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ORIGINAL RESEARCH

Spleen Volume Dynamics and Survival Outcomes in HCC Patients Undergoing Immune Checkpoint Inhibitors: A Retrospective Analysis

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Purpose: The spleen serves as an important immune organ which influences the anti-tumor immune response by modulating the immune microenvironment. This study investigated the prognostic impact of spleen volume (SV) on the survival in hepatocellular carcinoma (HCC) patients receiving immune checkpoint inhibitors (ICIs).

Patients and Methods: This retrospective study included 224 HCC patients treated with ICIs, categorized into Higher and Lower SV groups by median SV and further into SV increased and Non-SV increased groups based on changes in SV at 3 months after ICIs. Kaplan-Meier curves and Cox regression models were used to evaluate the influence of SV and clinical indicators on progression-free survival (PFS) and overall survival (OS). Independent prognostic factors identified via multivariate analysis were incorporated into nomograms, with their accuracy assessed using concordance index (C-index), time-dependent receiver operating characteristic (ROC) and calibration curves. Restricted cubic spline (RCS) analysis was conducted to assess the relationship between baseline SV and survival.

Results: The Higher SV and SV increased groups demonstrated shorter PFS and OS compared to the Lower SV and Non-SV increased groups, respectively. These results were consistent with different regimens in the Child A. The C-index of nomogram for PFS were 0.700 (0.678–0.721) and OS 0.733(0.709–0.757). The ROC and calibration curves confirmed robust discrimination and predictive accuracy of models. RCS analysis revealed a nonlinear association between baseline SV and survival risk, providing a more comprehensive overview of SV in relation to survival in HCC patients treated with ICIs.

Conclusion: The baseline SV and its relative change at three months after treatment are expected to become routine imaging makers for predicting survival in HCC patients receiving ICIs, which consequently contributes to their clinical management.

Keywords: spleen volume, hepatocellular carcinoma, immune checkpoint inhibitors, survival, nomogram

Introduction

Hepatocellular carcinoma (HCC) is the most common tumor of primary liver cancer, accounting for approximately 75–85% of all cases.[1] The incidence of HCC has increased rapidly in recent years, and currently more than half of the new cases diagnosed worldwide are from China.[2] With the advancement of therapeutic technology, tumor immunotherapy has made important breakthroughs, and a variety of immune checkpoint inhibitors (ICIs) have become an important therapeutic option for patients with advanced HCC.[3] The ICIs, such as anti-PD-1, anti-PD-L1, and anti-CTLA-4, are monoclonal antibodies that help the immune system target tumors by blocking inhibitory signals between tumor and

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immune cells. Despite their effectiveness in HCC patients, there is considerable individual variability in response, with some patients experiencing immune resistance or adverse immune-related events. Thus, finding markers to predict ICI efficacy is crucial for optimizing patient treatment plans.[4] Therefore, identifying markers that are able to predict the efficacy of ICIs is essential to optimize the treatment regimen for patients.

The spleen is an important organ in the immune system and contains several subpopulations of immune cells which participate in a variety of immune responses and pathophysiologic processes.[5] Previous studies showed that patients with splenomegaly may have splenic dysfunction and imbalance of the immune microenvironment.[6] There were many previous studies that showed the importance of SV in predicting the prognosis of tumor patients undergoing curative and palliative treatments.[7–9] The SV is an important predictor of postoperative survival and risk of postoperative liver failure in patients with HCC.[8] Splenomegaly predicts survival in patients with advanced primary liver cancer treated with ICIs.[10] Furthermore, increased SV predicted overall survival (OS) and progression-free survival (PFS) in metastatic renal cancer patients receiving ICIs.[11]

Computed tomography (CT) is a routine imaging technique for HCC patients in clinical practice,[12] enabling easy measurement of SV. There is potential to fully integrate splenic volumetric measurements into routine radiologic workflow. However, few studies explored the clinical manual measurement of SV on the prognosis of HCC patients receiving ICIs. Therefore, the main objective of this study was to explore the prognostic value of manual measurement of SV at baseline and 3 months of ICIs treatment in patients with HCC, in the expectation of providing new perspectives for clinical individualized treatment.

Materials and Methods

Patients

This study retrospectively included 224 hCC patients receiving ICIs during July 2020 to December 2023 at Union Hospital of Tongji Medical College, China. Inclusion criteria comprised (1) patients diagnosed with HCC according to the European Association for the Study of the Liver (EASL) criteria;[13] (2) Child-Pugh A or B; (3) older than 18 years of age; (4) all patients receiving first-line therapy including ICIs; (5) follow up period more than 3 months, and (6) undergoing an abdominal CT examination prior to the first treatment with ICIs. Exclusion criteria were (1) combination with other primary tumors, (2) having undergone splenectomy, (3) Child-Pugh class C, and (4) incomplete clinical or imaging data. The study was approved by the Research Ethics Committee of Tongji Medical College, Huazhong University of Science and Technology (Institutional Review Board No. S188). The study followed good clinical practice and the declaration of Helsinki. The methodology of this study was in accordance with the guidelines for Strengthening the Reporting of Observational Studies in Epidemiology (STROBE).[14]

CT Imaging Strategies

All patients underwent CT scanning of the abdomen before and at three months of treatment with ICIs. The patients were required to fast for 8 hours before scanning. The scans were performed using a 128-row CT scanner (SIEMENS SOMATOM Definition AS+, Siemens Healthcare, Germany) with the following parameters: electron tube voltage of 120 kV, automatic milliamp technology for electron tube current, layer thickness and layer spacing of 1.5 mm, and matrix of 512×512. During the scanning process, the patient was placed in the supine position, and the scanning area included the upper abdomen.

