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【 ORIGINAL ARTICLE 】

Enlargement of the Spleen Index Is a Predictor of the Occurrence of Esophageal Varices and Hepatocellular Carcinoma after Administering Direct-acting Antiviral Agents

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Abstract:

Objective Direct-acting antiviral agents (DAAs) can eliminate hepatitis C virus at a high rate, although the long-term incidence of portal hypertension and hepatocellular carcinoma (HCC) has not yet been elucidated. In this observational study, we clarified the predictors associated with the incidence of esophageal varices (EVs) and HCC after DAAs treatment based on ultrasound findings and blood examinations.

Methods A total of 78 patients treated with DAAs were enrolled in this study. The primary endpoint was to identify the predictors associated with EVs and HCC occurrence using univariate and multivariate analyses. Secondary endpoints were to extract the cutoff values for EVs and HCC occurrence and clarify the changes in liver stiffness (LS), spleen stiffness (SS), spleen index (SI), portal venous flow volume (PVF), and blood examination at 12 weeks after the end of DAAs treatment.

Results The mean observation period was 1,402±546 days. SI change (SI after DAAs-SI before DAAs) was a predictor of EVs occurrence in multivariate analysis (p=0.045). The treatment history of HCC, albumin value before DAAs, and SI change were predictors of HCC occurrence in multivariate analysis (p=0.002, p=0.032, and p=0.009, respectively). LS, SS, PVF, SI, and liver function significantly improved after DAAs treatment.

Conclusion Portal hypertension seems to improve after DAAs treatment over a long period. Patients with splenomegaly deterioration after DAAs treatment need to be carefully monitored for the occurrence of EVs and HCC.

Key words: DAAs, spleen index, splenomegaly, esophageal varices, hepatocellular carcinoma

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Introduction

The recent development of direct-acting antiviral agents (DAAs) has enabled a sustained virological response (SVR) to be achieved in more than 95% of patients with liver cirrhosis (LC) and hepatitis C virus (HCV) (1). However, it remains unknown whether viral eradication using DAAs improves portal hypertension and reduces the long-term occurrence of hepatocellular carcinoma (HCC). The incidence of ascites (decompensation) after DAAs treatment of patients with LC and Child-Pugh grade A disease was reported as 0.7% over 4 years of follow-up (2). They concluded that nonselective β-blocker treatment for preventing clinically significant portal hypertension (CSPH) was no longer unnecessary after effective DAAs treatment. However, another report showed that esophageal varices (EVs) were aggravated and that portosystemic encephalopathy occurred even after achieving SVR with DAAs (3). Knop et al. reported that liver stiffness (LS) as calculated by transient elastogra-

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phy (TE) improved until 3 years after DAAs treatment, but spleen stiffness (SS) did not change (4). SS is known to reflect splenic fibrosis depending on portal hypertension (5, 6) and shows a positive correlation with the hepatic venous pressure gradient (7). Thus, the effect of portal hypertension after DAAs treatment is controversial. There are no reports on the predictors associated with the occurrence of EVs after DAAs treatment.

Conversely, regarding the occurrence of HCC, there have been many reports showing that the achievement of SVR by DAAs reduced the risk of HCC development during short-term observation (8). However, few reports have examined the long-term risk of HCC occurrence. Nagaoki et al. reported that the cumulative HCC development rate at 5 years was 7% in the DAAs-SVR group, which was significantly lower than the 18% in the non-SVR group (3). Mawatari et al. reported an HCC occurrence rate of 4.7% at 4 years (9). Tosetti et al. reported that 18 of 148 patients (12.1%) developed HCC (11 de novo HCC, seven recurrences) during a median follow-up period of 49 months (2). Thus, the incidence of HCC varies with each report, and the predictors associated with the occurrence of HCC varices also vary.

In this study, we clarified the predictors associated with the occurrence of EVs and HCC after SVR by DAAs during long-term observation.

Materials and Methods

Study design

This was a retrospective longitudinal observational cohort study. The primary endpoint was to extract independent predictors associated with EVs and HCC occurrence. The secondary endpoints were to clarify the changes in LS, SS, spleen index (SI), portal venous flow volume (PVF), and blood test results after DAAs treatment and to identify the cutoff values to predict the occurrence of EVs and HCC. The time points for ultrasonography (US) and blood tests to be investigated were set as before DAAs treatment and 12 weeks after the end of DAAs medication (SVR 12).

The protocol of this study was approved by the Clinical Research Ethics Committee of our institution (study approval no.: 2017-031, 2017-327) and conformed with the principles of the Helsinki Agreement. A public announcement about this study was posted at our hospital before starting the research.

