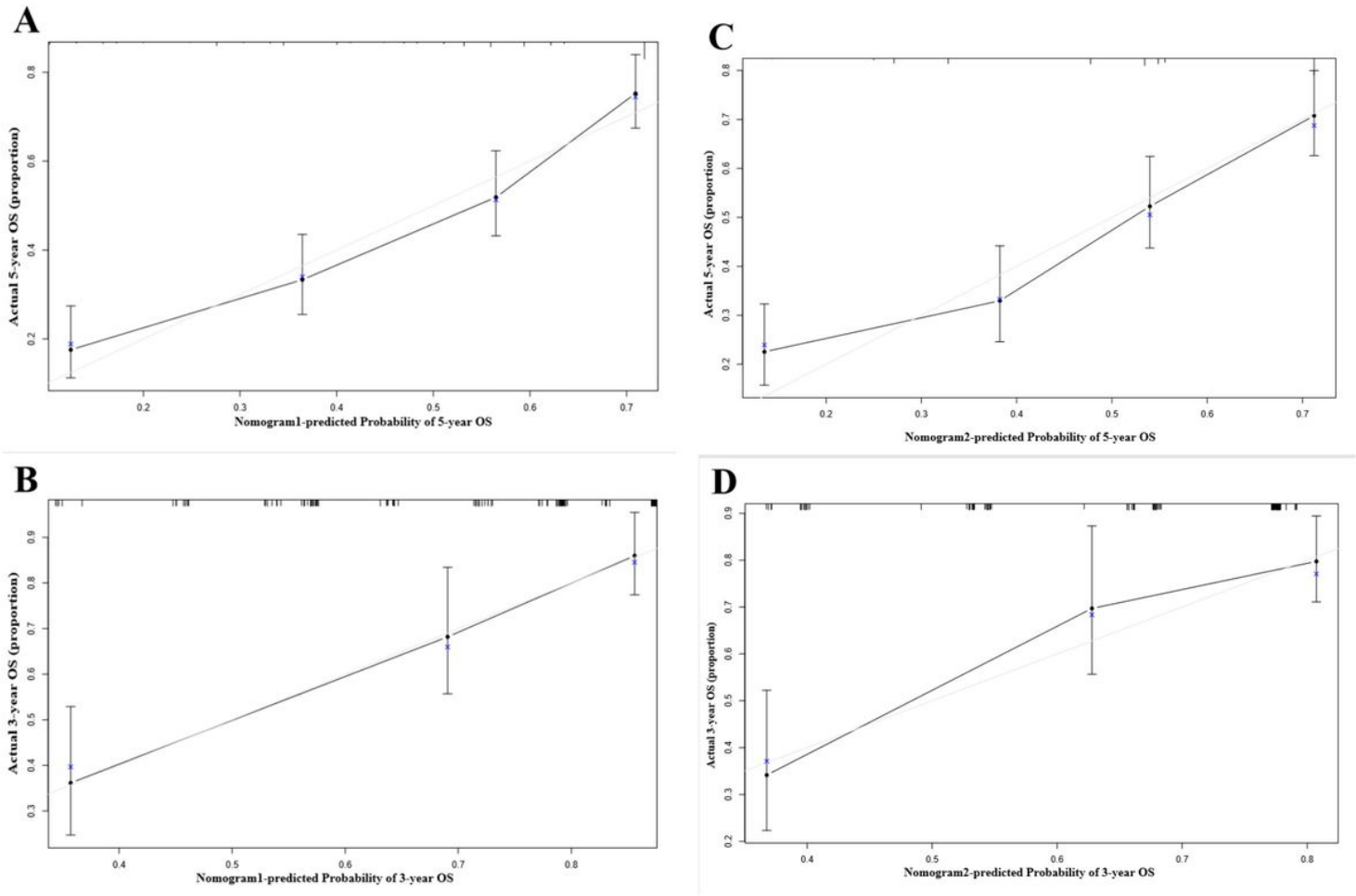

Figure 4

Calibration curve of the nomogram1 and nomogram2 in the primary and validation cohort, with the x axes are actual survival estimated by the nomogram, the y axes are observed survival calculated by the Kaplan Meier method. A. Five year survival OS in the primary cohort nomogram1. B. Three year OS in the validation cohort nomogram1. C. Five year survival OS in the primary cohort nomogram2. D. Three year OS in the validation cohort nomogram2.