SV Measurement

The gold standard definition of splenomegaly is based on spherical weight, but it is difficult to accurately assess splenic weight in clinical practice. CT and magnetic resonance imaging (MRI) provide easy and accurate methods of assessing splenic size. Two radiologists with 10 years of experience in abdominal radiology used the PACS system to measure spleen size and calculated SV using the following formula:[15]

$$SV\,(mL) = 30 + 0.58 \times (width\,(W) \times length\,(L) \times thickness\,(T))$$

The W is the maximum diameter of the spleen in any cross-section. T is the maximum distance between the inner and outer edges of the spleen in a plane perpendicular to the widest part of the spleen. L is the distance between the inner and outer edges of the spleen in a plane perpendicular to the width of the spleen and through the splenic hilum (Figure 1). The final SV result was the average of measurements by two radiologists, with consistency checked using the intraclass correlation coefficient (ICC). Discrepancies were resolved through discussion and consensus. The change in SV at three months after the first ICIs treatment was assessed by the formula, and A change in SV of >10% is considered an increase or decrease.

$$\Delta SV = \frac{(posttreatment\ SV - pretreatment\ SV)}{pretreatment\ SV}$$

Data Collection and Follow-up

Patients' electronic medical records provided baseline data on age, sex, body mass index (BMI), smoking history, drinking history, HBV infection history, Barcelona Clinical Liver Cancer (BCLC) stage, Child-Pugh classification, Eastern Cooperative Oncology Group physical status (ECOG PS), portal vein tumor thrombosis (PVTT), and laboratory tests. Based on the Modified Response Evaluation Criteria in Solid Tumors (mRECIST) guideline,[16] tumor response was classified as complete response (CR), partial response (PR), stable disease (SD), or disease progression (PD). PFS was the time from treatment initiation to disease progression which was determined by reviewing the electronic medical record, while OS was the time from treatment initiation to death or the last follow-up visit which was determined by telephone follow-up. The main indication for ICIs treatment is Child A patients, and the study also simply explored the prognosis significance of SV using different therapy regimens in this population.

Statistical Analysis

Continuous variables were summarized as mean (SD) or median (IQR) and compared using the *t*-test or Wilcoxon rank-sum test. Categorical variables were presented as counts (percentages) and compared with the chi-square test. Baseline SV was divided using the median. PFS and OS differences between groups were assessed with Kaplan-Meier curves and Log rank tests. Variables with $P < 0.1$ in univariate analysis were included in the multivariate Cox regression model. Nomograms for 6, 9, and 12-month PFS and OS were developed using multivariate Cox regression analysis. Their accuracy was assessed with the concordance index (C-index) and time-dependent ROC curves. Calibration curves compared predicted and observed survival probabilities. A forest plot showed PFS and OS hazard ratios by subgroup. Restricted cubic spline (RCS) analyzed the link between baseline SV and survival. All tests were two-tailed with significance at $P<0.05$. All analyses in this study were performed using the SPSS software (version 25.0, SPSS Inc., Chicago, IL, USA) and R version 4.3.0 (R Foundation).

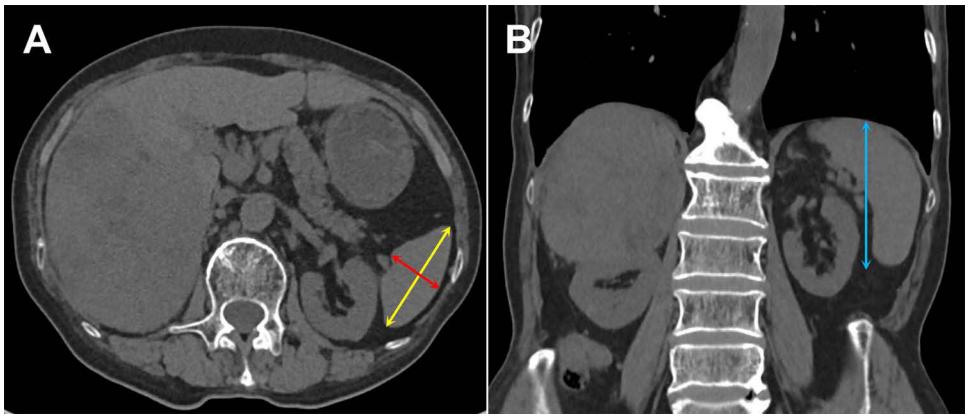

Figure 1 An 84- year-old man with hepatocellular carcinoma receiving ICIs. The (**A**) shows the width (yellow) and thickness (red) of the spleen, and the (**B**) shows the length (blue).

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Results

Baseline Characteristics of Patients

The study ultimately included 224 hCC patients treated with ICIs (including 112 Lower SV and 112 Higher SV). Table 1 demonstrates that there were no significant differences in either their baseline demographic or clinical characteristics (all P>0.05). Of the HBV (-) patients in this study, 24 were infected with HCV, 32 with nonalcoholic steatohepatitis/ nonalcoholic fatty liver disease, 22 with alcoholic liver disease, 20 with other diagnosed etiologies (eg, diabetes mellitus or autoimmune hepatitis), 7 were exposed to aflatoxins, and 30 had no known etiologies. The specific treatment regimens were ICIs combined with tyrosine kinase inhibitors (TKIs) in 137 patients and ICIs combined with bevacizumab in 87

Table 1 Baseline Characteristics of Patients

Characteristic	Total (n=224)	Lower SV (n=112)	Higher SV (n=112)	*P* value
Age (years)				0.169
<60	138 (61.6%)	64 (57.1%)	74 (66.1%)	
≥60	86 (38.4%)	48 (42.9%)	38 (33.9%)	
Sex				0.611
Female	43 (19.2%)	20 (17.9%)	23 (20.5%)	
Male	181 (80.8%)	92 (82.1%)	89 (79.5%)	
BMI (kg/m^2)	23.1 (20.8, 25.4)	23.2 (20.5, 26.1)	23.1 (20.9, 24.9)	0.842
HBV infection				0.339
No	135 (60.3%)	71 (63.4%)	64 (57.1%)	
Yes	89 (39.7%)	41 (36.6%)	48 (42.9%)	
Tumor diameter (cm)	7.4 (4.4, 10.7)	6.7 (3.8, 10.2)	8.35 (5, 10.8)	0.111
Tumor number				0.863
≤3	183 (81.7%)	92 (82.1%)	91 (81.2%)	
>3	41 (18.3%)	20 (17.9%)	21 (18.8%)	
PVTT				0.092
No	167 (74.6%)	89 (79.5%)	78 (69.6%)	
Yes	57 (25.4%)	23 (20.5%)	34 (30.4%)	
Lymph node metastasis				0.067
No	179 (79.9%)	95 (84.8%)	84 (75%)	
Yes	45 (20.1%)	17 (15.2%)	28 (25%)	
BCLC stage,				0.228
B	105 (46.9%)	57 (50.9%)	48 (42.9%)	
C	119 (53.1%)	55 (49.1%)	64 (57.1%)	
Child-Pugh				0.837
A	197 (87.9%)	98 (87.5%)	99 (88.4%)	
B	27 (12.1%)	14 (12.5%)	13 (11.6%)	

(*Continued*)

Table 1 (Continued).