Patient selection and inspection

The inclusion criteria were as follows: 1) age >20 years; 2) DAAs treatment between August 2015 and January 2019; and 3) US examination involving elastography before DAAs treatment and SVR 12. The exclusion criteria were as follows: 1) emergency cases with a history of EVs bleeding, 2) portal venous obstruction, 3) high bilirubin level >3 mg/dL, and 4) liver tumor, including HCC. The decision of potential HCC nodules was performed with double conformation by two inspectors based on evidence from US, computed tomography (CT) with contrast agent, or magnetic resonance imaging (MRI) with Gd-EOB-DTPA before DAAs treatment. All imaging examinations of HCC inspection were performed within 2 months before the start of DAAs treatment. After DAAs treatment, abdominal examination using US, CT, or MRI was performed at an interval of 3-6 months. For endoscopic surveillance, the first confirmation endoscopy was performed within 1 year before DAAs commencement, and subsequent endoscopies were performed at least every 1 year (6 months to 1 year). If EVs or gastric varices (GVs) with an appearance of F1 (or more) or a positive red color sign were observed, it was defined as the occurrence (or recurrence).

A total of 96 patients were enrolled in the study. Thirteen patients were excluded if they had not undergone US examination after DAAs treatment, and five patients were excluded because they interrupted their hospital visit. Finally, data from 78 patients were analyzed (Table 1).

Ultrasound examination and blood tests

The Aplio 500 and i800™ (Canon Medical Systems Corporation, Tochigi, Japan) were used for the US inspections. To calculate elastography parameters, the region of interest was set at a depth of 3 cm beneath the body surface. The propagation velocity of the shear wave speed (SWs), which is generated by acoustic push pulses, was calculated in the stable propagation wave area. SWs were measured five times in both the liver and spleen of each patient. The PVF was measured three times. The SI was calculated as the length from the tip of the spleen to the splenic hilum in the longitudinal view×maximum width in the longitudinal view.

The parameters investigated in the blood test were the platelet count (PLT), prothrombin time-international normalized ratio (PT-INR), levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, albumin, cholinesterase, hyaluronic acid, type IV collagen 7s, macrophage galactose-specific lectin-2 binding protein glycosylation isomer (M2BPGi), branched-chain amino acids/tyrosine molar ratio (BTR), Child-Pugh score, fibrosis-4 index (FIB4-index), aspartate aminotransferase to platelet ratio index (APRI), alpha-fetoprotein (AFP), and protein induced by vitamin K absence or antagonists-II (PIVKA-II).

Primary and secondary endpoints

Laboratory data, LS, SS, PV flow volume, and SI were compared before DAAs treatment and at the time point of SVR 12 (secondary endpoint) (Table 1). The patients were divided into EVs occurrence and nonoccurrence groups. Liver function values from the blood test and US elastography findings before and after DAAs administration, age, sex, etiology of liver cirrhosis, and underlying disease were compared between the two groups (Table 2). Similarly, patients were divided into HCC occurrence and nonoccurrence groups, and the values were compared (Table 3). The factors that demonstrated a statistically significant difference be-

Table 1. Patient Characteristics.

Characteristic (n=78)			
Age, yrs (±SD)	64.9±12.3		
Men, n (%)	21 (61.8)		
Ascites (presence/absence)	0/78		
History of EVs (presence/absence)	9/69		
History of HCC (presence/absence)	9/69		
Blood test data	**Before DAAs**	**After DAAs (SVR 12)**	**p value**
Platelet (×10^4/μL)	17.8±6.4	18.8±6.7	0.007
PT-INR	1.0±0.1	1.0±0.1	0.335
AST (IU/L)	53.6±36.2	23.4±10.0	<0.001
ALT (IU/L)	64.8±20.5	16.6±9.2	<0.001
Total bilirubin (mg/dL)	0.8±0.3	0.7±0.4	0.462
Albumin (g/dL)	4.0±0.4	4.1±0.4	0.005
Cholinesterase (IU/L)	292±100	283±82	0.439
Child-Pugh score	5.1±0.4	5.1±0.3	0.199
Hyaluronic acid (ng/mL)	210±319	142±202	<0.001
Type IV collagen 7s (ng/mL)	6.3±2.7	5.7±2.3	<0.001
BTR (μmol/L)	5.7±1.8	5.7±1.4	0.575
M2BPGi (C.O.I.)	2.6±2.9	1.5±1.7	<0.001
FIB4-index	3.1±2.3	0.8±1.1	<0.001
APRI	1.0±0.9	0.1±0.2	<0.001
AFP	63.8±401	14.1±64.6	0.388
PIVKA- II	22.8±11.0	23.6±15.2	0.583
Abdominal ultrasound			
LS (kPa)	11.5±8.0	9.1±5.8	<0.001
SS (kPa)	22.6±11.1	18.7±7.8	<0.001
PVF (mL/min)	559±237	667±327	0.001
SI (cm^2)	16.9±8.1	15.7±7.8	0.004

Data are expressed as the mean±standard deviation (SD).

