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ORIGINAL RESEARCH

Changes in Posttreatment Spleen Volume Associated with Immunotherapy Outcomes for Advanced Hepatocellular Carcinoma

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Purpose: We investigated whether spleen volume (SV) changes were associated with treatment outcomes in advanced hepatocellular carcinoma (HCC) patients who received immunotherapy or first-line sorafenib.

Patients and Methods: Patients with advanced HCC who underwent immunotherapy or first-line sorafenib at our institute were retrospectively analyzed. CT was used to measure SV before and within 3 months of treatment initiation. Tumor assessment followed Response Evaluation Criteria in Solid Tumors version 1.1. The association between SV change and tumor response or progression-free survival (PFS) was analyzed. The inverse probability of treatment weighting (IPTW) was used to adjust for differences in baseline characteristics.

Results: The immunotherapy group comprised 143 patients (124 men, mean age, 59.8 years ± 11.2 [standard deviation]), while the sorafenib group had 57 (47 men, mean age, 59.6 years ± 9.9). SV increased in 108 (75.5%) immunotherapy and 21 (36.8%) sorafenib patients. In the immunotherapy group, patients with increased SV were more likely than those with decreased SV to have a higher disease control rate (76.9% vs 57.1%, $p = 0.024$) and durable clinical benefit (52.8% vs 25.7%, $p = 0.005$). It was also associated with extended PFS in the immunotherapy group in both the univariate ($p = 0.028$) and multivariate ($p = 0.014$) analysis. By contrast, in the sorafenib group, an increased in SV was not associated with treatment response but was presumably associated with reduced PFS ($p = 0.072$) in the multivariate analysis. After IPTW adjustment, the increase in SV remained a significant predictor for DCB and PFS in the immunotherapy group.

Conclusion: Most patients exhibited an increase in SV after the initiation of immunotherapy, which may be used to predict response and prognosis. However, this association was not observed in patients who received sorafenib.

Plain Language Summary: The study provides significant evidence that an increase in spleen volume is associated with better treatment outcomes in advanced hepatocellular carcinoma patients undergoing immunotherapy. These findings offer oncologists a new potential biomarker for optimizing treatment strategies. Specifically, increased spleen volume could be used to predict higher rates of disease control and durable clinical benefits, allowing for more personalized care.

Keywords: hepatocellular carcinoma, immunotherapy, sorafenib, response, survival

Introduction

Hepatocellular carcinoma (HCC) remains a substantial global health concern because of its increasing incidence and limited therapeutic options, especially in its advanced stages.[1] Sorafenib is a potent multikinase inhibitor that was first

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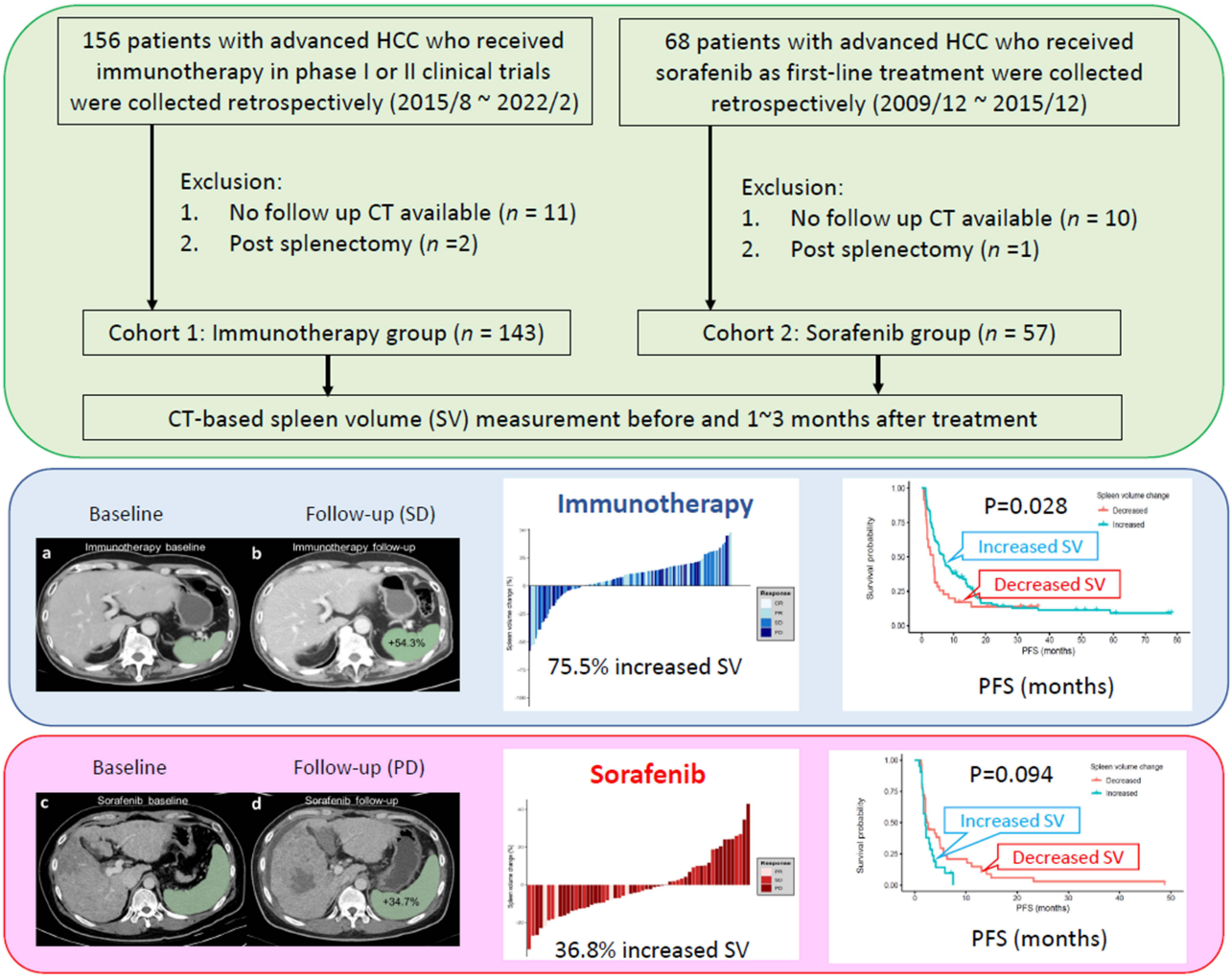
Graphical Abstract



introduced as a standard first-line treatment in two Phase III clinical trials.[2,3] Recently, immune checkpoint inhibitors (ICIs) have revolutionized the treatment methods for HCC. Compared with standard systemic therapy, combination therapy involving ICIs has demonstrated improved survival rates in patients with advanced HCC.[4–8] However, not all patients benefit from ICIs.[9] Therefore, reliable markers are urgently required to facilitate treatment selection and monitor therapeutic response.[10,11]

Many biomarkers that are potentially useful in predicting the treatment efficacy of ICIs in other types of cancer do not exhibit a similar efficacy in advanced HCC. For example, in patients with advanced HCC, tumor staining for programmed death ligand 1 (PD-L1) exhibits only a borderline or marginal association with tumor response to nivolumab treatment.[12] Tumors with a mismatch repair deficiency respond well to ICIs,[13] but this deficiency is rare in HCC.[14] Although early α-fetoprotein (AFP) response may assist in predicting treatment response,[15] its usefulness is limited to patients with baseline abnormal AFP levels.