Characteristic	Total (n=224)	Lower SV (n=112)	Higher SV (n=112)	*P* value
ECOG PS				0.633
0	173 (77.2%)	88 (78.6%)	85 (75.9%)	
≥1	51 (22.8%)	24 (21.4%)	27 (24.1%)	
Smoking				0.123
No	168 (75%)	89 (79.5%)	79 (70.5%)	
Yes	56 (25%)	23 (20.5%)	33 (29.5%)	
Drinking				0.847
No	193 (86.2%)	96 (85.7%)	97 (86.6%)	
Yes	31 (13.8%)	16 (14.3%)	15 (13.4%)	
Types of ICIs				
PD-1	208 (92.9%)	106 (94.6%)	102 (91.1%)	0.299
PD-L1	16 (7.1%)	6 (5.4%)	10 (8.9%)	
NLR	2.4 (1.7, 3.3)	2.3 (1.6, 3.2)	2.4 (1.8, 3.5)	0.187
PLR	117.9 (90.2, 161.7)	118.2 (89.8, 164.5)	117.9 (91.8, 160.5)	0.984
ALT (U/L	34 (22, 59.3)	35.5 (21.8, 62)	33 (22, 53.5)	0.611
AST (U/L)	43 (29, 73.3)	44 (29.8, 76.5)	42 (28.8, 67.3)	0.275
Hemoglobin (g/L)	129 (116, 141)	129 (114.8, 140.3)	129 (118.8, 142.3)	0.727
PT (s)	13.7 (13.1, 14.5)	13.6 (13.2, 14.5)	13.7 (13.1, 14.5)	0.787
Creatinine (μmol/L)	65.7 (56.5, 74.3)	65.4 (56.5, 73.9)	67 (56.5, 74.5)	0.577
Urea nitrogen (mmol/L)	4.6 (3.5, 5.8)	4.5 (3.4, 5.7)	4.8 (3.5, 5.9)	0.278
ALBI score	−2.3 (0.5)	−2.3 (0.5)	−2.3 (0.5)	0.187
AFP level (ng/mL)				1.000
<400	146 (65.2%)	73 (65.2%)	73 (65.2%)	
≥400	78 (34.8%)	39 (34.8%)	39 (34.8%)	

Abbreviations: SV, Spleen volume; BMI, Body mass index; HBV, hepatitis B virus; PVTT, portal vein tumor thrombosis; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Physical Status; ICIs, immune checkpoint inhibitors; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; ALT, Alanine transaminase, AST, Aspartate transaminase; PT, prothrombin time, ALBI, Albumin-Bilirubin, AFP, alpha-fetoprotein.

patients. Of the Child A patients, 120 received ICIs in combination with TKIs, 77 underwent ICIs combined with bevacizumab. In addition, Table S1 showed no significant differences in clinical and laboratory indicators between 150 Non-SV increased and 74 SV increased patients after 3 months of ICIs treatment.

Baseline SV Threshold and Changes in SV

The baseline SV (ICC: 0.98, 95% CI, 0.96–0.99) and SV at three months posttreatment (ICC: 0.96, 95% CI, 0.95–0.97) showed excellent interobserver agreement. The median cutoff value for SV in this study was 245.85 mL, which ultimately divided 112 patients in the Lower SV group and 112 patients in the Higher SV group. The median SV change rate was 3.31% at three months post-ICI treatment. An SV change of ≥10% indicated an increase or decrease,

with 74 patients (33.0%) showing increased SV, 21 patients (9.4%) showing decreased SV, and 129 patients (57.6%) showing no significant change. Patients with no significant SV change and decreased SV were categorized as the Non-SV increased group.

Tumor Response

The Table S2 showed the tumor response in the Lower SV group and the Higher SV. The ORR (15.2% vs 42.9%, P< 0.001) and DCR (58% vs 89.3%, P< 0.001) were lower in the Higher SV group compared with the Lower SV group. In addition, there was no significant difference in ORR and DCR between Non-SV increased group and SV increased group (Table S3). Meanwhile, Figure 2A showed the waterfall plot of percent change of SV in HCC patients.

Survival Analysis

A total of 129 patients had died by the time of analysis, the median PFS of 6.9 months (95% CI [6.1–8.7]) and a median survival time of 14 months (95% CI [12.8–16.8]) in all patients. The KM curves showed shorter PFS (4.5 vs 9.9 months, P<0.001, Figure 3A) and OS (12.6 vs 17 months, P=0.010, Figure 3B) in the Higher SV group compared to the Lower SV group. The KM curves also indicated shorter PFS (4.6 vs 8.5 months, P<0.001, Figure 3C) and OS (11.9 vs 16 months, P<0.001, Figure 3D) in the SV increased group compared to the Non-SV increased group after three months of ICIs treatment. Meanwhile, Figure 2B demonstrated the heatmap of the relationship between SV and clinical characteristics with OS for each patient.