DAAs: direct-acting antiviral agents, EV: esophageal varices, HCC: hepatocellular carcinoma, yrs: years, PT-INR: prothrombin time-international normalized ratio, AST: aspartate aminotransferase, ALT: alanine aminotransferase, BTR: branched-chain amino acids/tyrosine molar ratio, M2BPGi: macrophage galactose-specific lectin-2 binding protein glycosylation isomer, FIB4: fibrin-4, APRI: aspartate aminotransferase to platelet ratio index, AFP: alpha-fetoprotein, PIVKA-II: vitamin K absence or antagonists-II, LS: liver stiffness, SS: spleen stiffness, PVF: portal venous flow volume, SI: spleen index

tween the two groups were subjected to univariate and multivariate analyses to predict the occurrence (recurrence) of EVs and HCC (primary endpoint). The cutoff values for predicting EVs and HCC occurrence rates were extracted, and the rates divided by this cutoff value were compared between the two subgroups (Fig. 1, 2).

Statistical analysis

A statistical analysis was performed using the SPSS 28.0 J software program (SPSS, Chicago, USA). Continuous values are presented as the mean±standard deviation. All clinical data had a normal distribution, and Student's *t*-test was used to compare values between the two groups. The chi-square test was used to compare sex and underlying diseases. To extract the independent predictors associated with EVs and HCC occurrence, background factors before and after DAAs treatment were analyzed using univariate and multivariate analyses (Cox proportional hazards model). For the significant factors, the receiver operating characteristic (ROC) curve was plotted to calculate the cutoff value to predict the occurrence of EVs and HCC. Cumulative occurrence rates were compared using the Kaplan-Meier method. The log-rank test was used to analyze statistical significance. A p value of less than 0.05 was considered to indicate a statistically significant difference among groups.

Results

Changes after DAAs treatment

In all patients, DAAs treatment was successful, and SVR 12 was obtained. The values of PLT, AST, ALT, albumin, hyaluronic acid, type IV collagen 7s, M2BPGi, FIB4-index, APRI, LS, SS, PVF, and SI all significantly improved after DAAs treatment (Table 1).

Table 2. Comparison between the Esophageal Varices Occurrence and Non-occurrence Groups.

Characteristic	EVs occurrence group (n=7)	Non-occurrence group (n=71)	p value
Duration of survival (days)	1,141±635	818±412	0.160
History of EVs (presence/absence)	5/2	4/67	<0.001
Before DAAs			
Platelet (×10⁴/μL)	12.2±5.4	18.3±6.2	0.013
PT-INR	1.2±0.1	1.0±0.1	0.008
Albumin (g/dL)	3.6±0.3	4.0±0.4	0.004
BTR (μmol/L)	4.4±2.1	5.9±1.7	0.042
M2BPGi (C.O.I.)	5.5±4.5	2.0±2.2	0.002
LS (kPa)	20.1±12.0	10.5±6.9	<0.001
After DAAs			
Platelet (×10⁴/μL)	11.1±1.5	19.5±6.6	<0.001
PT-INR	1.2±0.1	1.0±0.1	<0.001
Cholinesterase (IU/L)	154±98	289±81	0.003
Type IV collagen 7s (ng/mL)	7.8±3.0	5.4±2.0	0.007
FIB4-index	0.5±0.1	0.8±1.1	0.014
SS (kPa)	25.3±10.5	18.1±7.3	0.018
SI change (cm²)	1.3±4.8	-1.4±3.3	0.047

Data are expressed as mean±standard deviation (SD). The Student's *t*-test is used for the comparison of values. A p value less than 0.05 is considered to indicate a statistically significant difference between groups. Only items for which a significant difference was obtained are listed.

Abbreviations are as explained in the legend in Table 1.

Table 3. Comparison between the Hepatocellular Carcinoma Occurrence and Non-occurrence Groups.

Characteristic	HCC occurrence group (n=9)	Non-occurrence group (n=69)	p value
Observation period (days)	807±507	1,187±600	0.073
Age (yrs)	483±555	483±555	0.007
History of HCC (presence/absence)	7/2	2/67	<0.001
Before DAAs			
Platelet (×10⁴/μL)	12.2±5.4	18.3±6.2	0.002
Albumin (g/dL)	3.6±0.3	4.0±0.4	0.002
BTR (μmol/L)	4.4±2.1	5.9±1.7	0.007
M2BPGi (C.O.I.)	5.5±4.5	2.0±2.2	0.030
Hyaluronic acid (ng/mL)	498±313	173±299	0.005
Type IV collagen 7s (ng/mL)	9.4±3.8	6.0±2.3	<0.001
FIB4-index	5.1±1.5	2.8±2.3	0.004
LS (kPa)	19.7±9.4	10.4±7.2	<0.001
After DAAs			
Platelet (×10⁴/μL)	12.6±3.5	19.6±6.7	<0.001
PT-INR	1.1±0.1	1.0±0.1	0.016
Albumin (g/dL)	3.9±0.4	4.1±0.4	0.025
AST (IU/L)	31.9±15.7	22.3±8.5	0.006
BTR (μmol/L)	4.4±1.2	5.9±1.3	0.004
Hyaluronic acid (ng/mL)	314±298	116±169	0.006
Type IV collagen 7s (ng/mL)	7.6±2.5	5.4±2.1	0.008
LS (kPa)	13.8±6.3	8.5±5.5	0.008
SI change (cm²)	21.9±9.8	32.4±9.9	0.018