The spleen is an organ that is intricately involved in immunomodulation and intriguingly associated with systemic inflammation and cancer progression. In general, immunotherapy exerts a systemic effect on multiple organs, including the spleen, which plays a vital role in hematopoiesis and immune response.[16–20] For instance, Susok et al[18] reported an increase in SV in patients treated with ICIs for melanoma. By contrast, Castagnoli et al[20] negated the predictive value of

SV in treatment response among patients who underwent immunotherapy for non–small-cell lung cancer. Muller et al[21] noted that an increase in SV before and throughout immunotherapy served as a major predictive factor of poor overall survival (OS) in patients with advanced HCC. However, few studies have compared the role of SV changes in patients undergoing treatment with ICIs or sorafenib for advanced HCC. To understand the effects of systemic therapy on patients with HCC, an in-depth exploration of the effect of systemic therapy on splenic dynamics is required.

SV changes may reflect shifts in splenic immune cells, and these shifts may influence the response of patients undergoing immunotherapy for HCC. The aim of this study is to investigate whether changes in SV during treatment would serve as a valuable prognostic marker. We examined the predictive value of SV changes in patients who underwent immunotherapy for advanced HCC. For comparison purposes, we included a group of patients who received sorafenib as a first-line treatment for advanced HCC.

Material and Methods

Study Population: Immunotherapy

This study was approved by the institutional review board. Because of the study's retrospective design, informed consent was not required.

Patients with advanced HCC who underwent immunotherapy in clinical trials between August 2015 and February 2022 at our institution were retrospectively analyzed (Figure 1). The immunotherapy regimens included anti-programmed cell death protein 1 (anti-PD-1), anti-PD-L1, and anticytotoxic T-lymphocyte-associated protein 4 (anti-CTLA-4) antibodies. ICIs combined with antiangiogenic targeted therapies and other agents currently under investigation were also included.

Comprehensive patient data, namely demographic information (age, sex), hepatitis etiology, liver function reserve (Child–Pugh class and albumin-bilirubin [ALBI] grade), tumor stage, Barcelona Clinic Liver Cancer (BCLC) stage, AFP level, tumor involvement extent, and number of previous systemic therapies, were retrieved from medical records.

The inclusion criteria were histologically confirmed or clinically diagnosed HCC, a baseline computed tomography (CT) scan performed within 4 weeks of treatment initiation, and at least one measurable lesion in accordance with Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1. Those with previous exposure to immunotherapy and the absence of pretreatment CT scans were excluded.

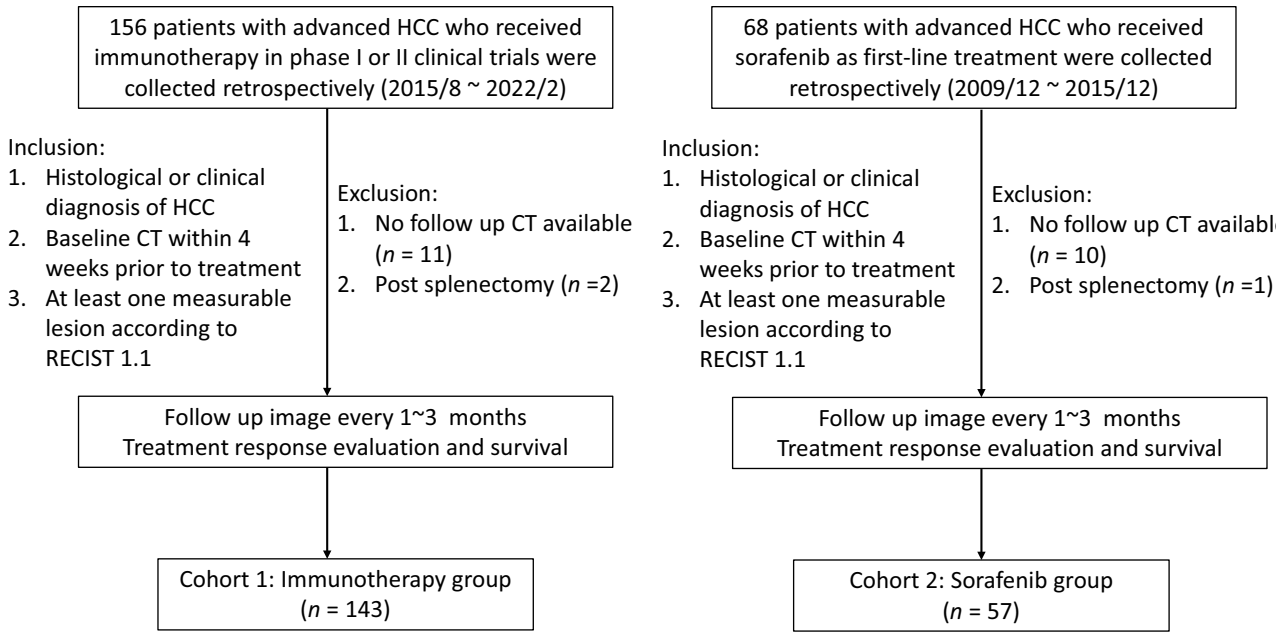


Figure 1 Study flowchart.

Follow-up CT scans were conducted every 1 to 3 months after the initiation of treatment. Tumor assessment was conducted in accordance with RECIST version 1.1. The disease control rate (DCR, including complete response, partial response, and stable disease) and objective response rate (ORR, including complete and partial response) were determined on the basis of optimal radiological response observed following immunotherapy. A durable clinical benefit (DCB) was defined as complete response, partial response, or stable disease lasting over 6 months.[11,22] Patient follow-up continued until December 31, 2022.

Study Population: Sorafenib

For patients who received sorafenib as a first-line treatment, a prospectively enrolled patient cohort was used (Figure 1).[23] This prospective study was approved by the institutional review board. Written informed consent was obtained from each patient before their inclusion in the study.

All relevant clinicopathological variables were prospectively collected from each patient's medical records.

