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Circulating Biomarkers Predict Immunotherapeutic Response in Hepatocellular Carcinoma Using a Machine Learning Method

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Background: Immune checkpoint inhibitor (ICI) therapy is a promising treatment for cancer. However, the response rate to ICI therapy in hepatocellular carcinoma (HCC) patients is low (approximately 30%). Thus, an approach to predict whether a patient will benefit from ICI therapy is required. This study aimed to design a classifier based on circulating indicators to identify patients suitable for ICI therapy.

Methods: This retrospective study included HCC patients who received immune checkpoint inhibitor therapy between March 2017 and September 2023 at Nanjing Drum Tower Hospital and Jinling Hospital. The levels of the 17 serum biomarkers and baseline patients' characters were assessed to discern meaningful circulating indicators related with survival benefits using random forest. A prognostic model was then constructed to predict survival of patients after treatment.

Results: A total of 369 patients (mean age 56, median follow-up duration 373 days,) were enrolled in this study. Among the 17 circulating biomarkers, 11 were carefully selected to construct a classifier. Receiver operating characteristic (ROC) analysis yielded an area under the curve (AUC) of 0.724. Notably, patients classified into the low-risk group exhibited a more positive prognosis (P = 0.0079; HR, 0.43; 95% CI 0.21–0.87). To enhance efficacy, we incorporated 11 clinical features. The extended model incorporated 12 circulating indicators and 5 clinical features. The AUC of the refined classifier improved to 0.752. Patients in the low-risk group demonstrated superior overall survival compared with those in the high-risk group (P = 0.026; HR 0.39; 95% CI 0.11–1.37).

Conclusion: Circulating biomarkers are useful in predicting therapeutic outcomes and can help in making clinical decisions regarding the use of ICI therapy.

Keywords: hepatocellular carcinoma, predictive model, immunotherapy, machine learning

Introduction

Liver cancer is the sixth most diagnosed cancer globally, accounting for over 800,000 deaths in 2020, constituting 8.3% of all cancer-related deaths. Hepatocellular carcinoma (HCC) constitutes around 90% of liver cancers, making it the predominant subtype.[1] More than 80% of patients were diagnosed in an unresectable state, resulting in poor prognosis with conventional treatment and rapid progression of the tumor.[2,3]

Immune checkpoint inhibitor (ICI) therapy is designed to target immune checkpoints, such as programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1), with the aim of activating the immune system to selectively eliminate cancer cells.[4] While several ICI drugs have shown efficacy in cancer treatment, the objective response rate in HCC remains relatively low, at around 30%.[5] One of the primary challenges in ICI treatment lies in

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recognizing patients who would benefit from it.[6,7] Currently, PD-L1 expression serves as a biomarker for predicting ICI treatment outcomes in certain cancer types, such as melanoma and non-small cell lung cancer.[8,9] However, a significant correlation between PD-L1 level and result of ICI treatment in HCC is lacking.[10,11] Recent studies have highlighted the potential predictive value of C-reactive protein (CRP) and alpha-fetoprotein (AFP) in determining the prognosis of HCC patients following atezolizumab and bevacizumab treatment.[12] Additionally, several clinical studies have shown that inflammation-related circulating biomarkers are closely associated with non-response to liver cancer immunotherapy and prognosis, including the neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR) and serum amyloid A. However, no validated biomarkers are found to be able to evaluate whether an HCC patient would benefit from immunotherapy. Therefore, further research is needed to explore the use of biomarkers to predict ICI treatment outcomes in HCC patients.

The Random Forest is an ensemble learning method that operates by constructing a multitude of decision trees during training and outputting the mode of the classes or the mean prediction of the individual trees. It generates each tree from a random sample of features and data points, which introduces diversity among the trees, making the model robust and insensitive to outliers. Therefore, we established a random forest model to generate a risk score from common circulating indicators in HCC, which was used to predict the outcomes of patients with HCC after immunotherapy.

Material and Methods

Experimental Design

The workflow of this study is illustrated in Figure 1. We measured the levels of certain circulating markers in the blood of patients before and after immunotherapy, and overall survival (OS) time was used to describe the outcomes of patients receiving immunotherapy. Subsequently, patients were randomly allocated to training and testing groups at a ratio of 7 3, with 258 and 111 patients in the training and test groups, respectively. Using the robust Random Survival Forest methodology, we identified biomarkers intricately linked to prognosis from the measured blood indicators. The selected

