【 ORIGINAL ARTICLE 】

The Association between the Platelet Count and Liver Volume in Compensated Cirrhosis Patients after the Eradication of Hepatitis C virus by Direct-acting Antivirals

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Abstract:

Objective Although most patients who obtain a sustained virological response (SVR) show an improved liver function, some show decreased platelet counts after the eradication of hepatitis C virus (HCV). The aim of this retrospective study was to clarify the association of the liver and spleen volumes with the platelet count after SVR achieved by direct-acting antiviral (DAA) treatment.

Methods This study enrolled 36 consecutive patients treated by DAAs who obtained an SVR between September 2014 and December 2018. The liver and spleen volumes were derived from computed tomography scans obtained at pretreatment, SVR, and 48 weeks after SVR. No patient developed hepatocellular carcinoma during this study.

Results Compared with pretreatment, the median aspartate aminotransferase, alanine aminotransferase, albumin serum levels, and platelet counts were significantly improved at SVR and 48 weeks after SVR. The liver/spleen volumes per body weight had decreased significantly from 22.5/4.2 mL/kg at baseline to 21.1/3.6 mL/kg at 48 weeks after SVR. The change in the liver volume was associated with the change in the platelet count, and the change in the spleen volume was negatively associated with the change in the serum albumin level. A multivariate analysis identified the change in the liver volume (≥95%, odds ratio 76.9, p=0.005) as the factor associated with improvement in the platelet count at 48 weeks after SVR. The patients with an increased liver volume at 48 weeks after SVR showed an increased platelet count.

Conclusion Both the liver and spleen volume decreased significantly after the eradication of HCV. The patients with a re-increased liver volume showed a rapid increase in the platelet count.

Key words: direct-acting antiviral (DAA), sustained virological response (SVR), liver volume, platelet count

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Introduction

Hepatitis C virus (HCV) infection is one of the most important public health concerns in the world. An estimated 270-300 million people are infected worldwide, and the prevalence of the infection is expected to peak in the next 10-20 years (1). However, the proportion of patients obtaining a sustained virological response (SVR) due to direct-acting antiviral (DAA) treatment has dramatically increased to more than 95% (2).

Most patients treated by DAAs show an improved liver function after obtaining an SVR. However, some patients have persistently abnormal liver function test results after SVR. Platelet counts also improve after SVR, and the changes in platelet counts were greater in patients obtaining an SVR than in those without SVR after interferon (IFN)-based therapy (3).

The liver and spleen volumes derived from computed tomography (CT) have been used to assess graft volumes and to predict operative outcomes in patients with decompensated liver disease (4, 5). Several reports have discussed the relationship between the liver volume and the functional reserve in patients with cirrhosis (6-8). In cirrhotic patients, the liver volume decreased in accordance with the progression of hepatic fibrosis, and the liver and spleen volumes were suggested to be potentially related to the degree of portal hypertension (9-11). However, the association between remodeling changes in the liver/spleen volume and changes in the platelet count in patients undergoing DAA therapy has remained unclear.

The present study assessed the changes in the liver/spleen volumes and platelet counts over a two-year period in patients who obtained an SVR by DAA therapy and clarified the association between the change in the platelet counts and liver volume.

Materials and Methods

Patients

We performed a retrospective single-center study of consecutive patients with HCV infections from the outpatient clinic at the Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, from September 2014 to December 2018. All patients had started an oral DAA regimen (daclatasvir (DCV), asunaprevir (ASV), sofosbuvir (SOF), ledipasvir (LDV), ombitasvir (OBV), ritonavir-boosted paritaprevir (PTV/r)] for 12 to 24 weeks. An SVR was defined as undetectable HCV RNA at 24 weeks after the end of treatment.

The exclusion criteria were any other liver diseases, including autoimmune hepatitis, drug-induced liver disease, and primary biliary cholangitis. Patients with Child-Pugh class B and C cirrhosis or who developed hepatocellular carcinoma (HCC) during this study period were excluded because only patients with Child-Pugh A can receive DAA treatment in Japan, and anticancer therapy, such as liver resection or radiofrequency ablation, influence the liver volume. Among 248 patients undergoing DAA therapy, 77 were diagnosed with Child-Pugh class A liver cirrhosis. We excluded 5 patients who developed HCC during the follow-up period and 36 who lacked CT imaging data. Subsequently, 36 patients with Child-Pugh class A who underwent CT imaging at pretreatment, SVR, and 48 weeks after SVR were enrolled in this study.