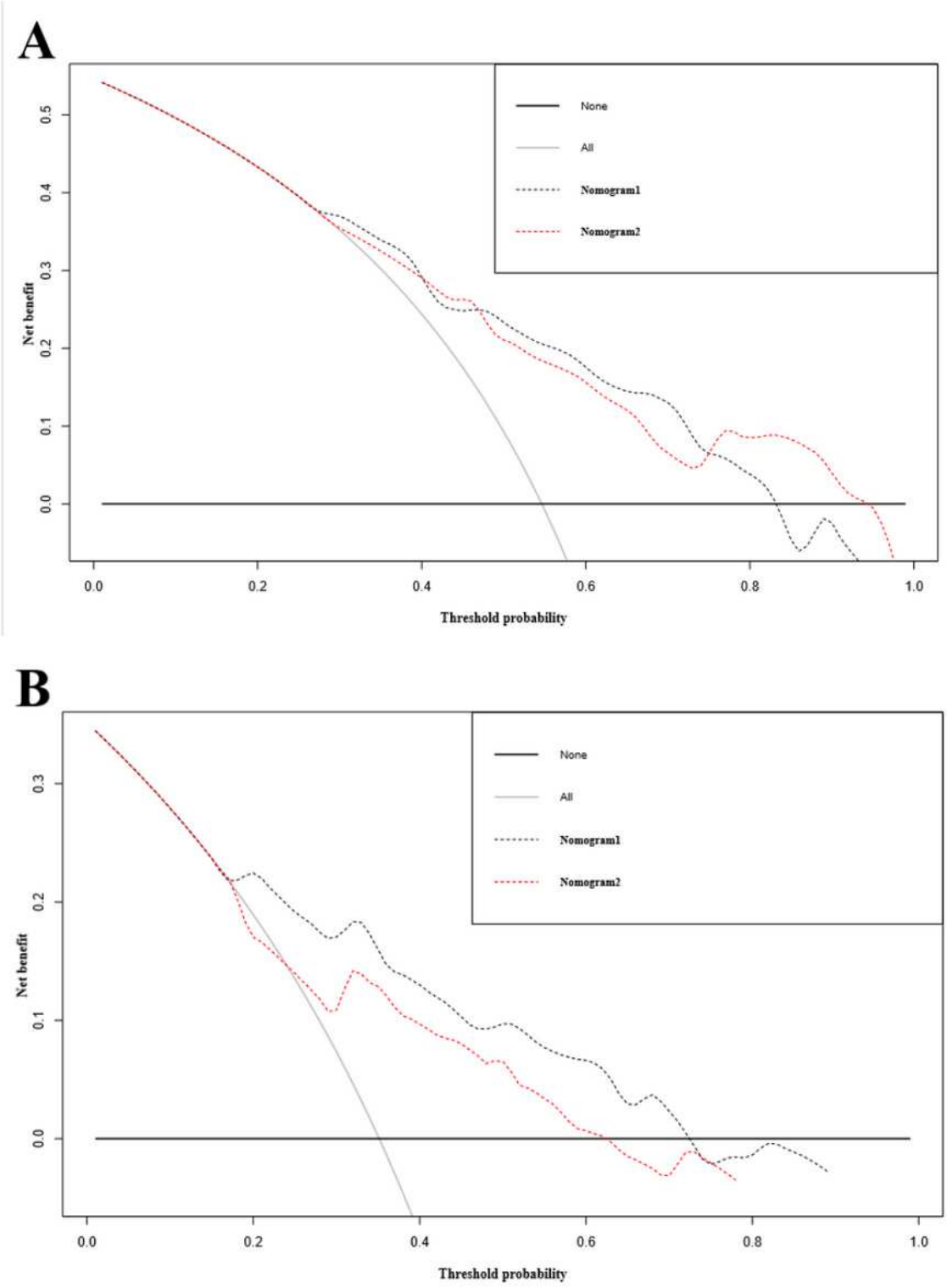

Figure 5

Decision curve analysis for overall survival in the primary and validation cohort. Black line: All patients dead. Gray line: None patients dead. Black dashed line: Model of nomogram1. Red dashed line: Model of nomogram2. A. Decision curve analysis f or overall survival in the primary. B. Decision curve analysis for overall survival in the validation cohort.