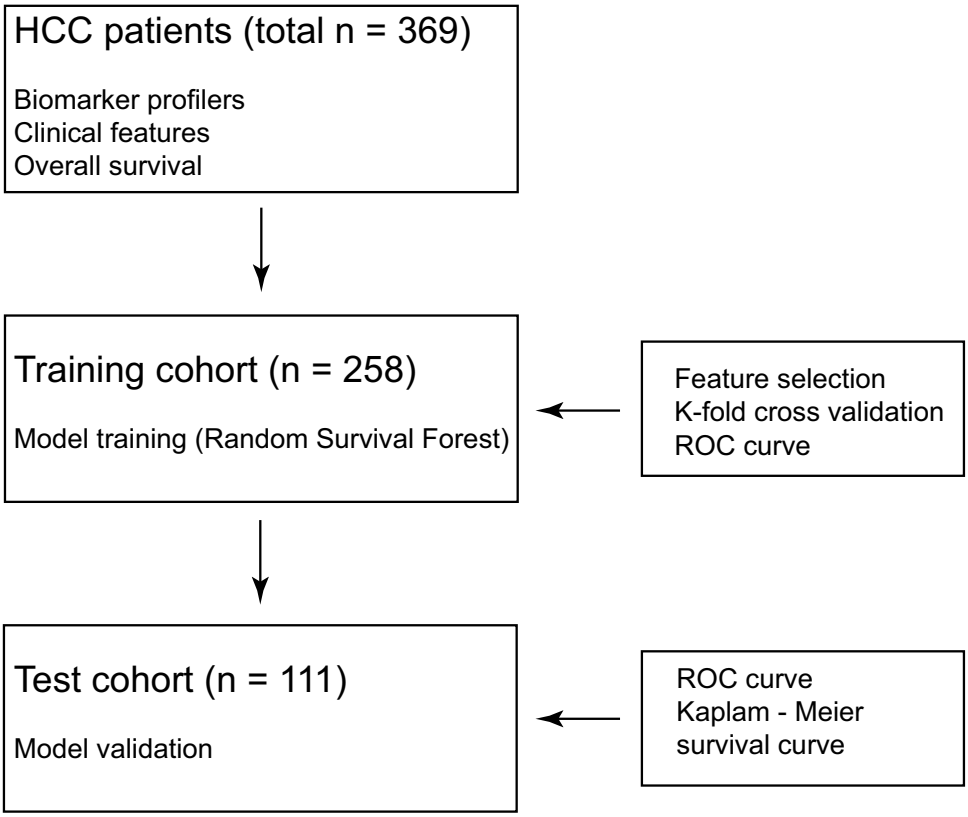

Figure 1 Workflow chart of the machine learning method to predict outcomes of HCC patients receiving ICI treatment.

biomarkers play a pivotal role in calculating a personalized risk score for each patient. Subsequently, this risk score was instrumental in constructing a predictive model to predict the prognosis after immunotherapy. To assess the accuracy of the model, we conducted 10-fold cross-validation within the training group and computed the concordance index (C-index) and AUC. Finally, the efficacy of the model was illustrated through survival analysis, providing a comprehensive evaluation of its predictive capabilities in delineating post-immunotherapy prognosis for individual patients.

Patients

We included 369 patients who received anti-PD-1 or anti-PD-L1 in this study. Patients who met the following criteria were included in this study: Diagnosed with hepatocellular carcinoma; received at least two cycles of immunotherapy; aged 18 or above; Eastern Cooperative Oncology Group Performance Status (ECOG PS) of 0–2; had imaging assessments before and after immunotherapy; and have sufficient liver and kidney function. Patients who met any of the following criteria were excluded: having other primary cancer comorbidities; receiving less than two cycles of immunotherapy; lacking Computed Tomography (CT) and/or Magnetic Resonance Imaging (MRI); lacking baseline clinical pathological data; and having no follow-up data.

Data Collection

The following serum data were collected at baseline and during treatment: AFP, carbohydrate antigen 19–9 (CA 19–9), D-Dimer, granulocytes, hemoglobin (HGB), Platelets, leukocytes, lymphocytes, CRP, CD8+ T Cells, CD4+ T Cells, NK, bilirubin, albumin, lactate dehydrogenase (LDH), protein induced by vitamin K absence (PIVKA-II) and international normalized ratio (INR). Clinical features were collected as following: age, sex, stage, liver metastasis, lymph node metastasis, other metastasis, tumor thrombus grade, surgery, radio therapy, liver cirrhosis and ICI therapy used.

Predictive Model Construction

We constructed a random forest model using the randomForestSRC package in R. Initially, we included all indicators in the model (ntree = 1000; node size = 3; mtry = 10; nsplit = 10) and calculated the minimal depth for each indicator. A reduced minimum depth suggested that the feature was positioned closer to the root, making it more important. Therefore, we defined importance as the reciprocal of the minimal depth and selected indicators with importance values greater than the mean of all indicators to construct the random forest. We then constructed the model again, using the training cohort and validated it using the test cohort, which were two mutually independent cohorts.