Patients underwent CT imaging every 6-12 months to determine the incidence of HCC. All patients provided information on demographic factors, medical history, and medication usage. Type 2 diabetes mellitus was diagnosed according to the Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. No patients had been administered growth factors, such as interleukin (IL)-11 agent.

All patients provided their written informed consent concerning this study at the start of DAA treatment, and this study was conducted in accordance with the 2013 Declaration of Helsinki and approved by the institutional ethics committee.

Laboratory imaging and clinical parameters

Laboratory assays included blood cell counts and measurements of serum concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transpeptidase (GGT), albumin, total cholesterol, triglycerides, and alpha-fetoprotein (AFP). The body mass index (BMI) was calculated as the weight (kg)/height (m^2). The FIB-4 index was calculated using the following formula: FIB-4 index = [(age (years) × AST (U/L)]/platelet count $(10^9/L)] \times [ALT (U/L)]^{1/2}$. After CT scanning, 1-mm portal vein phase reconstruction images obtained from raw data were divided and transferred to workstations (Ziosoft, Tokyo, Japan). The liver and spleen volume assessments of CT examinations were calculated automatically by the workstation software program.

All parameters were evaluated at pretreatment, SVR, and 48 weeks after SVR. All of the CT studies were performed using the same type of scanner with the same protocol.

Statistical analyses

The liver volume per body weight (LV/BW) and spleen volume per body weight (SV/BW) were calculated at each point. The change in biological and imaging parameters at 48 weeks after SVR from those at baseline are shown as the delta (%). Distributions of the characteristics of the study patients were assessed by the chi-squared test or Mann-Whitney U-test, as appropriate. Pearson's coefficient was assessed to analyze the factors associated with changes in the liver and spleen volume. In the multivariate logistic regression analysis, the platelet count, albumin, total bilirubin, and change in LV/BW at baseline to 48 weeks after SVR were included as confounders. Odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. Statistical comparisons were performed using the SPSS software program (SPSS, Chicago, USA). All p values less than 0.05 by the two-tailed test were considered significant.

Results

Patients' characteristics

Table 1 shows the characteristics of the patients at the start of DAA therapy who obtained an SVR. This study included 36 patients [23 (63.9%) men] with a median age of 68 years old. The distributions of DAA therapies were as follows: DCV/ASV n=12 (33.3%), SOF/LDV n=22 (61.1%), and SOF/ribavirin n=2 (5.6%). At the start of treatment, 22.2% of patients were alcohol drinkers, 11.1% had fatty liver, and 25.0% had varices. Among the 36 patients, 22 (61.1%) had a history of HCC. Seven patients had been

Table 1.   Characteristics of 36 Patients at Start of DAA Therapy.

	n=36
HCV genotype (1a/1b/2a/2b)	1/33/1/1
Gender (male/female)	23/13
Age (years)	68 (48-85)
BMI (kg/m^2)	21.7 (16.4-30.1)
Alcohol intake	8 (22.2%)
Diabetes mellitus	11 (30.6%)
Hypertension	15 (41.7%)
Fatty liver	4 (11.1%)
Esophageal varices	9 (25.0%)
Past history of HCC	22 (61.1%)
Total bilirubin, mg/dL	0.89 (0.32-1.66)
AST, U/L	44 (20-291)
ALT, U/L	35 (10-175)
GGT, g/dL	28 (12-132)
Albumin, g/dL	3.9 (2.7-4.6)
Platelet count, ×10^4/μL	10.3 (4.5-29.9)
Total cholesterol, mg/dL	154 (115-231)
Triglyceride, mg/dL	82 (46-204)
LDL-C, mg/dL	88 (58-170)
AFP, ng/mL	7.6 (1.8-90.0)
FIB-4 index	5.08 (1.50-32.52)
Liver volume/body weight, mL/kg	22.5 (14.5-37.9)
Spleen volume/body weight, mL/kg	4.2 (1.1-14.4)

Results are presented as n (%) for qualitative data or as median (range) for quantitative data.
DAA: direct acting antivirals, BMI: body mass index, HCC: hepatocellular carcinoma, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, LDL-: low-density lipoprotein cholesterol, AFP: alpha-fetoprotein

treated with radiofrequency ablation, and 15 had been treated with transcatheter arterial chemoembolization. The median LV/BW was 22.5 mL/kg, and the median SV/BW was 4.2 mL/kg.