To provide additional clinical insights, additional exploration was performed in the Child A patients. Patients were divided into SV increased and SV decreased groups using 0% SV change as a cut point. In Child A patients, PFS and OS were shorter in the Higher SV and SV increased groups than in the Lower SV and Non-SV increased/SV decreased groups using >10% or 0% SV change as cut points (all P values <0.05, Figure S1). Figures S2 and S3 showed that using different treatment regimens in the Child A patients. Higher SV and SV increased groups demonstrated shorter PFS compared to the Lower SV (ICIs combined with TKIs, 6.2 vs 8.9 months, P=0.008, ICIs combined with bevacizumab, 2 vs.10.2 months, P<0.001) and Non-SV increased (ICIs combined with TKIs, 5.1 vs 8.7 months, P=0.024, ICIs combined with bevacizumab, 3.3 vs 8.3 months, P=0.004) groups, respectively. Higher SV and SV increased groups demonstrated shorter OS compared to the Lower SV (ICIs combined with TKIs, 14 vs 17.8 months, P=0.023, ICIs combined with bevacizumab, 11.9 vs.15.6 months, P=0.015) and Non-SV increased (ICIs combined with TKIs, 12.9 vs 17.4 months, P=0.002, ICIs combined with bevacizumab, 9.7 vs 15.6 months, P=0.005) groups, respectively. Meanwhile, in Child A patients with different treatment regimens using 0% SV change as cut point, it was found that PFS (ICIs combined with TKIs, 6.4 vs 11 months, P=0.008, ICIs combined with bevacizumab, 4.8 vs 10.2 months, P=0.009) and OS (ICIs combined with TKIs, 14.6 vs 17.6 months, P=0.012, ICIs combined with bevacizumab, 10.6 vs 15.6 months, P=0.016) were shorter in the SV increased group than in the SV decreased group, respectively.

Univariate and Multivariate Analyses

Univariate analysis showed that age, PVTT, Lymph node metastasis, BCLC stage, smoking, AFP level, baseline SV group and change of SV group were potential predictors of PFS in HCC patients treated with ICIs (Table S4). PVTT, BCLC stage, AFP level, baseline SV group and change of SV group were potential predictors of their OS (Table 2). Multivariate analysis showed that BCLC-C stage, AFP level ≥400ng/mL were independent risk factors for PFS and OS. Both higher SV group and increased SV group correlated with shorter PFS (HR: 2.45,95% CI: 1.77–3.38, P< 0.001; HR: 2.04, 95% CI: 1.45–2.86, P< 0.001) and OS (HR: 1.94,95% CI: 1.34–2.80, P< 0.001; HR: 2.34, 95% CI: 1.61–3.40, P< 0.001). In Child A patients with 0% SV change as a cut point, multivariate analysis also showed that both higher SV group and SV increased group correlated with shorter PFS (HR: 2.10,95% CI: 1.49–2.96, P< 0.001; HR: 1.97, 95% CI: 1.34–2.89, P< 0.001) and OS (HR: 1.60,95% CI: 1.08–2.38, P= 0.020; HR: 2.55, 95% CI: 1.59–4.08, P< 0.001) (Tables S5 and S6).

Nomograms and Accuracy Evaluation

Independent prognostic factors identified via multivariate analysis were incorporated into nomograms of PFS (Figure 4A) and OS (Figure 4B). The C-indexes of the nomograms for PFS and OS prediction were 0.700 (0.678–0.721) and OS

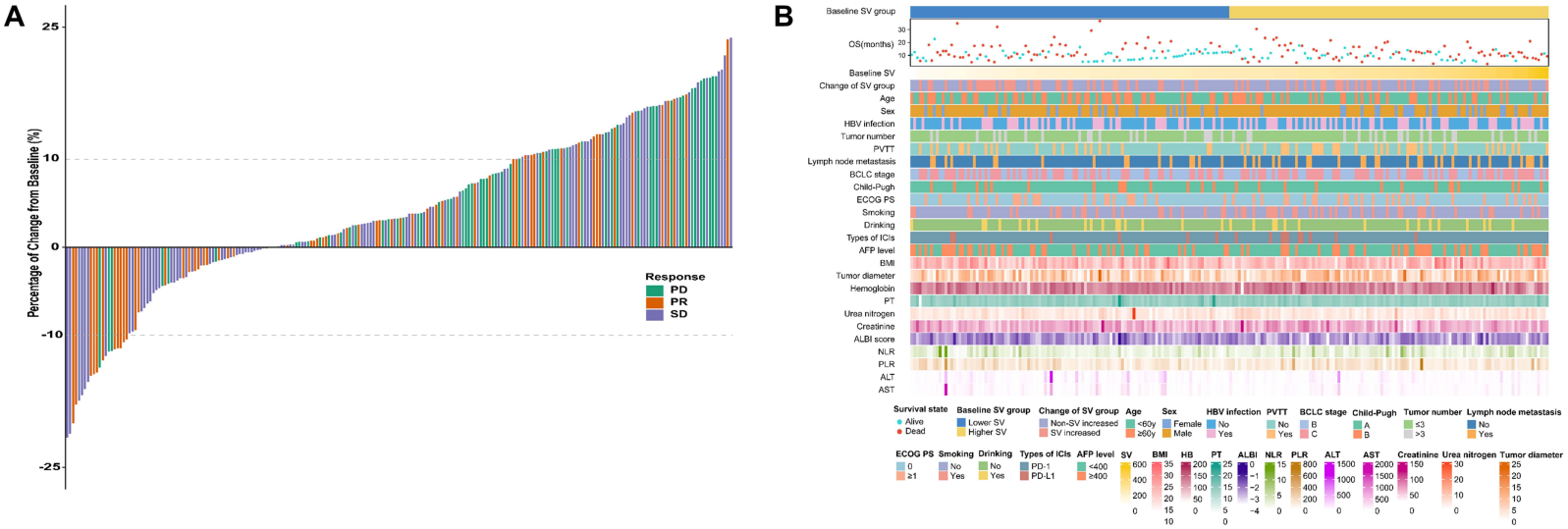

Figure 2 Waterfall plot (**A**) of percent change of spleen volume and heatmap (**B**) of the relationship between SV and clinical characteristics with OS for each patient.