Statistical Analysis

Statistical analyses were performed using the R programming language. For assessing differences in indicators between the two cohorts, the Wilcoxon rank-sum test was applied for continuous variables, whereas the $\chi 2$ test was used for categorical variables. The R package survival ROC facilitated calculation of the ROC curve. The criterion for differentiating between the high-risk and low-risk groups was established through the application of Youden's method to the ROC curve for 1-year outcomes. The ROC curves were generated utilizing the R packages survival and survminer. Differences in biomarker level between the two groups were evaluated using the Wilcoxon rank-sum test. Notably, for circulating features with a substantial number of missing values, we treated missing status as a distinct category in our analysis.[13]

Result

Profile of Patient Data

From March 2017 to September 2023, 369 hepatocellular carcinoma patients who received first-line anti-PD-1 or anti-PD -L1 treatment at Nanjing Drum Tower Hospital and Jinling Hospital were included in this study. The baseline characteristics were summarized in Table 1. Mean age of 57(15.4) were female and 309(83.7) were male; The median age of the patients was 56; Most of the patients were in stage 3(n=233, 63.1%); 79.9%(295) of the patients have multiple

Table 1 Summary of Patient Characters

	Total (n=369)	Train (n=258)	Test (n=111)	P Value
AFP (ng/mL)	394	661.95	81.45	0.01
CA 19–9 (u/mL)	41.3	42.65	39	0.35
D-Dimer (mg/L)	2.11	2.13	1.86	0.99
Granulocytes (10^9/L)	3.12	3.09	3.175	0.56
HGB (g/L)	124	126	118.5	0.2
Platelets (10^9/L)	123.5	127	112	0.11
Leukocytes (10^9/L)	4.65	4.6	4.7	0.7
Lymphocytes (10^6/L)	0.93	0.95	0.92	0.89
CRP (mg/L)	7.2	6.8	12.9	0.12
CD8+ T Cells (10^6/L)	24.3	25.2	10.3	0.15
CD4+ T Cells (10^6/L)	42	43	5.7	0.14
NK (10^6/L)	13.7	13.7	9.6	0.69
Bilirubin (μmol/L)	16.4	16.7	15.3	0.15
Albumin (g/L)	37.7	37.7	37.1	0.37
INR	1.180	1.18	1.135	0.5
LDH (u/L)	219	218.5	219	0.65
PIVKA2(mAU/mL)	1480.59	1368.37	7147.37	0.35
Age	56	55.5	57	0.86
Stage (n, %)				0.07
I	1(0.3)	0	1(0.9)	
2	61(16.5)	49(20)	12(10.8)	
3	233(63.1)	155(60)	78(70.2)	
4	74(20.1)	54(21)	20(18.1)	
Liver metastasis (n,%)				0.14
Yes	295(79.9)	212(82.1)	83(74.7)	
No	74(20.1)	46(17.9)	28(25.3)	
Lymph node metastasis (n, %)				0.94
Yes	129(35)	91(35.2)	38(34.3)	
No	240(65)	167(64.8)	73(65.7)	
Other metastasis (n, %)				0.78
Yes	52(14.1)	35(13.6)	17(15.4)	
No	317(85.9)	223(86.4)	94(84.6)	
Tumor Thrombus Grade (n, %)				0.38
0	210(57)	156(60.4)	54(48.6)	
I	4(1)	3(1.1)	1(0.9)	
2	7(1.9)	4(1.6)	3(2.7)	
3	49(13.3)	33(12.8)	16(14.5)	
4	97(26.3)	61(23.7)	36(32.4)	
Unknown	2(0.5)	1(0.4)	1(0.9)	
Sex (n, %)				0.96
Female	309(83.7)	39(15.1)	18(16.3)	
Male	57(15.4)	217(84.1)	92(82.8)	
Unknown	3(0.9)	2(0.8)	1(0.9)	
Surgery (n, %)				0.34
Yes	162(44)	118(45.8)	44(39.6)	
No	205(55.5)	138(53.4)	67(60.4)	
Unknown	2(0.5)	2(0.8)	0	
Radio Therapy (n, %)				0.29
Yes	102(27.6)	76(29.5)	26(23.5)	
No	267(72.4)	182(70.5)	85(76.5)	

(*Continued*)

Table 1 (Continued).

	Total (n=369)	Train (n=258)	Test (n=111)	P Value
TACE (n, %)				0.46
Yes	97(26.3)	72(28)	27(24.3)	
No	272(73.7)	186(72)	84(75.7)	
Liver Cirrhosis (n, %)				0.65
Yes	256(69.5)	179(69.3)	77(69.4)	
No	111(30)	77(29.9)	34(30.6)	
Unknown	2(0.5)	2(0.8)	0	
Immunotherapy drugs (n, %)				0.62
1	221(60)	151(58.5)	70(63)	
2	17(4.6)	11(4.2)	6(5.4)	
3	26(7)	17(6.5)	9(8.1)	
4	9(2.4)	8(3.1)	1(0.9)	
5	70(19)	54(21)	16(14.5)	
6	10(2.7)	6(2.3)	4(3.6)	
7	7(1.9)	4(1.5)	3(2.7)	
8	9(2.4)	7(2.7)	2(1.8)	
Follow-up duration (median)	373	411.5	313	0.18