Image Data Acquisition and Analysis

To evaluate treatment response, four-phase dynamic contrast-enhanced CT scans were conducted using 16- or 64-channel CT scanners. All scans were conducted in an axial plane with a tube voltage ranging from 100 to 130 kV and a slice thickness of 5 mm. SV was manually delineated using 3D Slicer software (version 4.10) on a venous-phase CT scan by an experienced radiologist, who was blinded to the clinical outcomes and had 15 years of abdominal imaging experience. The splenic areas of each axial image were then aggregated to calculate the total SV (Figure 2). Splenomegaly was defined as an SV greater than 314.5 cm^3.[24,25]

Statistical Analysis

Data are reported as means ± standard deviations for continuous variables and as absolute numbers and percentages for categorical variables. The distribution of continuous variables was evaluated for normality by using the Shapiro–Wilk test. The characteristics of patients exhibiting increased or decreased SV were compared using Pearson's chi-square or Fisher's exact test for categorical data, Student's *t*-test for normally distributed data, and the Mann–Whitney *U*-test for non–normally distributed data.

Progression-free survival (PFS) was calculated as the period from the date of immunotherapy or sorafenib treatment to the date of disease progression, death, or the last follow-up. It was estimated using the Kaplan–Meier method and compared between different groups by using a Log rank test in univariate analysis. Sex, age, hepatitis etiology, tumor involvement extent, AFP level, liver function reserve, and splenomegaly were adjusted in logistic regression and a Cox proportional hazards model to examine the effect of SV changes on DCB and PFS, respectively. Immunotherapy regimens and treatment lines were also adjusted in the immunotherapy group. In addition, to reduce the effect of potential confounding factors, we used weighted logistic and Cox proportional hazards regression models to adjust for differences in baseline characteristics, employing inverse probability of treatment weighting (IPTW). A two-sided p value of <0.05 was considered statistically significant. All statistical analyses were conducted using SAS software version 9.4 (SAS Institute, Cary, NC, USA) and R (version 4.3.3; http://www.R-project.org).

Results

Patient Characteristics in the Immunotherapy and Sorafenib Groups

The immunotherapy group comprised 143 patients. Table 1 lists the demographic, clinical, and imaging characteristics of the patients. At the time of immunotherapy initiation, most of the patients had chronic hepatitis B virus infection (75%), a Child–Pugh score of 5 (84%), BCLC stage C disease (83%), and extrahepatic metastasis (77%). Before immunotherapy was initiated, approximately half of the patients (71 out of 143) had undergone other systemic therapies. The median follow-up period was 36.3 months (95% confidence interval [CI], 29.1–43.5 months), and the ORR was 29.4% (42 out of 143 patients), with a DCR of 72% (103 out of 143 patients). A total of 66 patients (46%) exhibited a DCB, with median PFS of 5.4 months (95% CI, 3.6–7.2 months).

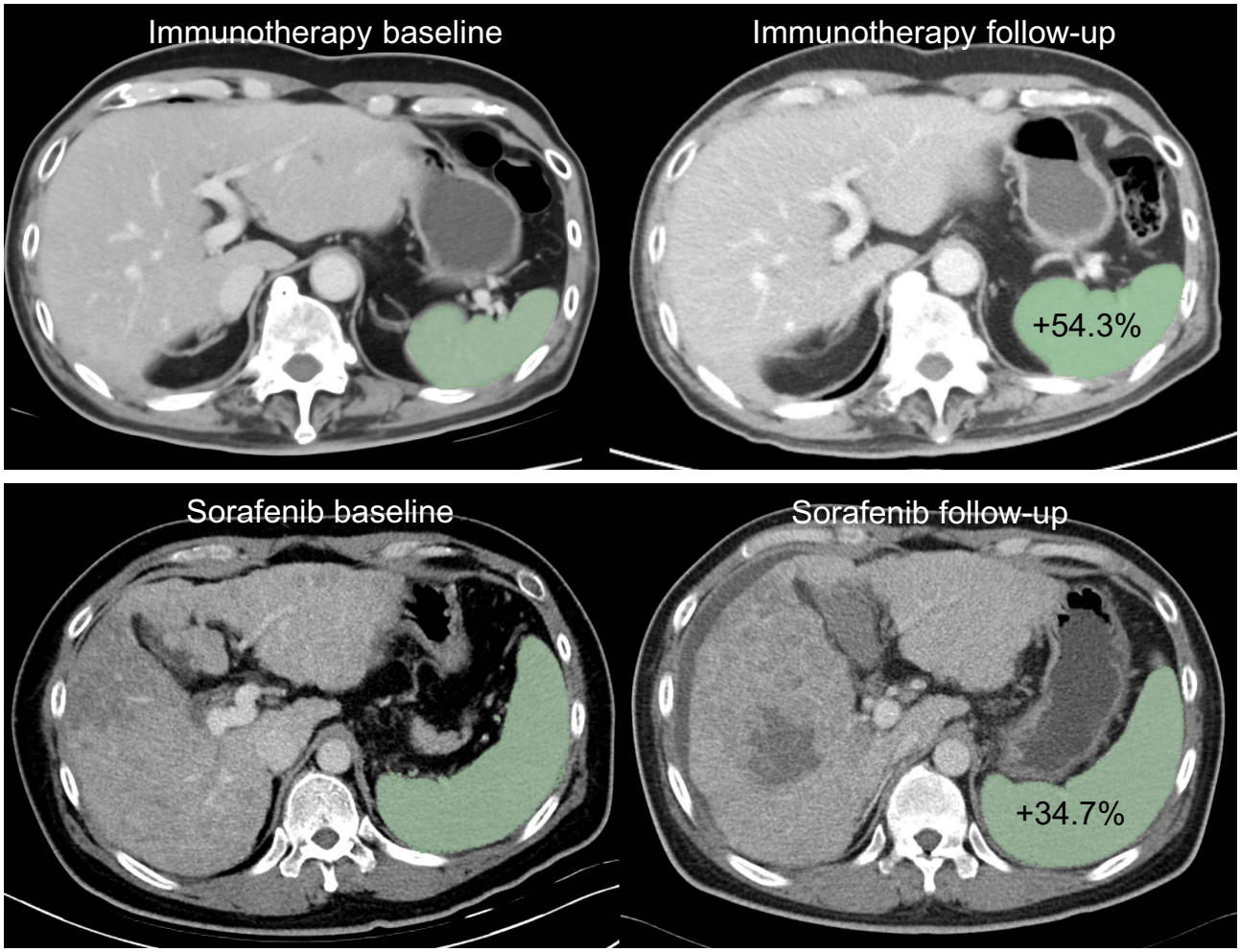


Figure 2 CT-based SV measurement. Upper panel displays the CT scans of a 66-year-old man who underwent therapy with atezolizumab, bevacizumab, and tocilizumab. His SV increased by approximately 54.3%, from 85 cm^3 at baseline to 132 cm^3 at his 41-day follow-up CT scan. His condition remained stable, and he experienced a DCB, with PFS of 13.8 months. Lower panel displays the CT scans of a 39-year-old man with advanced HCC treated with sorafenib. His SV increased by approximately 34.7%, from 656 cm^3 at baseline to 884 cm^3 at his 42-day follow-up CT scan. Despite treatment, his condition worsened, and he exhibited PFS of 1.4 months.