Changes in biochemistry and liver/spleen volumes

The median serum levels of AST and ALT were significantly lower at SVR and 48 weeks after SVR than at baseline (Fig. 1). These values did not change significantly between SVR and 48 weeks later (Fig. 1). The median platelet count and serum albumin level were significantly increased at SVR and 48 weeks after SVR than at baseline (Fig. 1). These values also did not change significantly between SVR and 48 weeks later (Fig. 1). The median values of LV/BW decreased significantly from 22.5 mL/kg at baseline to 21.1 mL/kg at SVR. The median value did not change significantly between SVR and 48 weeks later (Fig. 2). However, the SV/BW decreased significantly between baseline (4.2 mL/kg) and SVR, between SVR and 48 weeks after SVR (3.6 mL/kg), and between baseline and 48 weeks after SVR (Fig. 2). The change in the LV/BW (%) correlated with change in the platelet count (p=0.007) (Fig. 3a) but not with the change in the albumin level (Fig. 3b). The change in the

SV/BW (%) correlated with the platelet count at baseline (p = 0.032) (Fig. 3c), and the SV/BW (%) the negatively correlated with the change in the albumin level (p=0.005) (Fig. 3d). However, the SV/BW(%) was not correlated with the change in the platelet count (Fig. 3e). The platelet count, LV/BW, and SV/BW were not markedly different between ribavirin-including and ribavirin-free regimens.

In the present study, nine patients had esophagus varices at baseline. The grade of their varices did not change after SVR.

Factors associated with improvements in platelet counts

The median platelet counts increased significantly from 10.3×10^4/μL at baseline to 11.6×10^4/μL at 48 weeks after SVR. In our study population, 10 (27.8%) patients showed a platelet count that decreased from baseline to 48 weeks after SVR. Table 2 lists the characteristics of study patients stratified by changes in their platelet counts. The differences in the gender, age, BMI, alcohol intake, and prevalence of complications between the patients whose counts increased and those whose counts decreased were not significant. The baseline liver function tests of the two groups also did not reflect the changes in the platelet counts. The median LV/BW values at baseline of the patients with a decreased platelet count and those with an increased platelet count were 24.0 mL/kg and 21.1 mL/kg, respectively (p=0.037). The difference in the median SV/BW values at baseline between the patients with a decreased platelet count and an increased platelet count was not significant. The change in the median LV/BW value of patients with decreased platelet counts between baseline and 48 weeks after SVR (87%) was significantly smaller than the change in the median value of patients with an increased platelet count (97%) (p<0.001). The differences between the changes in the SV/BW values were not significant.

To identify factors significantly associated with improved platelet counts, we performed a multivariate logistic regression analysis. The following factors were evaluated in the multivariate analysis: platelet count, albumin, total bilirubin, and change in LV/BW at baseline to 48 weeks after SVR. The change in the LV/BW at 48 weeks after SVR (≥95%) was shown to be an independent factor associated with an improved platelet count (adjusted OR 76.9, 95% CI 3.78-1,000; p=0.005) (Table 3).