Data are expressed as mean±standard deviation (SD). The Student's *t*-test is used for the comparison of values. A p value less than 0.05 is considered to indicate a statistically significant difference between groups. Only items for which a significant difference was obtained are listed.

Abbreviations are as explained in the legend in Table 1.

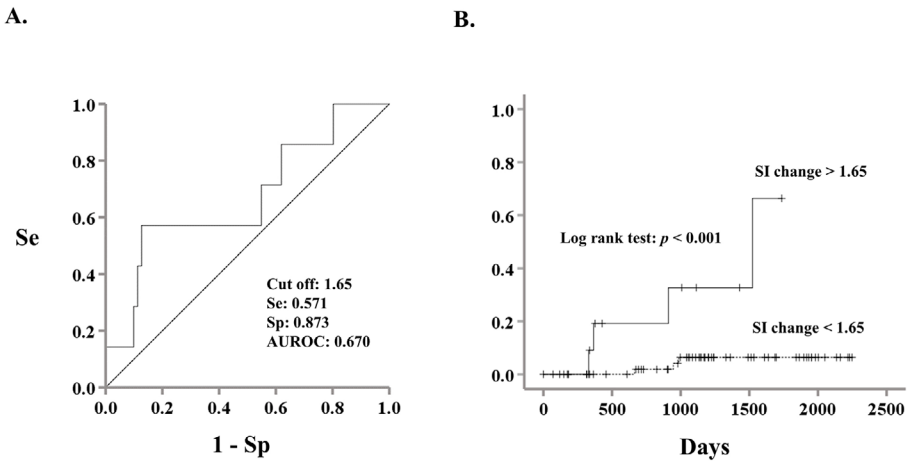

Figure 1. **Receiver operating characteristic curves and occurrence rates of esophageal varices after direct-acting antiviral agents. A: Cutoff value of the spleen index change. The cutoff value of spleen index (SI) change (=value after DAAs-value before DAAs) is 1.65 cm^2 to predict the occurrence of esophageal varices. B: Differences in the occurrence rate of esophageal varices with high and low spleen index changes. The occurrence rate of esophageal varices is significantly higher in patients with an SI change of more than 1.65 than in patients with an SI change of less than 1.65. DAAs: direct-acting antiviral agents, Se: sensitivity, Sp: specificity, AUROC: area under the receiver operating characteristic curve**

Comparison between the EVs occurrence and non-occurrence groups

EVs occurrence (or recurrence) was observed in seven patients. All items for which significant differences were obtained in Table 1 were newly compared between the EVs occurrence and nonoccurrence groups. Significant differences were observed in PLT, PT-INR, albumin BTR, M2BPGi, and LS levels before DAAs treatment. Moreover, significant differences were also observed in PLT, PT-INR, cholinesterase, type IV collagen 7s, FIB4-index, SS after DAAs treatment, and SI change (SI after DAAs-SI before DAAs) (Table 2).

Comparison between the HCC occurrence group and the nonoccurrence group

HCC occurrence (or recurrence) was observed in nine patients. All items for which significant differences were obtained in Table 1 were compared between the HCC occurrence and nonoccurrence groups. Significant differences were observed in PLT, albumin BTR, hyaluronic acid, type IV collagen 7s, and LS levels before and after DAAs treatment. Moreover, significant differences were also observed in M2BPGi, FIB4 index before DAAs, PT-INR, and AST after DAAs (Table 3).

Predictors associated with EVs and HCC occurrence rate

To identify the predictors associated with EVs (and HCC) occurrence, a univariate analysis was performed on the factors with a significant difference between the EVs (HCC) occurrence and nonoccurrence groups (Table 2-4), and a multivariate analysis was performed on the independent factors identified by a univariate analysis (Table 4).

As predictors of EVs occurrence, history of EVs, PLT, PT-INR, albumin, LS before DAAs, SS after DAAs, and SI change were identified by a univariate analysis. Finally, SI change was identified as an independent predictor by a multivariate analysis.