The sorafenib group comprised 57 patients. At the time of treatment initiation, most of the patients had chronic hepatitis B virus infection (63%), a Child–Pugh score of 5 (93%), BCLC stage C disease (91%), and extrahepatic metastasis (63%). The median follow-up period was 24.5 months (95% CI, 20.1–28.9 months), and the ORR was 7% (4 out of 57 patients), with a DCR of 45.6% (26 out of 57 patients). A total of 10 patients (18%) exhibited a DCB, with median PFS of 2.2 months (95% CI, 1.8–2.6 months).

Table 1 Patient Characteristics and Treatment Response in the Immunotherapy and Sorafenib Groups

Characteristics	Immunotherapy	Sorafenib	*p*
Total	143 (100%)	57 (100%)	
Age (years)	59.8 (11.2)	59.6 (9.9)	0.506
Sex (male)	124 (87%)	47 (82%)	0.440
Immunotherapy drug			
PD-1 blockade	45 (31%)		
PD-1 blockade + antiangiogenic therapy	68 (48%)		
Others	30 (21%)		

(*Continued*)

Table 1 (Continued).

Characteristics	Immunotherapy	Sorafenib	*p*
HBsAg (+)	107 (75%)	36 (63%)	0.099
Anti-HCV (+)	21 (15%)	10 (18%)	0.614
Portal vein thrombosis	35 (24%)	29 (51%)	**<0.001**
AFP > 400 ng/mL	53 (37%)	33 (58%)	**0.007**
Platelet (10^9/L)	195 (78)	184 (131)	**0.03**
Morphology			**0.014**
Uninodular and ≤50% of the liver	41 (29%)	19 (33%)	
Multinodular and ≤50% of the liver	94 (66%)	28 (49%)	
>50% of the liver	8 (5.6%)	10 (18%)	
Macrovascular invasion	50 (35%)	38 (67%)	**<0.001**
Extrahepatic spread	110 (77%)	36 (63%)	**0.048**
Child–Pugh score			0.090
5	120 (84%)	53 (93%)	
6	23 (16%)	4 (7.0%)	
ALBI			**0.016**
1	94 (66%)	27 (47%)	
2	49 (34%)	30 (53%)	
BCLC stage C	119 (83%)	52 (91%)	
Best response			**<0.001**
CR	2 (1.4%)	0 (0%)	
PR	40 (28%)	4 (7.0%)	
SD	61 (43%)	22 (39%)	
PD	40 (28%)	31 (54%)	
ORR (CR/PR)	42 (29%)	4 (7.0%)	**<0.001**
DCR (CR/PR/SD)	103 (72%)	26 (46%)	**<0.001**
DCB (CR/PR/SD ≥ 6 months)	66 (46%)	10 (18%)	**<0.001**
Splenomegaly (> 314.5 cm^3)	32 (22%)	35 (61%)	**<0.001**
Baseline spleen volume (cm^3)	267 (144)	392 (222)	**<0.001**
Spleen volume increased after treatment	108 (76%)	21 (37%)	**<0.001**
Spleen volume change (%)	11.0 (25)	−1.2 (16)	**<0.001**
Portal hypertension	9 (6.3%)	22 (39%)	**<0.001**
Time from first treatment to 1st follow-up CT (days)	47.3 (8.6)	50 (15.9)	0.233

Notes: All data are presented as N (%) or mean (standard deviation). A bold typeface signifies that the P value is less than 0.05.

Abbreviations: PD-1, programmed cell death protein 1; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; ORR, objective response rate; DCB, durable clinical benefit.

Compared with patients who received sorafenib, a larger proportion of those who underwent immunotherapy exhibited an increase in SV after treatment (75.5% vs 36.8%, $p < 0.001$). Figure 3 depicts waterfall plots illustrating the relationship between therapeutic response and SV change in the immunotherapy (Figure 3A) and sorafenib (Figure 3B) groups.

Changes in SV and Treatment Outcomes in the Immunotherapy Group

In the immunotherapy group, patients who exhibited an increase in SV after treatment ($n = 108$) were significantly younger, more likely to undergo immunotherapy as a first-line treatment, and more likely to have ALBI grade 1 liver function reserve compared with those who exhibited a decrease in SV after treatment (n = 35, Table 2). Neither baseline SV nor splenomegaly was associated with an increase or decrease in SV. Posttreatment changes in ALBI scores were not significantly different between patients with increased and decreased SV ($p = 0.871$).

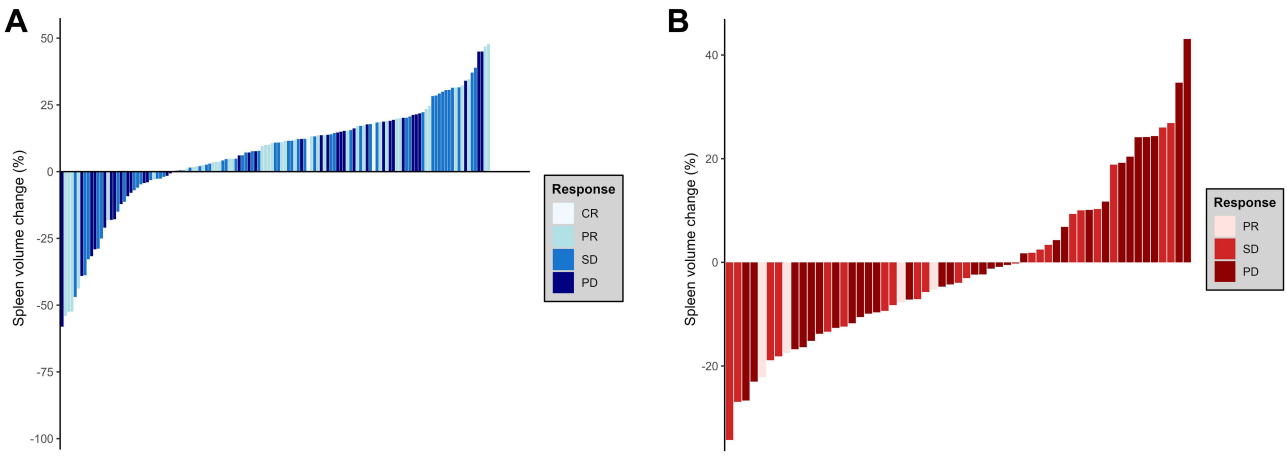


Figure 3 Waterfall plots of optimal tumor response versus SV changes. (**A**) immunotherapy group. (**B**) sorafenib group.