Association between the liver volume and platelet count

To evaluate the relationships between the liver volume and platelet counts, we followed the changes in the LV/BW values according to the changes in the platelet counts from baseline to 48 weeks after SVR (Fig. 4). In the patients with increased platelet counts, the LV/BW at SVR and 48 weeks later compared to baseline was 0.98 and 1.02, respectively. In patients with decreased counts at 48 weeks after SVR, the LV/BW at SVR and 48 weeks later compared to base-
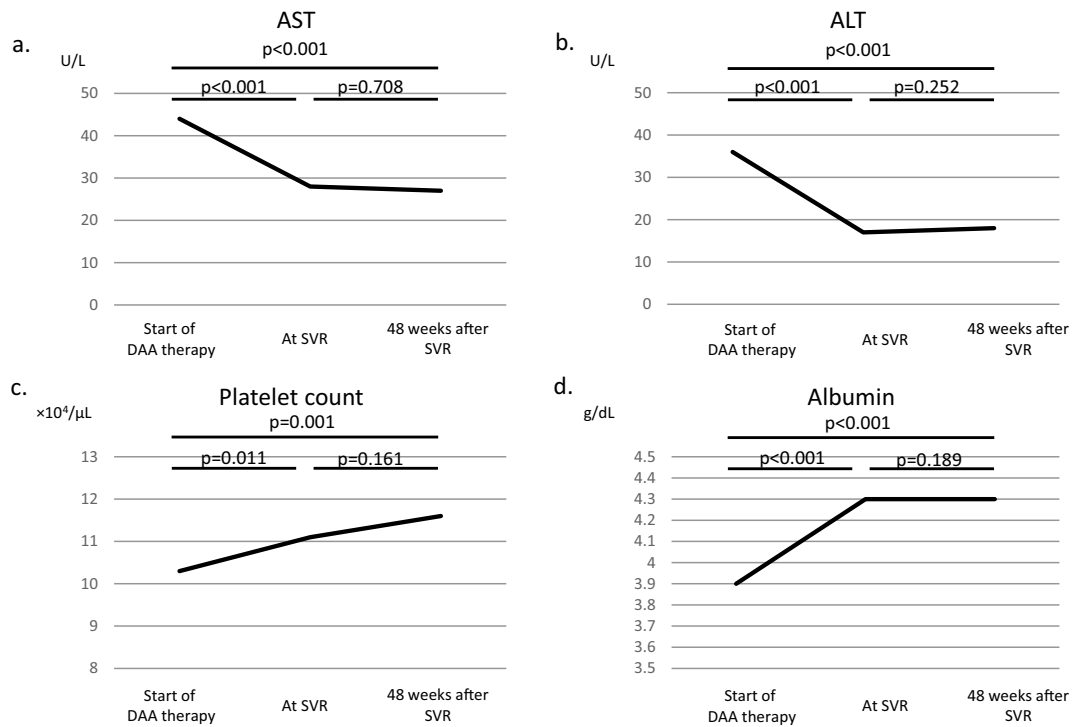
Figure 1. The clinical parameters of patients treated with direct-acting antivirals (a: AST, b: ALT, c: Platelet Counts, d: Albumin) at the start of treatment, sustained virological response (SVR), and 48 weeks after SVR.
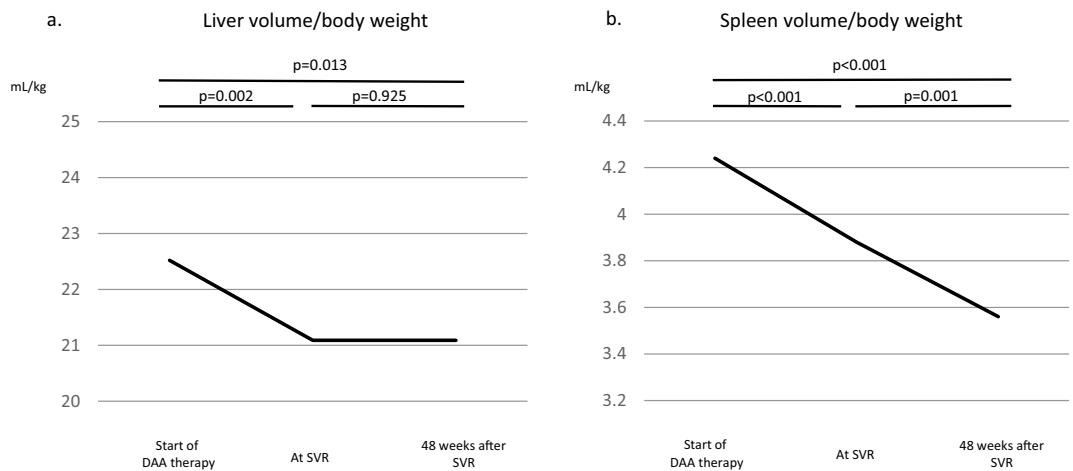