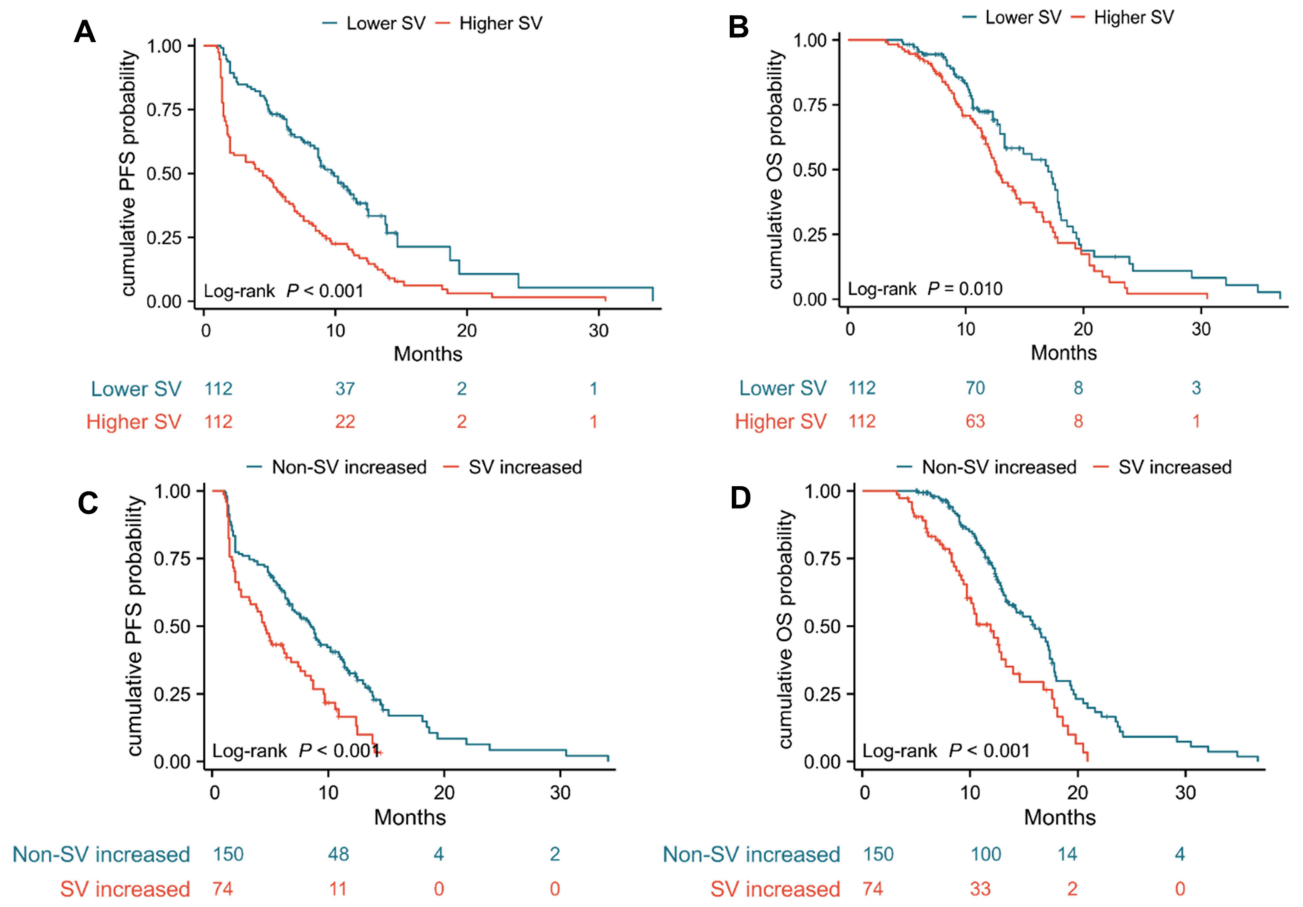

Figure 3 Kaplan-Meier curves of baseline SV and relative change in SV at three months after treatment. The KM curves of PFS (**A**) and OS (**B**) for Lower SV group (blue) and Higher SV group (red). The KM curves of PFS (**C**) and OS (**D**) for Non-SV increased group (blue) and SV increased group (red).

0.733(0.709–0.757), respectively. The performance of nomogram for clinical prediction was evaluated using the area under the time-dependent receiver operating characteristic (ROC) curve (AUC). The AUCs were 0.760 (95% CI: 0.696–0.825), 0.747 (95% CI: 0.675–0.818), and 0.698 (95% CI: 0.601–0.794) at 6, 9 and 12 months of PFS (Figure 4C), respectively. The AUCs were 0.870 (95% CI: 0.780–0.960), 0.830 (95% CI: 0.762–0.899), and 0.741

Table 2 Univariate and Multivariate Cox Proportional Hazards Analyses of OS

Characteristic	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	*P* value	Hazard Ratio (95% CI)	*P* value
Age (years)				
<60	Reference			
≥60	0.898 (0.619–1.304)	0.573		
Sex				
Female	Reference			
Male	1.390 (0.869–2.225)	0.170		
BMI (kg/m^2)	1.028 (0.974–1.085)	0.321		
HBV infection				
No	Reference			
Yes	0.885 (0.619–1.267)	0.505		

(*Continued*)

Table 2 (Continued).