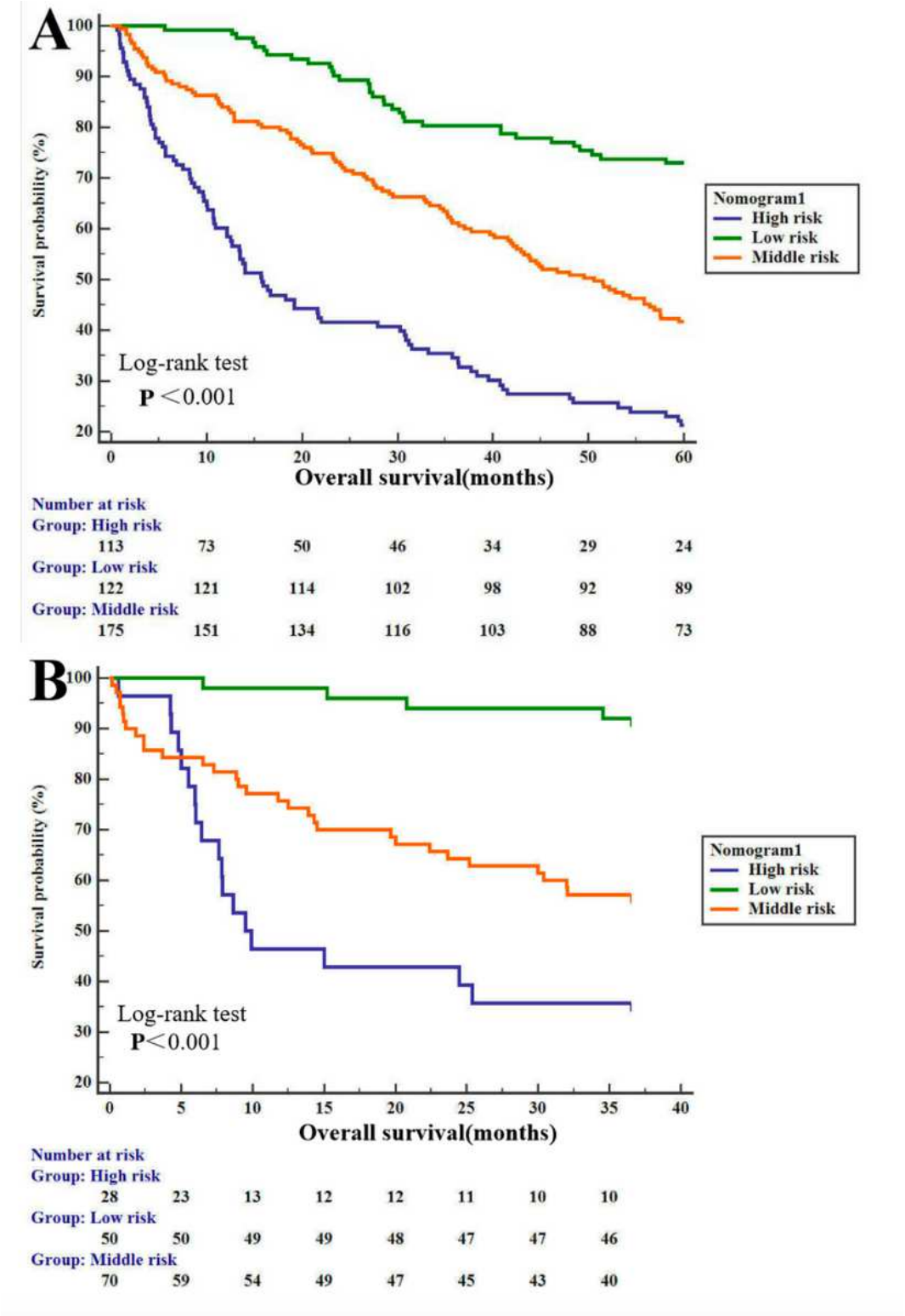

Figure 6

Kaplan Meier survival curves o f nomogram1. A. In the primary cohort. B. In the validation cohort.