Compared with patients who exhibited a decrease in SV after treatment, those who exhibited an increase in SV after treatment were more likely to have a higher DCR (76.9% vs 57.1%, $p = 0.024$) and greater DCB (52.8% vs 25.7%, $p = 0.005$; Table 2). After patient demographics, hepatitis etiology, tumor involvement extent, liver function reserve, AFP level, and immunotherapy regimens or lines were adjusted for, increased SV after treatment remained a significant predictor of DCB in multivariate analysis (odds ratio, 6.94; 95% CI, 2.07–23.27, $p < 0.001$; Table 3).

Patients who exhibited an increase in SV after treatment had significantly longer PFS than did those who exhibited a decrease in SV after treatment (median: 6.9 vs 3.6 months, $p = 0.028$; Figure 4A). After other potential predictors were adjusted for, increased SV after treatment remained an independent predictor of improved PFS (hazard ratio [HR], 0.51; 95% CI, 0.3–0.87, $p = 0.014$; Table 4).

Changes in SV and Treatment Outcomes in the Sorafenib Group

In the sorafenib group, an increase in SV was not associated with any clinical variables, including age and liver function reserve (Table 5). In patients who received sorafenib, an increase in SV was not associated with a DCB in univariate analysis and multivariate analysis ($p = 0.232$, Table 3). Compared with patients who exhibited a decrease in SV after

Table 2 Comparison of SV Changes in the Immunotherapy Group

Characteristics	Decreased SV	Increased SV	*p*
Total	35 (100%)	108 (100%)	
Age (years)	65.2 ± 9.4	58.1 ± 11.3	**0.001**
Sex (male)	32 (91.4%)	92 (85.2%)	0.408
Immunotherapy drug			
PD-1 blockade	12 (34.3%)	33 (30.6%)	0.534
PD-1 blockade + antiangiogenic therapy	18 (51.4%)	50 (46.3%)	
Others	5 (14.3%)	25 (23.1%)	
HBsAg (+)	24 (68.6%)	83 (76.9%)	0.327
Anti-HCV (+)	6 (17.1%)	15 (13.9%)	0.636
Portal vein thrombosis	7 (20%)	28 (25.9%)	0.479
AFP > 400 ng/mL	13 (37.1%)	40 (37%)	0.991
Platelet (10^9/L)	171.5 (67.6)	202.2 (79.6)	**0.031**
Morphology			0.844
Uninodular and ≤50% of the liver	10 (28.6%)	31 (28.7%)	
Multinodular and ≤50% of the liver	24 (68.6%)	70 (64.8%)	
>50% of the liver	1 (2.9%)	7 (6.5%)	
Macrovascular invasion	9 (25.7%)	41 (38%)	0.187
Extrahepatic spread	28 (80%)	82 (75.9%)	0.619

(*Continued*)

Table 2 (Continued).

Characteristics	Decreased SV	Increased SV	*p*
Child–Pugh score			0.209
5	27 (77.1%)	93 (86.1%)	
6	8 (22.9%)	15 (13.9%)	
ALBI			**0.040**
1	18 (51.4%)	76 (70.4%)	
2	17 (48.6%)	32 (29.6%)	
BCLC stage C	28 (80%)	91 (84.3%)	0.558
Immunotherapy line (≥ 2nd line)	28 (80%)	43 (39.8%)	**<0.001**
Best Response			0.109
CR	0 (0%)	2 (1.9%)	
PR	6 (17.1%)	34 (31.5%)	
SD	14 (40%)	47 (43.5%)	
PD	15 (42.9%)	25 (23.1%)	
ORR (CR/PR)	6 (17.1%)	36 (33.3%)	0.068
DCR (CR/PR/SD)	20 (57.1%)	83 (76.9%)	**0.024**
DCB (CR/PR/SD ≥ 6 months)	9 (25.7%)	57 (52.8%)	**0.005**
Splenomegaly (> 314.5 cm^3)	9 (25.7%)	23 (21.3%)	0.586
Baseline spleen volume	279.5 ± 163.9	262.6 ± 137.9	0.716
Spleen volume change (%)	−20.1 ± 18.3	21.1 ± 17.9	**<0.001**
Portal hypertension	2 (5.7%)	7 (6.5%)	1.00

Notes: All data are presented as N (%) or mean (standard deviation). A bold typeface signifies that the P value is less than 0.05.

Abbreviations: PD-1, programmed cell death protein 1; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; ORR, objective response rate; DCB, durable clinical benefit.

Table 3 Multivariate Analysis of DCB by Logistic Regression in the Immunotherapy and Sorafenib Groups

Characteristic	Immunotherapy			Sorafenib		
	OR	95% CI	*p*	OR	95% CI	*p*
Increased spleen volume	6.94	2.07, 23.27	**<0.001**	0.29	0.04, 2.22	0.232
Age	1.04	0.99, 1.09	0.088	0.97	0.88, 1.07	0.541
Sex (Male)	0.69	0.21, 2.33	0.552	0.78	0.10, 6.17	0.817
Immunotherapy drug						
PD-1 blockade	2.23	0.66, 7.57	0.616			
PD-1 blockade + antiangiogenic therapy	8.03	2.01, 32.04	**0.003**			
Others	–					
HBsAg (+)	2.69	0.77, 9.40	0.122	0.29	0.03, 2.67	0.272
Anti-HCV (+)	2.29	0.47, 11.11	0.303	0.60	0.04, 8.25	0.703
Portal vein thrombosis	0.70	0.15, 3.21	0.651	1.36	0.04, 43.5	0.861
AFP > 400 ng/mL	1.09	0.47, 2.55	0.839	1.63	0.27, 9.7	0.594
Multinodular or >50% of the liver (vs Uninodular and ≤50% of the liver)	0.31	0.12, 0.84	**0.021**	1.43	0.09, 21.69	0.796
Macrovascular invasion	1.40	0.34, 5.73	0.644	1.79	0.04, 74.23	0.760
Extrahepatic spread	0.38	0.10, 1.42	0.151	0.94	0.17, 5.36	0.946
Child–Pugh score 6 (vs 5)	0.86	0.24, 3.03	0.809	1.03	0.05, 23.2	0.983
BCLC stage C (vs B)	3.29	0.79, 13.68	0.102	0.82	0.04, 17.8	0.902
ALBI 2 (vs 1)	1.15	0.47, 2.85	0.756	1.80	0.29, 11.12	0.527
Immunotherapy line ≥ 2nd line (vs 1st line)	1.61	0.53, 4.92	0.401			
Splenomegaly	1.03	0.33, 3.22	0.956	2.46	0.28, 21.9	0.418

Notes: A bold typeface signifies that the P value is less than 0.05. An odds ratio greater than one implies that the predictor variable is positively associated with DCB, even after controlling for other variables in the model.