Notes: Immunotherapy drugs 1~8 represent 8 different anti-PD1 therapy. P value for continuous variables were calculated using the Wilcoxon rank-sum test, and $\chi 2$ for categorical variables.

hepatic tumors; About 57%(210) of the patients did not develop tumor thrombus; Around half of the patients (n=162, 44%) have undergone surgery and 27.6%(102) of the patients have received radiotherapy; 97(26.3%) patients have received TACE treatment. As shown in Table 1, no differences were observed in immune drug therapy, stage, tumor thrombus grade, age, sex, surgery, radiotherapy, transcatheter arterial chemoembolization (TACE), liver cirrhosis, liver metastases, lymph metastases, and other metastases between the training and test sets. In addition, no significant difference was observed in OS between the two groups of patients.

Prediction of Immunotherapeutic Outcomes Based on Baseline Circulating Indicators Level

We established a machine learning method utilizing a random forest algorithm to predict OS in immunotherapy based on the analysis of circulating indicators at baseline. Initially, we assessed and ranked the importance of the 19 baseline indicators in the training set using their minimal depth in the algorithm. Subsequently, 11 key markers [HGB, CA 19–9, AFP, LDH, albumin, platelets, granulocytes, bilirubin, CRP, lymphocytes, and leukocytes] were selected from a pool of 19 circulating indicators (Figure 2A). Selected markers were used to construct an OS prediction model. The model generated a risk score for each patient, based on the chosen indicators. To validate the predictive performance of the model, we conducted ten-fold cross-validation within the training group, achieving a mean C-index of 0.59 (Figure 2B). Subsequently, we assessed the accuracy of the model through survival analysis, illustrating its classification performance with respect to patient survival status. To further evaluate the discriminatory ability of the model, we plotted an ROC curve for the one-year survival rate. Employing Youden's method, we determined an optimal threshold, thereby categorizing the patients into high-risk and low-risk groups (Supplementary Figure 1A). In the training cohort, 176 patients were categorized into the low-risk group and 80 patients were placed in the high-risk group. A survival analysis employing the Kaplan-Meier method revealed a significantly poorer clinical prognosis for patients in the high-risk group than for those in the low-risk group ($P < 0.0001$; HR 0.12; 95% CI 0.07–0.21). The median OS for the high-risk group was 582 days (95% CI, 526–734 days), in contrast to 1215 days for the low-risk group (95% CI, 1134–1555 days) (Figure 2D).

We applied the model to a test group to assess the accuracy of the model predictions. The C-index for the validation set showed an improvement of 0.6, surpassing that of the training cohort (Figure 2B). The ROC curve at one-year mark