As predictors of HCC occurrence, the factors of history of HCC, PLT, albumin, FIB4-index, LS before DAAs, PLT, PT-INR, AST, LS after DAAs, and SI change were identified by a univariate analysis. Regarding the PLT and LS factors before and after DAAs, the item with a smaller p value was incorporated into the multivariate analysis to exclude any confounding factors (in this case, PLT before DAAs and LS before DAAs were used). Finally, such factors as a history of HCC, the albumin level, and SI change were identified as independent predictors by a multivariate analysis (Table 4).

In addition, we analyzed whether ALT and AFP changes and a history of fatty liver could be predictors of EVs and HCC occurrence. A univariate analysis showed that ALT change and fatty liver were not predictors of EVs occurrence (p=0.347 and 0.136, respectively), but AFP change was identified as a predictor (p=0.027). Regarding HCC occurrence, ALT change and fatty liver were not predictors according to a univariate analysis (p=0.639 and 0.295, respectively). However, AFP change had a tendency to be a predictor (p=0.090) (data not shown).

Receiver operating characteristic curve for predicting EVs and HCC occurrence

Regarding the predictors obtained by the multivariate analysis shown above, ROC curves for predicting EVs and

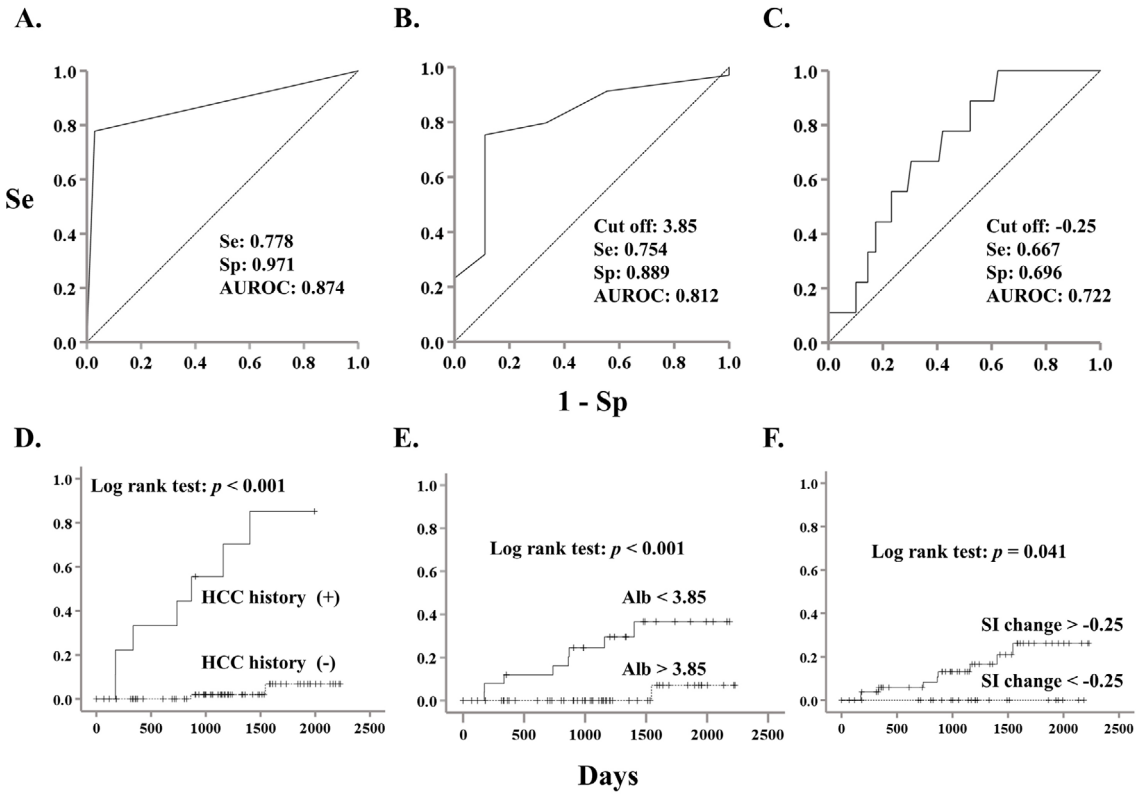

Figure 2. **Receiver operating characteristic curves and occurrence rates of hepatocellular carcinoma after direct-acting antiviral agents. A: Receiver operating characteristic curves for treatment history of hepatocellular carcinoma. B: Cutoff value of albumin before direct-acting antiviral agents. The cutoff value of albumin is 3.85 for predicting the occurrence of hepatocellular carcinoma (HCC). C: Cutoff value of spleen index change. The cutoff value of the spleen index (SI) change is -0.25 cm^2 for predicting the occurrence of HCC. D: Differences in the occurrence rate with or without a history of hepatocellular carcinoma. The occurrence (recurrence) rate is significantly higher in patients with a history of HCC. E: Difference in the occurrence rate with high and low albumin values. The OS occurrence (recurrence) rate of HCC is significantly higher in patients with an albumin value of less than 3.85 than in patients with an albumin value >3.85. F: Differences in the occurrence rate with high and low spleen index changes. The OS occurrence (recurrence) rate of HCC is significantly higher in patients with spleen index (SI) changes of more than -0.25 cm^2 than in patients with SI changes of less than -0.25 cm^2. Se: sensitivity, Sp: specificity, AUROC: area under the receiver operating characteristic curve**