Figure 2. The clinical parameters of patients treated by direct-acting antivirals (a: liver volume/body weight, b: spleen volume/body weight) at the start of treatment, sustained virological response (SVR), and 48 weeks after SVR.

line was 0.90 and 0.83, respectively. The changes at each point in the LV/BW values of the patients with increased platelet counts were significantly greater than those in the patients with decreased platelet counts (p=0.017, p<0.001, respectively). We followed the LV/BW value of 24 patients at 96 weeks after SVR. The remaining 12 patients were excluded due to a lack of data or the development of HCC (Fig. 4). The LV/BW value in the patients with decreased counts at 48 weeks increased from 0.83 to 0.91 at 96 weeks after SVR. The median platelet count increased significantly from 8.0×10⁴/μL at 48 weeks after SVR to 10.4×10⁴/μL at

96 weeks after SVR (p=0.020).

Discussion

We investigated the relationship between the liver volume and the changes in platelet counts of Japanese patients with HCV infection treated with DAAs who obtained an SVR. The patients with decreased platelet counts at 48 weeks after SVR showed a greater reduction rate in the liver volumes than those with increased platelet counts. The reduction rate in the liver volume was correlated with the change in the
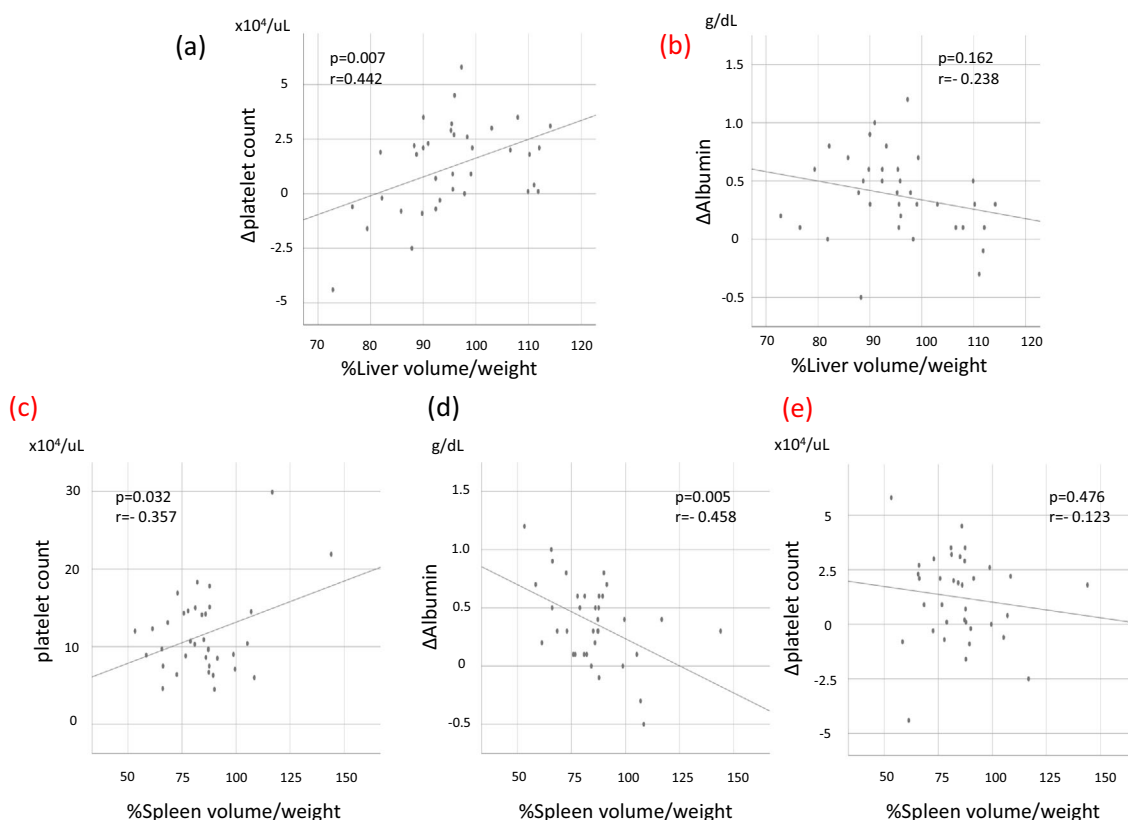
Figure 3. The association between a: the change in the liver volume and platelet count, b: the change in the liver volume and albumin level, c: the change in the spleen volume and platelet count at baseline, d: the change in the spleen volume and albumin level, and e: the change in the spleen volume and platelet count.