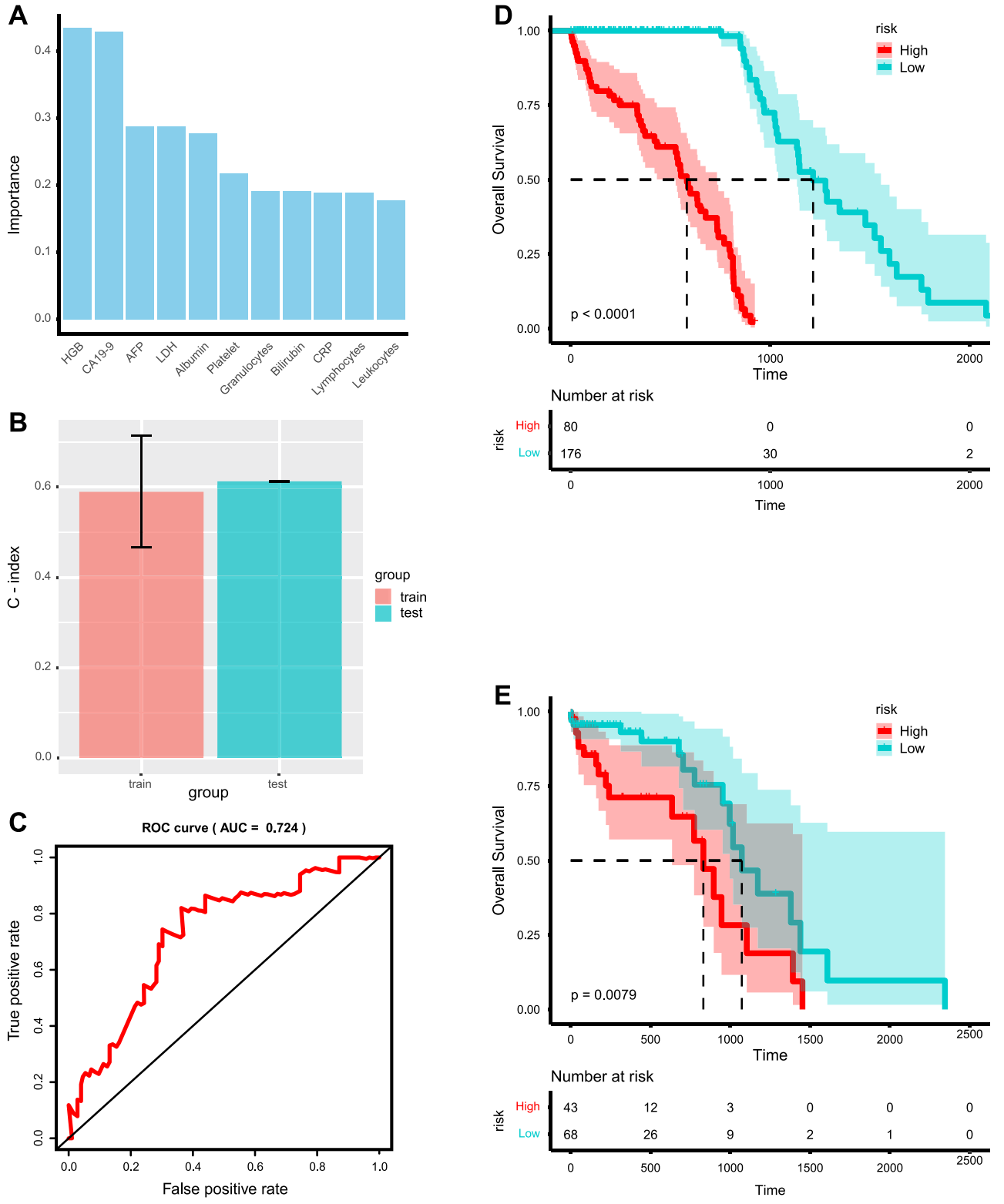

Figure 2 Performance of the model using baseline biomarker level. (**A**) The importance of selected circulating biomarkers in the random forest model. Importance refers to the reciprocal of the minimal depth, which indicates how much influence the variable has on final decision. (**B**) C-index of the model for training and test cohorts. (**C**) Receiver operating characteristic curve (ROC) of the model on test cohort; Kaplan–Meier curves for overall survival of "high risk" and "low risk" groups for training (**D**) and test (**E**) set. The "high risk" and "low risk" groups were defined using cut-off value of risk score optimized by training set, the risk scores stand for risk of event occurrence.

exhibited satisfactory results, with an AUC value of 0.724 (Figure 2C). In the test cohort, 43 patients were classified as high-risk, whereas 68 patients were classified as low-risk. Subsequent observations of survival periods revealed significant differences. Survival analysis indicated that patients in the high-risk group had a markedly worse prognosis than those in the low-risk group ($P = 0.0079$; HR, 0.43; 95% CI 0.21–0.87). The median OS for the high-risk and low-risk groups was 831 days (95% CI, 635–NA) and 1072 days (95% CI, 996–NA), respectively (Figure 2E).

Prediction of Immunotherapeutic Outcomes Based on Baseline Circulating Indicators Level and Clinical Features

To enhance the efficacy of the model, we incorporated 11 clinical features and developed a parallel model to predict patient prognosis by integrating circulating indicators and pertinent clinical features that could influence immunotherapy outcomes. In this iteration, we carefully selected 12 circulating indicators — HGB, CA19-9, LDH, Albumin, AFP, Platelets, CRP, Bilirubin, Granulocytes, Leukocytes, Lymphocytes, INR — alongside 5 clinical features: age, radiotherapy, stage, surgery, and tumor thrombus grade to construct the model (Figure 1A). This updated model exhibited commendable performance in predicting patient survival using circulating indicators. The average C-index in the training group for k-fold cross-validation stood at 0.56, while the C-index in the test group showed a slight improvement, reaching 0.63 (Figure 3B). Notably, the ROC curve at the one-year mark in the test group demonstrated a superior outcome compared to the model without clinical features, with an AUC of 0.752 (Figure 3C). To further refine the risk stratification, we applied Youden's method to the ROC curve at one year in the training group, deriving a threshold value to categorize patients into high-risk and low-risk groups (Supplementary Figure 1B). In the training set, we allocated 42 and 216 patients to the high-and low-risk groups, respectively. Similarly, in the test cohort, 11 patients were allocated to the high-risk group, while 100 patients to the low-risk group. Survival analysis revealed that high-risk patients exhibited significantly poorer outcomes compared to the low-risk group in both the training set ($P < 0.001$; HR 0.08; 95% CI 0.03–0.19) and the validation set ($P = 0.026$; HR 0.39; 95% CI 0.11–1.37). The median OS for the high-risk group in the training cohort was 342 days (95% CI, 193–553) and 831 days (95% CI, 220–NA) in the validation set. In contrast, the median OS for the low-risk group in the training cohort was 1040 days (95% CI 949–1284), and in the validation set, it was 1017 days (95% CI 945–1439) (Figure 3D and E).