Characteristic	Univariate Analysis		Multivariate Analysis	
	Hazard Ratio (95% CI)	***P* value**	**Hazard Ratio (95% CI)**	***P* value**
Tumor diameter (cm)	1.008 (0.971–1.047)	0.668		
Tumor number				
≤3	Reference			
>3	0.723 (0.459–1.138)	0.161		
PVTT				
No	Reference		Reference	
Yes	1.555 (1.086–2.227)	**0.016**	0.910 (0.598–1.384)	0.660
Lymph node metastasis				
No	Reference			
Yes	1.122 (0.746–1.686)	0.581		
BCLC stage				
B	Reference		Reference	
C	1.988 (1.350–2.927)	**< 0.001**	1.882 (1.208–2.932)	**0.005**
Child-Pugh				
A	Reference			
B	1.338 (0.775–2.310)	0.296		
ECOG PS				
0	Reference			
≥1	1.164 (0.754–1.795)	0.493		
Smoking				
No	Reference			
Yes	1.368 (0.929–2.015)	0.113		
Drinking				
No	Reference			
Yes	0.834 (0.510–1.361)	0.467		
NLR	1.058 (0.968–1.155)	0.213		
PLR	1.001 (0.999–1.003)	0.249		
ALT(U/L)	1.001 (1.000–1.002)	0.217		
AST(U/L)	1.001 (1.000–1.001)	0.193		
Hemoglobin (g/L)	0.996 (0.988–1.005)	0.383		
PT (s)	1.041 (0.906–1.196)	0.568		
Urea nitrogen (mmol/L)	0.938 (0.846–1.040)	0.224		
Creatinine (μmol/L)	1.001 (0.988–1.015)	0.885		
AFP level (ng/mL)				
<400	Reference		Reference	
≥400	2.407 (1.687–3.435)	**< 0.001**	2.395 (1.641–3.496)	**< 0.001**
ALBI score				
Baseline SV group				
Lower SV	Reference		Reference	
Higher SV	1.588 (1.112–2.267)	**0.011**	1.937 (1.339–2.800)	**< 0.001**
Change of SV group				
Non-SV increased	Reference		Reference	
SV increased	2.200 (1.518–3.188)	**< 0.001**	2.339 (1.612–3.395)	**< 0.001**

Abbreviations: SV, Spleen volume; OS, overall survival; BMI, Body mass index; HBV, hepatitis B virus; PVTT, portal vein tumor thrombosis; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Physical Status; ICIs, immune checkpoint inhibitors; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; ALT, Alanine transaminase, AST, Aspartate transaminase; PT, prothrombin time, ALBI, Albumin-Bilirubin, AFP, alpha-fetoprotein; CI, confidence interval.

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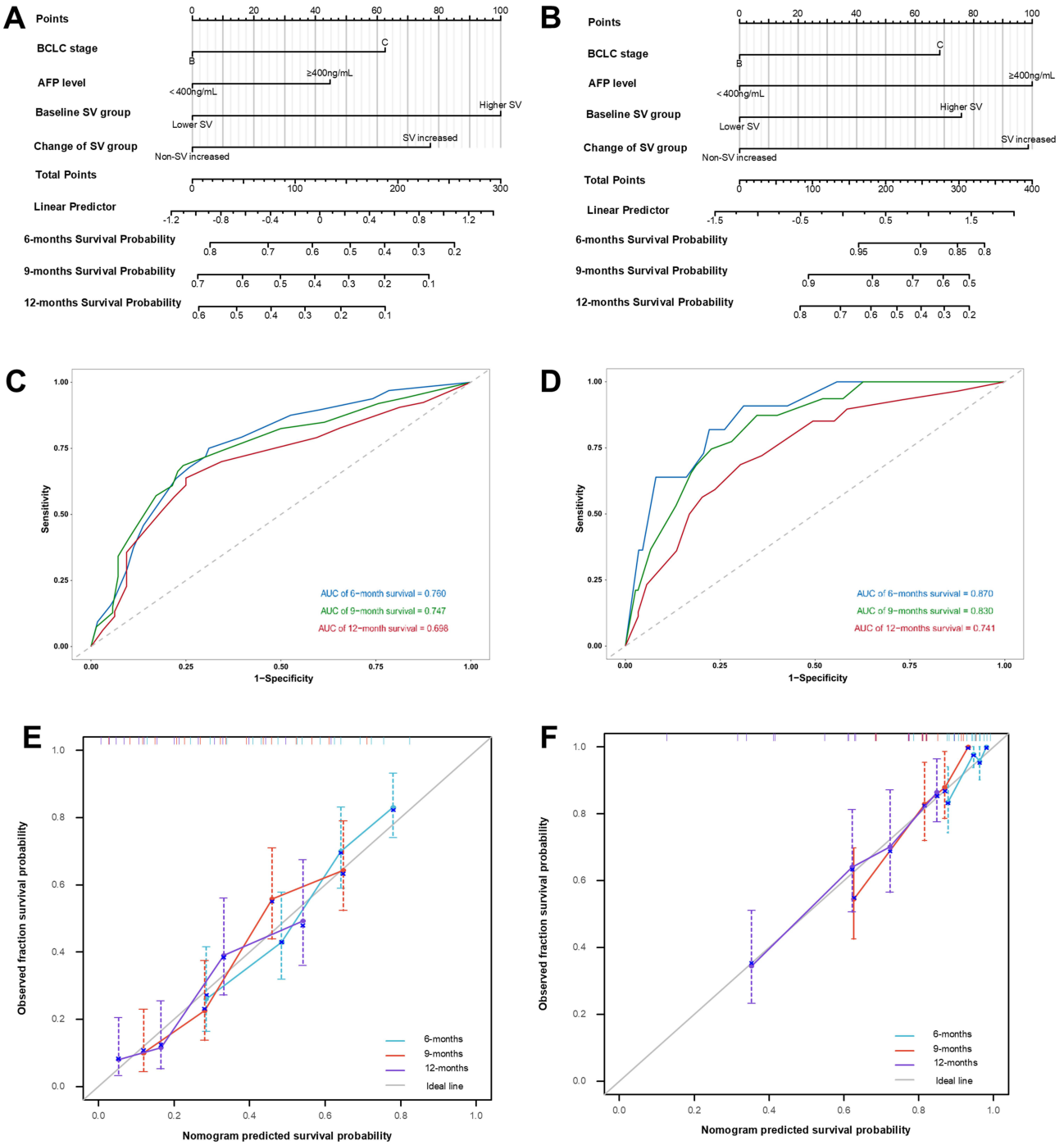

Figure 4 Nomograms predicting PFS (**A**) and OS (**B**) at 6, 9, and 12 months. Time-dependent ROC curves to assess the discrimination for PFS (**C**) and OS (**D**) of nomograms. Calibration curves for PFS (**E**) and OS (**F**) of nomograms.