Abbreviations: PD-1, programmed cell death protein 1; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; OR, odds ratio; CI, confidence interval.

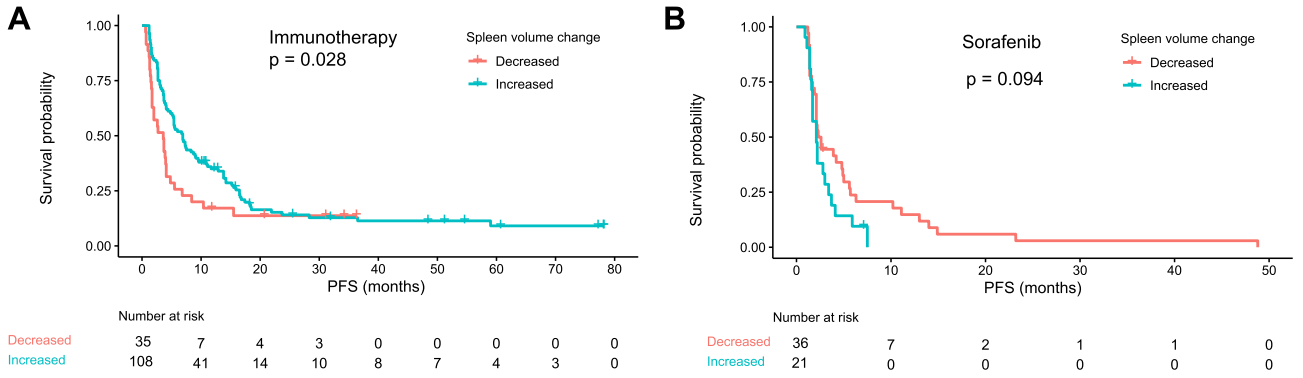


Figure 4 Kaplan–Meier curves of PFS versus SV changes. (**A**) immunotherapy group. (**B**) sorafenib group. All p values were determined using a Log rank test.

treatment, those who exhibited an increase in SV after treatment tended to have shorter PFS (median: 2.1 vs 2.5 months, $p = 0.094$; Figure 4B). In patients who received sorafenib, an increase in SV after treatment exhibited borderline significance in predicting poor PFS in multivariate analysis (HR, 1.87; 95% CI, 0.95–3.71, $p = 0.072$; Table 4).

Comparison of SV Change in Immunotherapy and Sorafenib Groups Using IPTW for DCB and PFS

After IPTW adjustment, increased SV remained a significant positive predictor for DCB (odds ratio, 3.42; 95% CI, 1.41–8.25, $p = 0.006$; Table 6) in the immunotherapy group and a significant negative predictor for DCB (odds ratio, 0.11; 95% CI, 0.01–0.8, $p = 0.03$; Table 6) in the sorafenib group. Besides, increased SV remained a significant predictor for longer PFS (HR, 0.52; 95% CI, 0.31–0.89, $p = 0.018$; Table 7) in the immunotherapy group, but it was not significant in the sorafenib group.

Table 4 Multivariate Analysis of PFS by Cox Regression in the Immunotherapy and Sorafenib Groups

Characteristic	Immunotherapy			Sorafenib		
	HR	95% CI	p	HR	95% CI	p
Increased spleen volume	0.51	0.30, 0.87	**0.014**	1.87	0.95, 3.71	0.072
Age	0.98	0.97, 1.00	0.087	0.99	0.95, 1.03	0.639
Sex (Male)	1.48	0.8, 2.74	0.210	0.86	0.35, 2.10	0.732
Immunotherapy						
PD-1 blockade	0.87	0.49, 1.56	0.639			
PD-1 blockade + antiangiogenic therapy	0.55	0.28, 1.09	0.088			
Others	–					
HBsAg (+)	0.61	0.33, 1.11	0.107	1.25	0.54, 2.90	0.596
Anti-HCV (+)	0.53	0.24, 1.15	0.106	1.14	0.40, 3.25	0.801
Portal vein thrombosis	1.11	0.55, 2.22	0.774	0.89	0.26, 3.07	0.852
AFP > 400 ng/mL	1.09	0.72, 1.64	0.679	0.83	0.45, 1.52	0.543
Multinodular or >50% of the liver (vs Uninodular and ≤50% of the liver)	1.59	0.96, 2.61	0.069	0.67	0.25, 1.78	0.424
Macrovascular invasion	1.03	0.52, 2.04	0.926	0.67	0.20, 2.30	0.524
Extrahepatic spread	1.33	0.74, 2.41	0.346	0.69	0.31, 1.54	0.360
Child Score 6 (vs 5)	1.25	0.70, 2.26	0.450	1.63	0.43, 6.19	0.473
ALBI 2 (vs 1)	1.15	0.76, 1.75	0.507	1.24	0.64, 2.39	0.527
BCLC stage C (vs B)	0.56	0.29, 1.09	0.088	2.29	0.42, 12.55	0.339
Immunotherapy line ≥ 2nd line (vs 1st line)	0.85	0.48, 1.51	0.568			
Splenomegaly	1.04	0.60, 1.80	0.891	0.85	0.38, 1.91	0.700

Notes: A bold typeface signifies that the P value is less than 0.05. A hazard ratio greater than one suggests that the variable is associated with shorter PFS, while accounting for other factors.

Abbreviations: PD-1, programmed cell death protein 1; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; HR, hazard ratio; CI, confidence interval.