We proceeded to develop a comparable model for predicting patient prognosis post-immunotherapy, incorporating circulating indicator levels post-ICI treatment and relevant clinical features that could impact immunotherapy outcomes. In this instance, two models were constructed. The first included 13 circulating markers: HGB, LDH, albumin, leukocytes, lymphocytes, CA19-9, AFP, granulocytes, CRP, platelet, INR, PIVKA-II, and bilirubin. In the second model, 11 circulating markers (HGB, LDH, albumin, leukocytes, lymphocytes, platelets, granulocytes, AFP, bilirubin, CA 19–9 and CRP) along with 6 clinical features (immune drug selection, age, stage, whether received radio therapy, whether received surgery and tumor thrombus grade) were selected to build the predictive model. However, the results predicted by these two models did not satisfy our expectations. Further investigations and refinement are necessary to enhance the predictive accuracy of these models for patient prognosis after immunotherapy. This could involve reassessing the feature selection and model parameters, or considering additional factors that may contribute to improved performance. (Supplementary Figures 2 and 3).

Differences of Selected Features Between High-Risk and Low-Risk Group

After combining the training and test cohorts into a unified cohort, we evaluated the selected circulating biomarkers between the high-risk and low-risk groups.

In the first model, which included 11 circulating biomarkers with potential predictive value for ICI treatment outcomes, the levels of six biomarkers showed significant changes between the high- and low-risk groups. Specifically, higher levels of HGB and bilirubin were noted in the high-risk group, whereas AFP, granulocyte, leukocyte, and platelet levels were elevated in the low-risk group (Supplementary Figure 4).

However, when patients were categorized based on both circulating biomarkers and clinical features, the impact of circulating biomarkers diminished. Only 2 of the 12 selected biomarkers demonstrated a significant difference between