(95% CI: 0.661–0.821) at 6, 9 and 12 months of OS (Figure 4D), respectively. The calibration plots of the prognostic nomograms in predicting 6, 9 and 12 months PFS (Figure 4E) and OS (Figure 4F) demonstrated good coincidences between the estimated risk and observed risk.

Subgroup Analysis

Subgroup analyses based on baseline characteristics of 224 patients before and after treatment, respectively, showed relatively consistent results for PFS and OS. Except for with HBV infection, with PVTT, and the PD-L1-usage

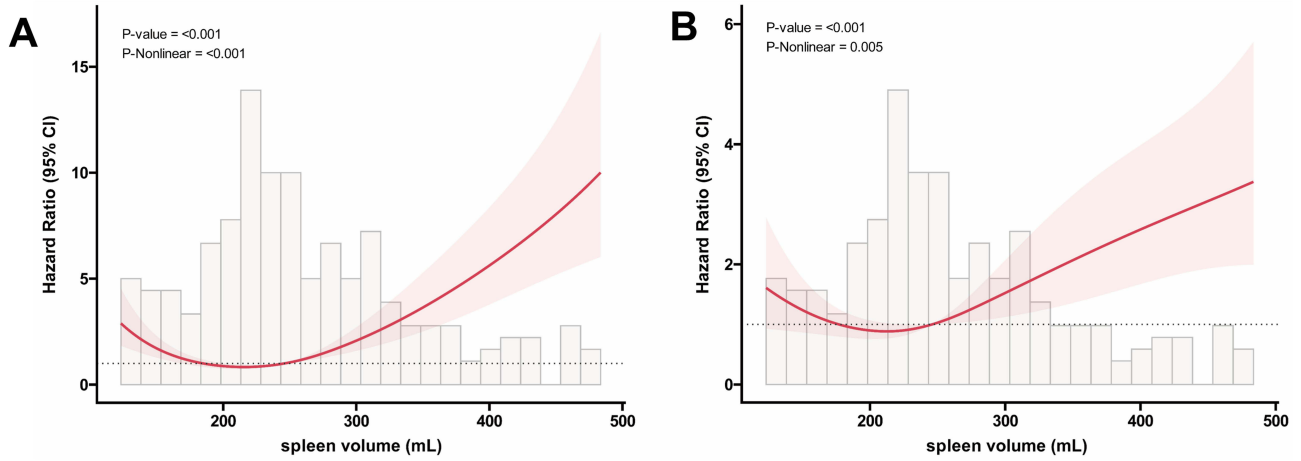

Figure 5 Association of baseline spleen volume with PFS (**A**) and OS (**B**) in patients with hepatocellular carcinoma treated with immune checkpoint inhibitors visualized by restricted cubic spline analysis. Hazard ratios were adjusted for age and sex.

subgroups, the risk of short PFS was higher in the Higher SV than in the Lower SV (Figure S4). In the subgroup with smoking, there was a trend toward a higher risk of short PFS in patients with Higher SV compared to Lower SV. Subgroup analysis of OS demonstrated that Higher SV was correlated with shorter OS in groups of age <60 years, females, without HBV infection, tumor number <3, with PVTT, with lymph node metastasis, BCLC-C stage, Child-Pugh A, ECOG PS of 0, no smoking, no drinking, PD-1useage, AFP ≥400ng/mL (Figure S5). In addition, the SV increased group showed a significantly higher risk of shorter PFS compared to the Non-SV increased group in most subgroups, with the exception of with HBV infection, with lymph node metastasis, Child-Pugh B, ECOG PS of ≥1, with drinking and PD-L1 usage (Figure S6). Except for the tumor number >3, Child-Pugh B, ECOG PS of ≥1, with drinking and PD-L1 usage groups, the risk of short OS was higher in the SV increased than in the Non-SV increased (Figure S7).

RCS Analysis

The RCS analysis showed a significant nonlinear relationship between baseline SV and risk of PFS (P for nonlinear <0.001, Figure 5A) and OS (P for nonlinear=0.005, Figure 5B) in HCC patients receiving ICIs. We could also observe that patients had an increased risk of survival when the baseline SV was 245.85mL.

Discussion

This study revealed that SV affected the survival of HCC patients treated with ICIs. The Higher SV and SV increased groups had shorter OS than the Lower SV and Non-SV increased groups, respectively. These results were consistent with different regimens in the Child A. And the nomograms developed on the independent prognostic factors identified by multivariate regression well predicted the survival of patients at 6, 9, and 12 months. There was a significant nonlinear relationship between baseline SV and survival of HCC patients treated with ICIs. Therefore, baseline SV and its relative changes were expected to be imaging markers for the prognosis of HCC patients treated with ICIs, which could guide the clinicians to make prompt adjustments for their treatment regimens.

To the best of our knowledge, this was the first study to explore the prognostic impact of manually measured baseline SV and SV changes at 3 months after initial ICIs treatment in HCC patients, integrating these parameters into nomograms. The spleen is rich in various immune cell subpopulations, with fluctuation of its volume reflecting change in the number of immune cells in the body.[6,17] Previous studies showed that baseline SV was a relevant prognostic factor for various treatment modalities in HCC patients,[7,8,18] and our study confirmed the above view. We found that baseline Higher SV group was associated with shorter PFS and OS. Although mildly enlarged spleen harbors more immune cells, especially T cells and natural killer (NK) cells, potentially enhancing anti-tumor responses during the early phases of ICIs treatment,[5] chronically enlarged spleen probably reflects chronic inflammatory and immune dysregulation. This may involve an increased presence of immunosuppressive cells within the tumor microenvironment, such as myeloid-derived

suppressor cells (MDSCs) and regulatory T (Treg) cells, which inhibit anti-tumor immunity.[19] The splenomegaly is usually associated with a pathological state of portal hypertension.[20] This pathologic state may lead to further deterioration of liver function, thereby affecting patient survival. In intermediate to advanced HCC patients, such as those in this study, higher SV may imply an increased systemic inflammatory response with predominant immunosuppression. Furthermore, while no statistically significant differences in tumor burden, PVTT incidence, or lymph node metastasis were observed between the higher and lower SV groups in this study, the high SV group exhibited trends toward greater tumor burden, higher PVTT incidence, and increased lymph node metastasis. These factors may contribute to heightened treatment resistance, ultimately resulting in shorter PFS and OS. These findings suggest that higher SV in intermediate and advanced HCC may represent a multifaceted pathology involving systemic inflammation, immunosuppression, and tumor progression, which collectively explain the possible association between splenomegaly and poorer OS and PFS.