HCC occurrence were calculated and plotted. The optimal cutoff value of SI change for predicting EVs was 1.65 cm^2 (Fig. 1A). Using these cutoff values, we divided all patients into two subgroups (low and high values). The EVs occurrence rate was significantly high in the high SI change group (p<0.001) (Fig. 1B). Regarding the HCC occurrence rate, the optimal cutoff values of albumin and SI change were 3.85 and -0.25 cm^2, respectively (Fig. 2A-C). The occurrence rate was significantly higher in the groups with a history of HCC, a low albumin level, and high SI change than in the other groups for each factor (p<0.001, p<0.001, and p<0.001, respectively) (Fig. 2D-F).

Correlations among the spleen index, elastography, and portal venous volume

The Pearson correlation coefficients between SI, LS, SS, and PVF were calculated (Table 5). There was a tendency toward a positive correlation between the LS before DAAs and the SI before DAAs (r=0.214, p=0.060) (Fig. 3A). A significant correlation was obtained between the LS after DAAs and the SI after DAAs (r=0.446, p<0.001) (Fig. 3B). There was a significant positive correlation between the SS after DAAs treatment and the SI after DAAs treatment (r=0.453, p<0.001) (Fig. 3C). There was a tendency toward a negative correlation between the PVF change and the SI change (r=-0.192, p=0.093) (Fig. 3D).

Discussion

This observational study demonstrated that the independent predictors associated with EVs occurrence after DAAs treatment were SI change, and those associated with HCC occurrence were a history of HCC, the albumin level, and SI change according to multivariate and ROC curve analyses.

Table 4. Predictors Associated with the Occurrence Rate of Esophageal Varices and Hepatocellular Carcinoma.

Factor	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Predictors associated with EVs occurrence rate				
History of EVs	16.8 (3.3-86.7)	<0.001	7.7 (0.7-80.6)	0.088
Platelet Bf	0.8 (0.7-1.0)	0.025	1.0 (0.8-1.3)	0.875
PT-INR Bf	437 (1.3-1.6×10^5)	0.043	0.1 (0.0-478)	0.583
Albumin Bf	0.1 (0.0-0.7)	0.016	0.1 (0.0-3.5)	0.232
LS Bf	1.1 (1.0-1.2)	0.005	1.1 (1.0-1.2)	0.238
SS Af	1.1 (1.0-1.1)	0.055	Not performed	
SI change	1.3 (1.0-1.5)	0.019	1.2 (1.0-1.5)	0.045
Predictors associated with HCC occurrence rate				
History of HCC	37.1 (7.6-180.9)	<0.001	2,377 (18-3.1×10^5)	0.002
Platelet Bf	0.8 (0.7-1.0)	0.011	1.1 (0.8-1.6)	0.618
Albumin Bf	0.1 (0.0-0.7)	0.014	422 (1.7-1.0×10^5)	0.032
FIB4-index Bf	1.2 (1.0-1.5)	0.026	3.1 (1.0-9.5)	0.054
LS Bf	1.1 (1.0-1.1)	0.007	1.0 (0.8-1.1)	0.945
Platelet Af	0.8 (0.7-1.0)	0.014	Not performed	
PT-INR Af	187 (2-20,371)	0.029	2,885 (0.1-1.2×10^8)	0.068
AST Af	1.0 (1.0-1.1)	0.027	1.0 (0.9-1.1)	0.681
LS Af	1.1 (1.0-1.2)	0.020	Not performed	
SI change	1.3 (1.1-1.5)	0.009	1.2 (1.0-1.5)	0.009

Data are analyzed using the Cox proportional hazard model for the occurrence of esophageal varices (EVs) and hepatocellular carcinoma (HCC) after direct-acting antiviral agents (DAAs). A p value less than 0.05 is considered to indicate a statistically significant difference between groups.

OR: odds ratio, CI: confidence interval, Bf: before DAAs, Af: after DAAs, PT-INR: prothrombin time-international normalized ratio, AST: aspartate aminotransferase, FIB4: fibrin-4, LS: liver stiffness, SS: spleen stiffness, SI: spleen index

Table 5. Correlation between Spleen Index, Elastography, and Portal Venous Volume.

Pearson correlation coefficient	r value	p value
LS Bf and SI Bf	0.214	0.060
LS Af and SI Af	0.446	<0.001
LS change and SI change	0.044	0.704
SS Bf and SI Bf	0.180	0.115
SS Af and SI Af	0.453	<0.001
SS change and SI change	0.044	0.704
PVF Bf and SI Bf	0.093	0.416
PVF Af and SI Af	-0.108	0.346
PVF change and SI change	-0.192	0.093

A p value less than 0.05 is considered to indicate a statistically significant difference.