Table 5 Comparison of SV Changes in the Sorafenib Group

Characteristics	Decreased SV	Increased SV	*p*
Total	36 (100%)	21 (100%)	
Age	59 ± 10	60 ± 10	> 0.999
Sex (Male)	29 (81%)	18 (86%)	0.730
HBsAg (+)	25 (69%)	11 (52%)	0.198
Anti-HCV (+)	6 (17%)	4 (19%)	> 0.999
Portal vein thrombosis	17 (47%)	12 (57%)	0.470
AFP > 400 ng/mL	20 (56%)	13 (62%)	0.640
Platelet (10^9/L)	172 (91)	206 (181)	0.433
Morphology			> 0.999
Uninodular and ≤50% of the liver	12 (33%)	7 (33%)	
Multinodular and ≤50% of the liver	18 (50%)	10 (48%)	
>50% of the liver	6 (17%)	4 (19%)	
Macrovascular invasion (+)	21 (58%)	17 (81%)	0.081
Extrahepatic spread (+)	23 (64%)	13 (62%)	0.881
Child–Pugh score			0.620
5	34 (94%)	19 (90%)	
6	2 (5.6%)	2 (9.5%)	
ALBI			0.259
1	15 (42%)	12 (57%)	
2	21 (58%)	9 (43%)	
BCLC stage C	31 (86%)	21 (100%)	0.146
Best Response			0.393
PR	4 (11%)	0 (0%)	
SD	13 (36%)	9 (43%)	
PD	19 (53%)	12 (57%)	
ORR (CR/PR)	4 (11%)	0 (0%)	0.285
DCR (CR/PR/SD)	17 (47%)	9 (43%)	0.750
DCB (CR/PR/SD ≥ 6 months)	8 (22%)	2 (9.5%)	0.295
Splenomegaly (> 314.5 cm^3)	23 (64%)	12 (57%)	0.614
Baseline spleen volume (cm^3)	399 (227)	379 (220)	0.787
Spleen volume change (%)	−11 (8)	16 (12)	**< 0.001**
Portal hypertension	15 (42%)	7 (33%)	0.531

Notes: All data are presented as N (%) or mean (standard deviation). A bold typeface signifies that the P value is less than 0.05.

Abbreviations: PD-1, programmed cell death protein 1; HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; PR, partial response; SD, stable disease; PD, progressive disease; DCR, disease control rate; ORR, objective response rate; DCB, durable clinical benefit.

Table 6 Multivariate Analysis of DCB by Logistic Regression in the Immunotherapy and Sorafenib Groups After Inverse Probability of Treatment Weighting Adjustment

	Immunotherapy			Sorafenib		
	OR	95% CI	*p*	OR	95% CI	*p*
Increased spleen volume	3.42	1.41, 8.25	**0.006**	0.11	0.01, 0.80	**0.030**
Age	1.06	1.02, 1.10	**0.001**	1.01	0.94, 1.08	0.830
Sex (Male)	0.79	0.28, 2.21	0.650	0.58	0.08, 4.23	0.595
HBsAg (+)	3.12	1.14, 8.55	**0.026**	0.13	0.02, 0.90	**0.039**
Anti-HCV (+)	1.78	0.47, 6.66	0.394	0.31	0.03, 2.80	0.298
Portal vein thrombosis	0.48	0.14, 1.67	0.248	4.68	0.33, 66.0	0.253

(*Continued*)

Table 6 (Continued).

	Immunotherapy			Sorafenib		
	OR	**95% CI**	***p***	**OR**	**95% CI**	***p***
AFP > 400 ng/mL	1.34	0.66, 2.69	0.416	1.90	0.32, 11.25	0.480
Platelet	8.00	1.76, 36.45	**0.007**	0.57	0.10, 3.33	0.528
Multinodular or >50% of the liver (vs Uninodular and ≤50% of the liver)	0.24	0.10, 0.59	**0.002**	0.49	0.07, 3.26	0.461
Macrovascular invasion	2.17	0.64, 7.34	0.212	0.87	0.05, 15.59	0.927
Extrahepatic spread	0.38	0.14, 1.04	0.060	0.37	0.07, 1.93	0.239
Child–Pugh score 6 (vs 5)	0.23	0.07, 0.82	**0.023**	0.64	0.03, 11.82	0.762
ALBI 2 (vs 1)	0.93	0.44, 1.97	0.859	0.82	0.19, 3.62	0.797
BCLC stage C (vs B)	2.69	0.77, 9.47	0.122	1.29	0.13, 13.05	0.827
Splenomegaly	1.28	0.50, 3.23	0.608	8.83	1.67, 46.56	**0.010**
Portal hypertension	1.00	1.00, 1.01	0.142	0.99	0.97, 1.00	**0.013**

Notes: A bold typeface signifies that the P value is less than 0.05. Inverse Probability of Treatment Weighting (IPTW) is adjusted for all variables other than spleen volume change.

Abbreviations: HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; HR, hazard ratio; CI, confidence interval.

Table 7 Multivariate Analysis of PFS by Cox Regression in the Immunotherapy and Sorafenib Groups After Inverse Probability of Treatment Weighting Adjustment

Characteristic	Immunotherapy			Sorafenib		
	HR	**95% CI**	***p***	**HR**	**95% CI**	***p***
Increased spleen volume	0.52	0.31, 0.89	**0.018**	1.53	0.75, 3.11	0.241
Age	0.98	0.96, 1.00	0.075	1.00	0.96, 1.05	0.948
Sex (Male)	1.58	0.83, 3.03	0.168	0.86	0.34, 2.34	0.815
HBsAg (+)	0.58	0.33, 1.04	0.069	1.25	0.80, 5.50	0.131
Anti-HCV (+)	0.59	0.28, 1.26	0.174	1.14	0.41, 5.87	0.521
Portal vein thrombosis	1.33	0.71, 2.50	0.377	0.89	0.12, 2.31	0.391
AFP > 400 ng/mL	1.07	0.71, 1.61	0.757	0.83	0.43, 1.7	0.656
Multinodular or >50% of the liver (vs Uninodular and ≤50% of the liver)	1.76	1.05, 2.95	**0.031**	0.81	0.31, 2.12	0.670
Macrovascular invasion	0.85	0.46, 1.55	0.592	0.58	0.16, 2.15	0.417
Extrahepatic spread	1.58	0.98, 2.55	0.062	0.69	0.18, 2.77	0.609
Child Score 6 (vs 5)	1.52	0.84, 2.75	0.164	1.63	0.36, 6.3	0.569
ALBI 2 (vs 1)	1.25	0.82, 1.89	0.300	1.24	0.7, 3.28	0.295
BCLC stage C (vs B)	0.55	0.79, 0.99	**0.048**	2.29	0.11, 56.82	0.572
Splenomegaly	0.66	1.00, 2.21	0.371	0.85	0.47, 2.59	0.585
Platelet	0.55	1.00, 1.00	**0.018**	1.00	1.00, 1.01	**0.004**
Portal hypertension	1.00	0.31, 1.65	0.210	1.63	0.47, 5.59	0.438

Notes: A bold typeface signifies that the P value is less than 0.05. Inverse Probability of Treatment Weighting (IPTW) is adjusted for all variables other than spleen volume change.

Abbreviations: HBsAg, hepatitis B surface antigen; anti-HCV, anti-hepatitis C antibody; AFP, α-fetoprotein; ALBI, albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; HR, hazard ratio; CI, confidence interval.