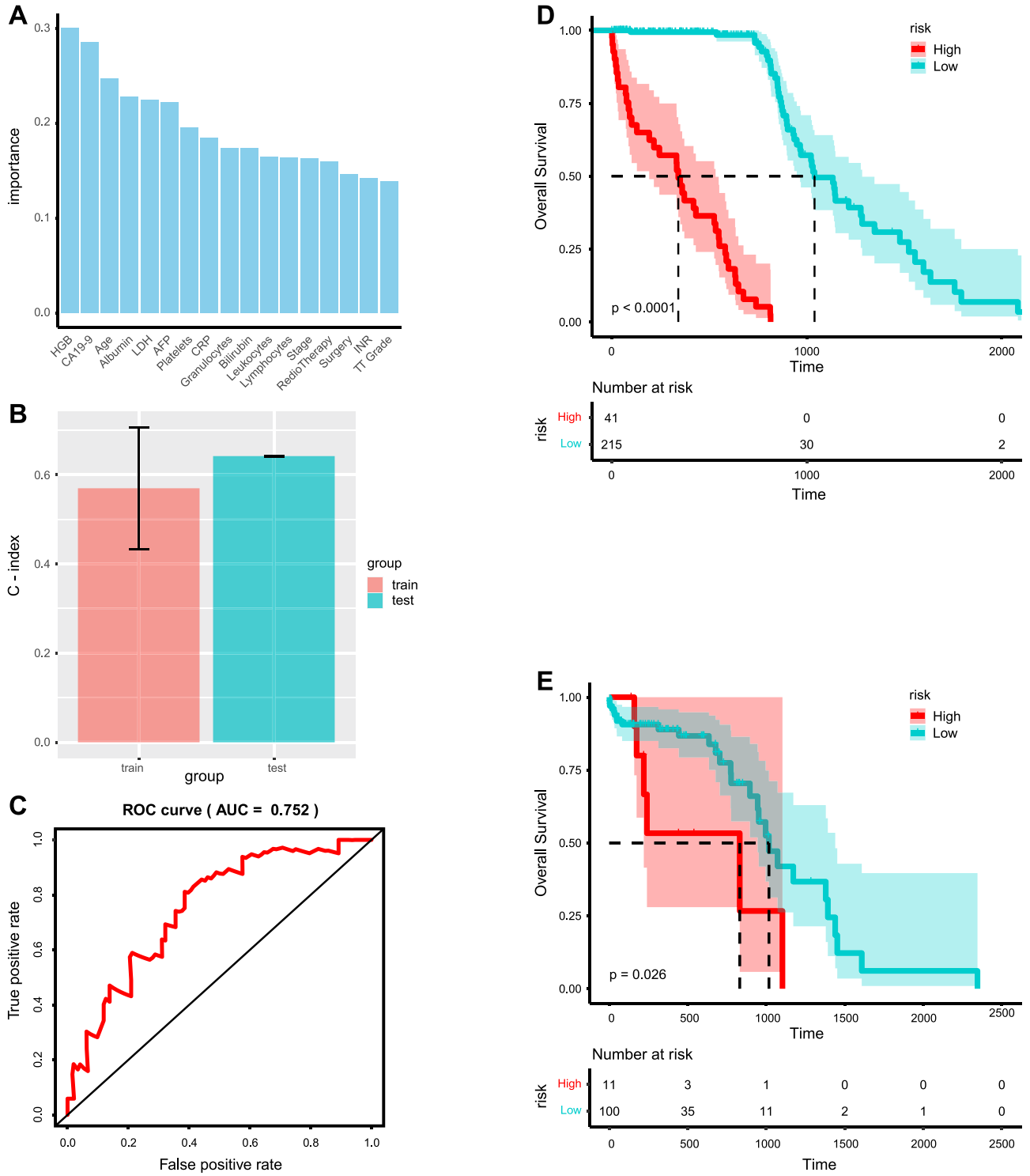

Figure 3 Performance of the model using baseline biomarker level and clinical features. (**A**) The importance of selected circulating biomarkers and clinical features in the random forest model. Importance refers to the reciprocal of the minimal depth, which indicates how much influence the variable has on final decision. (**B**) C-index of the model for training and test cohorts. (**C**)Receiver operating characteristic curve (ROC) of the model on test cohort; Kaplan–Meier curves for overall survival of "high risk" and "low risk" groups for training (**D**) and test (**E**) set. The "high risk" and "low risk" groups were defined using cut-off value of risk score optimized by training set, the risk scores stand for risk of event occurrence.

the high-risk and low-risk groups. Remarkably, the high-risk group showed higher HGB levels, while the group with lower risk exhibits elevated platelet levels (Supplementary Figure 5).

This analysis underscores the complex interplay between circulating biomarkers and clinical features in predicting outcomes after ICI treatment, highlighting the need for a comprehensive understanding of both factors for an accurate prognosis.

Discussion

In the present work, we built an ensemble learning classifier using a random survival forest to identify immunotherapeutic-related circulating biomarkers to predict the outcomes of ICI therapy in HCC patients. Random survival forests have been used in several studies to explore prognostic values.[14,15] In the present study, 11 serum indicators were selected to build the model and succeed in patients with prolonged survival after ICI treatment. Twelve serum indicators and five clinical features were selected to generate the model in the same manner and showed great effectiveness. Our findings may assist doctors in identifying patients who may benefit from ICI treatment.

As shown in Supplementary Figures 4 and 5, level of AFP, bilirubin, granulocyte, HGB, leukocyte and platelet are significantly different between high-risk and low-risk groups, which means these indicators may have a substantial impact on patients' survival conditions. Among which, AFP and bilirubin are well-known biomarkers that have prognostic value to HCC patients.12 however, the impact of HGB, leukocyte and platelet has not been deeply studied. In our study, we found that high level of leukocyte and platelet, along with low level of HGB are related to worse prognosis. Thus, clinicians need to pay more attention to these indicators to assist in determining whether patients are suitable for ICI therapy. Also, our study also suggests further studies to explore the influence these serum indicators have on prognosis of patients receiving ICI therapy.

HGB appeared to be the most important indicator provided by the algorithm for predicting the outcomes of patients with HCC in response to ICI therapy. Our result shows that low level of HGB may indicate poor survival. HGB levels has been confirmed in several study to have an influence on prognosis of different cancer types.[16–18] However, little is known about whether or how HBG affects the efficacy of immunotherapy. A study showed that serum HGB level is correlated with occurrence and survival of bone metastasis in HCC patients.[19] Another research demonstrated that preoperative anemia (HGB < 90 g/L) is a significant determinant of intra-abdominal bacterial infections for HCC patients who went through liver transplantation.[20] Anemia is reported to be a common symptom in HCC patients, which is caused by blood loss, nutritional deficiencies and insufficient iron stores.[21] Together, these studies indicate that HGB level is an important factor in HCC progress, but the mechanism behind it is to be discovered.

CA 19–9 is another important indicator for predicting the outcomes of ICI therapy in patients with HCC. Traditionally, CA19-9 has not been recognized as a highly sensitive biomarker for HCC. However, it is noteworthy that CA19-9 immunoreactivity is detectable in the bile ductules and interlobular bile ducts within non-neoplastic areas surrounding HCC.[22] In normal individuals, CA19-9 is primarily synthesized by pancreatic and bile duct cells, as well as the stomach and colon, and is present in minimal amounts in serum. However, in the presence of a tumor, plasma CA19-9 levels can be significantly elevated. CA19-9 is commonly acknowledged as a marker for early diagnosis of intrahepatic cholangiocarcinoma (ICC).[23] Moreover, a high level of CA19-9 in combined hepatocellular-cholangiocarcinoma (cHCC-CCA) indicates a poorer prognosis, suggesting the possibility that some patients within our cohort may have cHCC-CCA.[24] The invasion of cancer tissue can result in bile duct injury, and damage to the epithelial cells of the bile ducts, along with cholestasis, can also lead to an increase in the plasma concentration of CA19-9.[25] CA 19–9 also serves as a marker for acute liver failure, implying that HCC patients with higher CA 19–9 levels may face an increased risk of liver failure, ultimately resulting in a worse prognosis.[26] Therefore, the reason CA 19–9 is important in our model could be some patients in our cohort have combined hepatocellular-cholangiocarcinoma or hepatocellular carcinoma with bile duct tumor thrombus, and the invasion of cancer tissue into the bile ducts would lead to an increase in CA19-9 levels. In future studies, we would pay more attention to bile duct injury and reduce its impact on the research.