LS: liver stiffness, SS: spleen stiffness, SI: spleen index, PVF: portal venous flow, Bf: before direct-acting antiviral agents (DAAs), Af: after DAAs

The best cutoff values of SI change for predicting EVs and HCC occurrence were 1.65 and -0.25 cm^2, respectively. This implied that EVs and HCC occurrence (or recurrence) were significantly observed in cases with spleen enlargement after DAAs treatment compared with those of patients before DAAs treatment. Hirooka et al. clarified that SI measurement by ultrasonography (including one-, two-, and three-dimensional methods) was strongly correlated with the spleen volume measured by CT (10). Therefore, SI change indicated splenic volumetric change in our study. Splenomegaly occurred in patients with portal hypertension. Although the pathogenic mechanisms causing spleen enlargement are still poorly understood, there are some architectural alterations, including pulp hyperplasia, congestion from an increased blood flow, and fibrosis in the spleen (11, 12). Davidov et al. reported that splenomegaly was an independent predictor of the aggravation of liver fibrosis stage at 1 year after DAAs treatment according to a multivariate analysis, and they showed that EVs occurred in 50% of patients in the liver fibrosis aggravation group during the follow-up period (13). The EVs occurrence rate was higher than that of other studies, in which the EVs occurrence rate was reported to be 4.5% in patients with F3 - F4 stage chronic liver disease after DAAs treatment during a 4-year follow-up (14). Therefore, the existence of splenomegaly, which indicates the congestion state in the splenic and portal veins, is important for predicting EVs occurrence. Cho et al. reported that the median spleen volume increased from 438.2 to 580.8 cm^3, and splenic enlargement occurred in 56% (15/27) of the patients after the treatment of GVs by balloon-occluded retrograde obliteration (BRTO) (15). This indicated that the elevation of portal venous pressure after BRTO caused splenic enlargement. In

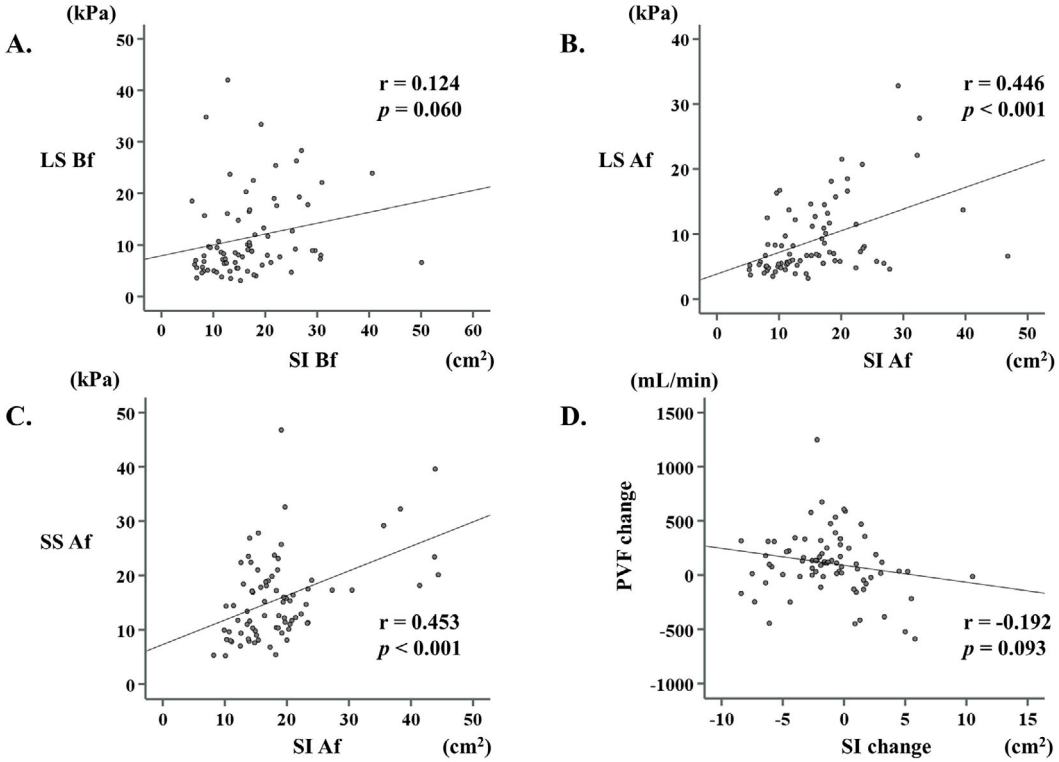

Figure 3. Correlation between spleen index, elastography, and portal venous volume. A: Correlation between spleen index and liver stiffness before DAAs treatment. There was a tendency toward a positive correlation between liver stiffness (LS) before DAAs treatment and the spleen index (SI) before DAAs treatment (r=0.214, p=0.060). B: Correlation between spleen index and liver stiffness after DAAs treatment. A significant correlation was obtained between the LS after DAAs and the SI after DAAs (r=0.446, p<0.001). C: Correlation between spleen index and spleen stiffness after DAAs treatment. There was a significant positive correlation between the SS after DAAs and the SI after DAAs (r=0.453, p<0.001). D: Correlation between portal venous flow volume change and spleen index change. There was a tendency toward a negative correlation between the portal venous flow volume change and the SI change (r=-0.192, p=0.093).