Discussion

In this study, we examined changes in SV as a potential indicator of treatment response in patients undergoing immunotherapy or receiving sorafenib for advanced HCC. During the initial follow-up, we observed a significant increase in SV (75.5%) in the immunotherapy group compared with the sorafenib group (36.8%). The increase in SV was not associated with the changes of liver function reserve. In the immunotherapy group, compared with patients who exhibited a decrease in SV, those who exhibited an increase in SV showed a higher DCR and greater DCB. In addition, an increase in SV predicted a greater DCB and extended PFS. By contrast, in the sorafenib group, an increase in SV was

not associated with treatment response but was presumably associated with reduced PFS. After IPTW adjustment, the increase in SV remained a significant predictor for DCB and PFS in the immunotherapy group.

Systemic chemotherapy has profound implications for hematopoiesis and immunocompetence.[16,26] Emerging evidence suggests that the spleen can be used as an indicator of systemic immune response during immunotherapy.[16] Animal studies have indicated that anti-PD-L1 and anti-PD-1 treatments may affect the exhaustion status and clonality of T lymphocytes in the spleen and increase the number of splenic $CD4^+$ and $CD8^+$ cells, monocytes, macrophages, and natural killer cells after treatment.[27–30] Interestingly, this response is associated with an increase in spleen size.[29] Therefore, determining the effects of immunotherapy and sorafenib on the spleen may provide an in-depth understanding of their underlying mechanisms and efficacy in patients with HCC.

Studies have reported contradictory findings regarding SV changes during chemotherapy and immunotherapy for different types of cancer. For example, Susok et al[18] investigated SV changes during treatment initiation in 49 patients with stage III and IV melanoma. After 3 months, they observed an increase in SV in 31 out of 44 patients (70.5%). However, they reported no significant relationship between this increase in SV and treatment response. Seith et al[31] used ^{18}F-fluorodeoxyglucose positron emission tomography/magnetic resonance imaging to examine 17 patients receiving ICIs for melanoma. They discovered that, compared with the nonresponder group, the responder group exhibited a marked increase in SV. Castagnoli et al[20] evaluated SV changes in 70 patients receiving pembrolizumab for locally advanced or metastatic non–small-cell lung cancer. Their results revealed no significant changes in SV with pembrolizumab treatment, thus indicating no correlation between SV changes and treatment outcomes. Furthermore, a recent study involving 45 patients with metastatic renal cell carcinoma receiving nivolumab as second-line or subsequent therapy reported that an increase in SV was associated with shorter PFS and OS.[32] These studies highlight the complex and potentially variable role of SV changes in predicting the efficacy of cancer treatments.

In this study, we observed a 75.5% increase in SV after the initiation of immunotherapy. This finding is consistent with that of Muller et al,[21] who reported a similar increase of 76% in 50 patients undergoing immunotherapy for HCC. In their study, they did not consider an increase in SV as a prognostic factor of OS. However, they did not examine the relationship between SV and DCB or PFS. In the present study, we focused solely on these two outcomes because of the complexity of OS, which is influenced by numerous variables, such as tumor stage at diagnosis, liver function, patient performance status, treatment history, additional treatments,[33] the presence of other medical conditions, the type of immunotherapy administered, sarcopenia, myosteatosis,[34] and nutritional status.[35] Further research is required to elucidate the effect of SV changes on OS while controlling for these confounding factors.

SV is a prognostic predictor in patients with HCC who undergo both curative and palliative treatment.[36–39] In this study, we discovered that splenomegaly also served as a negative predictor of PFS in patients undergoing immunotherapy. As an indirect measure of drug-induced hepatotoxicity, CT-quantified SV expansion is used to capture the increase in portal pressure associated with liver injury.[17,19,40–42] In our immunotherapy group, changes in SV during the initial follow-up served as a favorable predictor of DCB and PFS. However, in the sorafenib group, SV increases were likely associated with a decrease in PFS, which may be indicative of liver decompensation and portal hypertension secondary to tumor enlargement. Recently, multiple deep learning techniques have been developed to achieve a fully automated evaluation of SV by relying on CT data,[39,43] thus indicating the potential of SV as a promising imaging biomarker for seamless integration into standard radiological workflows.

This study has some limitations. First, this study was retrospectively conducted at a single institution on a relatively small number of patients who received various immunotherapeutic agents. Because of the limited sample size, no subgroup analysis per agent was conducted. Subsequent studies should validate the role of SV as a prognostic factor in patients' response to different immunotherapeutic agents and treatment regimens. Second, this study included only patients who underwent follow-up abdominal CT scans, which may have introduced a degree of selection bias. Third, because CT examinations were performed with clinical discretion, the frequency and interval of these scans were not uniform throughout the study population.

Conclusion

In conclusion, this study highlights the potential of SV changes as a prognostic marker for patients who undergo immunotherapy for advanced HCC. In patients who undergo immunotherapy, a substantial increase in SV correlates with

improved treatment response. However, in patients who receive sorafenib, an increase in SV may predict poor PFS. Despite these correlations, the clinical importance of SV changes in terms of OS requires further investigation.

Abbreviations

HCC, Hepatocellular carcinoma; ICI, Immune checkpoint inhibitor; PD-L1, Programmed death ligand 1; AFP, α-Fetoprotein; SV, Spleen volume; PD-1, Programmed cell death protein 1; CTLA-4, Cytotoxic T-lymphocyte-associated protein 4; ALBI, Albumin-bilirubin; BCLC, Barcelona Clinic Liver Cancer; CT, Computed tomography; RECIST, Response Evaluation Criteria in Solid Tumors; DCR, Disease control rate; ORR, Objective response rate; DCB, Durable clinical benefit; PFS, Progression-free survival; CI, Confidence interval; HR, Hazard ratio.

Data Sharing Statement

For ethical reasons, the data are not publicly available. The data sets generated and analyzed in this study are available from the corresponding author upon reasonable request.

Ethics Approval and Informed Consent

Institutional Review Board approval of National Taiwan University Hospital was obtained.

This study was conducted in full compliance with the ethical principles outlined in the Declaration of Helsinki.

For patients receiving immunotherapy, written informed consent was waived by the Institutional Review Board because of retrospective analysis. Besides, stringent measures have been implemented to ensure the confidentiality and privacy of patient data. All medical records were anonymized and securely stored, accessible only to authorized research personnel. The data was handled in accordance with applicable privacy laws and guidelines to protect patient identities and maintain the integrity of the research.

For patients receiving sorafenib, written informed consent was obtained from all patients receiving sorafenib.

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Author Contributions

Study design and concept: Bang-Bin Chen, Yu-Yun Shao.

Data collection: All authors.

Study analysis: Bang-Bin Chen, Yu-Yun Shao.

Data interpretation and review and approval the manuscript submission: All authors.

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