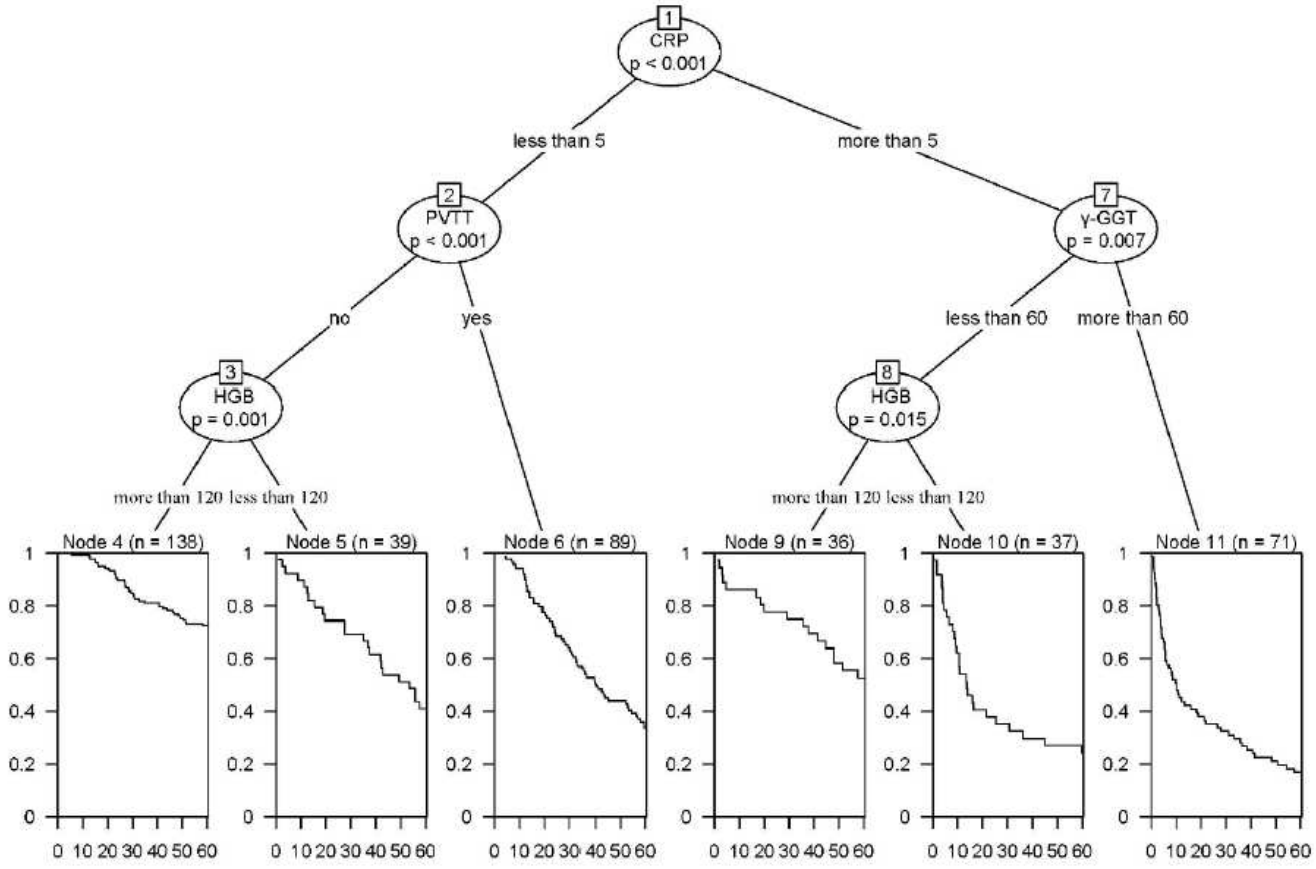

Figure 7

Decision Tree of nomogram1 five years OS in the primary cohort including CRP, PVTT, γ GGT and HGB.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TABLE.pdf