our study, patients with increased SI after DAAs administration could potentially demonstrate a state of high portal venous pressure. Very recently, the consensus on portal hypertension of the Baveno VII workshop was published (16). In this consensus study, it is stated that splenic TE ≥50 kPa has a risk of CSPH and that splenic TE ≤40 kPa has a low probability of high-risk EVs. In advance, SS has been proposed as a good marker for predicting EVs compared with LS. Additionally, in a study using point SWs, the area under the receiver operating characteristic curve (AUROC) of splenic SWs for predicting CSPH and high-risk EVs was higher than that of hepatic SWs (17). In our previous study using a carbon tetrachloride rat model, the length of the spleen was positively correlated with the SS (6). Therefore, we analyzed the correlation between the SI, LS, SS, and PVF in the present study. The LS and SS after DAAs treatment were significantly correlated with the SI after DAAs treatment, and there was a negative tendency between PVF change and SI change. From this result, it seems that small changes in SI may improve the PH status and increase PVF. Furthermore, SI was significantly correlated with both LS and SS after virus elimination, thus suggesting that liver and spleen fibrosis (not inflammation) may be a factor correlating with SI.

A history of HCC treatment was one of the independent predictors of HCC occurrence. This result is consistent with that of our previous study (8). Albumin was also a predictor, and the same result was observed in a multivariate analysis by Tahata et al. (18). The albumin-bilirubin (ALBI) score, calculated by albumin and bilirubin values, is a strong marker of the prognosis of patients with LC and the liver function (19). A report showed that the ALBI score was a predictor of the development of HCC and EVs in an observational study of the natural history of advanced chronic liver disease (20). The reason for the suppression of HCC development by high albumin levels remains unknown. However, Nojiri et al. clarified that the presence of albumin in the serum reduced the phosphorylation of retinoblastoma proteins and enhanced the expression of p21 and p57 in Hep3B cells. The G0/G1 cell population was increased, and they demonstrated that albumin suppressed HCC proliferation (21). Regarding the relationship between splenomegaly and HCC occurrence, a report showed that a high splenic volume was an independent predictor of posthepatectomy HCC recurrence and overall survival (22). Hashimoto et al.

reported that patients with splenomegaly have a greater number of splenic CD4+ regulatory T cells and programmed death ligand 1- and ligand 2-expressing cells than control patients. In this report, it was suggested that patients with splenomegaly had poorer tumor immunity than those without splenomegaly (23). Therefore, SI change is considered to be an independent predictor of HCC occurrence in our study.

We previously reported that AFP was a predictor of HCC occurrence after DAAs treatment in a retrospective study of 234 HCV patients (8). However, in the present study, a significant difference was not obtained in the AFP value between the HCC occurrence group and the nonoccurrence group (AFP values before DAAs were 409.8±1,058.3 and 7.9±14.0, p=0.354. AFP values after DAAs treatment were 76.1±167.0 and 3.8±5.4, p=0.296). Although the AFP value is clearly high in the occurrence group, the reason why a significant difference cannot be obtained seems to be that the populations of the two groups are not equally distributed (there are many outliers). We also performed a univariate analysis with the Cox proportional hazards model for AFP before and after DAAs treatment. As a result, the p values were 0.052 and 0.004, suggesting that AFP after DAAs treatment may be a predictor of HCC occurrence (data not shown). However, the AFP factor was not included in the multivariate analysis in this study because no significant difference was obtained in the two-group comparison. A poor reduction of ALT and AFP and the presence of fatty liver have been reported as carcinogenic risk factors after SVR (24-26). Therefore, we checked whether changes in ALT and AFP and the existence of a fatty liver are risk factors for HCC occurrence according to a univariate analysis. As a result, only AFP change had a tendency to be a predictor, but there was no significant difference.

This study is associated with several limitations. First, this was a single-center observational study. Second, the number of blood examinations was limited. Third, the number of patients was too small to obtain any verifiable scientific evidence, so this study can only be considered preliminary research. However, to the best of our knowledge, this is the first study to demonstrate that SI change is a predictor of EVs and HCC occurrence after DAAs administration.

The authors state that they have no Conflict of Interest (COI).

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