At three months after ICIs treatment, the median rate of SV change for all patients was 3.31%. The median rate of SV change was 14% and 0.3% in the SV increased and Non-SV increased groups, respectively. The present study also found SV increased group at three months after ICIs treatment had significantly shorter PFS (4.6 vs 8.5months, P<0.001) and OS (11.9 vs 16 months, P<0.001) than Non-SV increased group, suggesting further that dynamic variations of SV possibly reflect the body's response to treatment. A study showed that in metastatic renal cancer patients receiving immunotherapy, SV change >10% group had short PFS and OS.[11] Müller et al[18] revealed that SV increase after immunotherapy was not associated with survival outcome in HCC patients. Whereas Chen et al[21] found that HCC patients in the immunotherapy group with increased SV were more likely to have longer PFS than those with decreased SV, the results were reversed for patients in the sorafenib group. These studies highlighted the complex and potentially variable role of SV changes in predicting the efficacy of cancer treatments. Previous studies used ICIs in combination with bevacizumab as the immunotherapy group. Therefore, we briefly explored the prognostic impact of SV in HCC patients of Child A using ICIs in combination with bevacizumab and SV change (0%). The results still found that PFS and OS were shorter in the SV increased group than in the SV decreased group. This may be attributed to larger baseline tumors in the SV increased group than in the SV decreased group in this treatment group (7.88 vs 5.18 cm, P=0.004). The larger tumor load not only implied more tumor cells need to be removed, but may also accompanied by more complex tumor microenvironment,[22] making the effectiveness of ICIs treatment unstable and thereby affecting the survival of patient. In addition, it is well known that NLR is one of the peripheral indicators of chronic inflammation.[23] The NLR was higher in the SV increased group than the decreased group in this treatment group of our study, possibly representing a higher level of chronic inflammation. In chronic inflammation, MDSCs increase with the effect of some cytokines. The elevated MDSCs inhibit the cellular activity of T cells and NK cells and promote tumor progression, leading to ICI resistance. In addition, MDSCs stimulate Treg cells production and increase the release of immunosuppressive cytokines, such as interleukin-10 (IL-10), which specifically inhibit CD4+ and CD8+ T cell activity and promote tumor growth.[24]

This study also found that certain indicators significantly affect the survival of HCC patients treated with ICIs. Specifically, the BCLC-C stage correlated with shorter PFS and OS. The BCLC stage system is an important tool for evaluating the prognosis of HCC patients and guiding treatment.[25] The higher BCLC stage, the higher tumor burden and more aggressive tumors, which in turn will affect survival. Additionally, AFP levels ≥400 ng/mL are strongly associated with shorter PFS and OS, as elevated AFP suggests a larger tumor burden and higher aggressiveness, reducing the effectiveness of ICIs.[26] In this study, we developed nomogram models with independent prognostic indicators in multivariate regression to predict PFS and OS in HCC patients treated with ICIs. The models demonstrated good discriminatory performance with C-indexes and AUCs of ≥ 0.7 for predicting 6, 9, and 12-month PFS and OS. Calibration plots confirmed good predictive accuracy. The nomogram combined the pretreatment BCLC stage, AFP level, baseline SV, and SV change at three months posttreatment, showing significant promise for clinical use in prognosis prediction. Additionally, RCS analysis highlighted a nonlinear relationship between baseline SV and survival, which contributed to a more comprehensive understanding of their association.

This study is limited by several reasons. First, this is a retrospective study possibly with selection bias, so in the future we need to conduct a large-scale multicenter prospective study employing varied SV change cutoff values and diverse ICIs treatment regimens to further validate the results of this study. But in order to eliminate the bias caused by patient heterogeneity, we ensured a stable baseline between groups and performed multivariate Cox regression. Second, although many previous studies used this formula to calculate SV, the accuracy of their measured SV still needs to be considered,

especially when compared to automatic or semi-automatic outline methods. Finally, this SV formula was derived from CT scans, but Yang et al[27] also applied it to pancreatic cancer patients who underwent MRI. Therefore, we could further verify the present results by combining CT and MR scans in the future.

Conclusion

The baseline SV and its relative change at three months after treatment were associated with the prognosis of HCC patients treated with ICIs. They are expected to become routine imaging markers for patients to assist in their clinical management.

Abbreviations

SV, Spleen volume; BMI, Body mass index; HBV, hepatitis B virus; PVTT, portal vein tumor thrombosis; BCLC, Barcelona Clinic Liver Cancer; ECOG PS, Eastern Cooperative Oncology Group Physical Status; ICIs, immune checkpoint inhibitors; NLR, Neutrophil to lymphocyte ratio; PLR, Platelet to lymphocyte ratio; ALT, Alanine transaminase, AST, Aspartate transaminase; PT, prothrombin time, ALBI, Albumin-Bilirubin, AFP, alpha-fetoprotein; PFS, progression-free survival; OS, overall survival; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; ORR, objective response rate; DCR, disease control rate; C-index, concordance index; RCS, Restricted cubic spline; receiver operating characteristic, ROC; ICC, intraclass correlation coefficient; CI, confidence interval.

Data Sharing Statement

The datasets used and/or analyzed in this study are available from the corresponding author on reasonable request.

Ethics Approval and Informed Consent

Approval for this study was obtained from the Ethics Committee of Union Hospital, Tongji Medical College, Huazhong University of Science and Technology (Institutional Review Board No. S188). Given its retrospective and anonymous nature, the institutional review board waived the requirement for informed consent.

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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 declare that there is no conflict of interest.

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