Our results show that patients with high level of AFP are at higher risk. Currently, AFP is the most widely used biomarker for the detection of HCC with approximately 70% sensitivity,[27] and a higher serum AFP level often indicates worse prognosis in HCC patients at all stages.[12] Recently, a study involving HCC patients receiving nivolumab or pembrolizumab showed that low serum AFP level at the start of ICI treatment leads to a good response as well as a significantly longer median PFS and OS.[28] One possible reason for this may be that AFP has the ability to affect the

microenvironment around the tumor by inhibiting the anti-tumor functions of several immune cells, including dendritic cells (DCs), T cells, and natural killer (NK) cells.[29,30] Researchers cultured peripheral blood monocytes with AFP and observed that DCs differentiation was significantly inhibited. With limited mature DCs and inflammatory mediators produced, T Cell activation would also be restrained, resulting in immune escape of HCC tumor cells.[31] Also, DCs are the main producers of IL-12, and loss of DCs functions leads to an insufficient amount of IL-12, which then suppresses the function of NK cells.[32] In addition, AFP plays an important role in the tumor VEGF pathway, and VEGF production is reported to be reduced when AFP is silenced.[33] VEGF can restrain immune cell activity and T-cell infiltration, thus reducing ICI effectiveness.[34]

In addition to HGB, CA 19–9 and AFP, several other biomarkers have demonstrated great potential for identifying patients who would benefit from ICI therapy. Researchers have found that albumin combined with bilirubin perform well in measuring liver function of patients with HCC and chronic liver disease, as well as classifying patients with different prognosis.[35,36] Platelets are essential contributors to both innate and adaptive immune responses, and are often considered a factor that contribute to tumor growth.[37] However, a recent study reported that platelets can inhibit HCC growth by releasing P2Y12-dependent CD40L, which indicated that platelets that the association between platelets and cancer is complicated.[38] CRP as well as AFP were used together to develop a CRAFITY score to predict outcomes of HCC patients undergoing ICI therapy, which has been proved valid under different conditions.[12,39,40]

In our study, the median OS reached 1000 days for low-risk group and 800 days for high-risk group. It seems too high for patients with advanced HCC. We believe this is due to the fact that about half of our patients had right censoring, and it can be seen from Figure 2D and E that the + representing censoring are concentrated before 500 days, with an even denser concentration before 300 days. This could lead to an overestimation of the median survival time. In future studies, we will improve the follow-up procedures and research plans to reduce the loss of survival data, ensuring that we can obtain more valid data.

This study has some limitations. First, incomplete data may have introduced bias into the results (Supplementary Figure 6). Although our findings suggest the potential predictive value of these blood indicators, more comprehensive data are required to obtain more accurate results. Second, this was a retrospective study, implying a lack of purposeful feature selection. More rigorously planned prospective studies are required to construct accurate models.

Conclusion

In conclusion, we established a random forest model to generate a risk score from common circulating indicators in HCC, which was used to predict the outcomes of patients with HCC after ICI therapy.

Abbreviations

AFP, alpha-fetoprotein; AUC, analysis yielded an area under the curve; CRP, C-reactive protein; C-index, concordance index; DCs, dendritic cell; HCC, hepatocellular carcinoma; ICC, intrahepatic cholangiocarcinoma; ICI, immune checkpoint inhibitor; INR, international normalized ratio; LDH, lactate dehydrogenase; NK, natural killer; OS, overall survival; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; PIVKA-II, protein induced by vitamin K absence; ROC, receiver operating characteristic; TACE, transcatheter arterial chemoembolization; VEGF, vascular endothelial growth factor.

Data Sharing Statement

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

Ethics Approval and Consent to Participate

This study was approved by the Medical Ethics Committee of Drum Tower Hospital affiliated with Nanjing University Medical School and Ethics Committee of Jinling Hospital. Procedures performed in this study was in compliance with the Declaration of Helsinki. Patients treated in our hospital will be informed of the potential research use of personal information. Written informed consent was signed by the patients before treatment.

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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.

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Disclosure

The authors report no conflicts of interest in this work.

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