platelet count, while the reduction rate in the spleen volume was correlated with the change in the albumin level. To our knowledge, this is the first study to estimate the changes in the liver and spleen volumes by CT and to investigate the relationships between the liver volume and platelet count in Japanese patients with HCV treated by DAAs after achieving an SVR.

In the present study, the liver volume significantly decreased from baseline to SVR and increased from 48 to 96 weeks after SVR in this study. The platelet counts correlated with the changes in the liver volume and increased with liver volume. In the decreased phase, the eradication of HCV by DAAs reduced hepatic inflammation, which might have led to a reduction in the liver volume.

The increase in the platelet count had several causes, including splenic sequestration, autoimmune destruction, and a decreased production of growth factors (12-14). A previous study reported that the spleen size decreased while the platelet count increased following IFN therapy (3). Interventional management for splenomegaly by methods such as partial splenic artery embolization (PSE) or splenectomy also leads to increased platelet counts and an improved liver function (15). In our study, the spleen volume decreased significantly, regardless of changes in the platelet count.

The platelet-associated immunoglobulin G (PA-IgG) is an important cause of thrombocytopenia in HCV infection.

Honma et al. reported that the level of PA-IgG was negatively correlated with the platelet count at baseline and significantly decreased after the eradication of HCV by DAA (16). They concluded that only the platelet count at baseline was associated with the normalization of PA-IgG. In the present study, the platelet count at baseline was not correlated with an improving platelet count at 48 weeks after SVR. This result suggests that the effect of decreasing PA-IgG was limited in patients with cirrhosis. Factors associated with vascular endothelial dysfunction, such as von Willebrand Factor and thrombomodulin, have also been found to be significantly correlated with the platelet count in patients with HCV-related hepatitis (13). However, there have been no reports analyzing the association between changes in these factors and the platelet counts after SVR.

The decreased production of thrombopoietin (TPO) in the liver is another mechanism underlying thrombocytopenia. TPO is a growth factor produced by the liver that stimulates the development of the megakaryocytic line in the bone marrow and has been found to decline with advancing HCV-related liver disease. No published reports have mentioned an association between TPO and liver volume. However, since the liver is the main organ that produces TPO, an increasing liver volume might lead to higher levels of TPO and an increased platelet count. The reduction in the spleen volume also affects the liver volume. Transforming growth

Table 2. Characteristics of 36 Patients according to Change in Platelet Count from Baseline to after 48 Weeks of SVR.

	Platelet count decreased n=10	Platelet count increased n=26	p
Gender (male/female)	5/5	18/8	0.440
Age (years)	72 (59-77)	70 (48-85)	0.876
BMI (kg/m^2)	21.4 (18.6-25.1)	22.2 (16.4-30.1)	0.741
Alcohol intake	1 (10.0%)	7 (26.9%)	0.397
Diabetes mellitus	3 (30.0%)	8 (30.8%)	1.000
Hypertension	4 (40.0%)	11 (42.3%)	1.000
Fatty liver	1 (10.0%)	3 (11.5%)	1.000
Esophageal varices	3 (30.0%)	6 (23.1%)	0.675
Past history of HCC	5 (50.0%)	17 (65.4%)	0.462
At baseline			
Total bilirubin, mg/dL	1.02 (0.61-1.66)	0.85 (0.32-1.26)	0.080
AST, U/L	41 (21-77)	42 (20 -291)	0.337
ALT, U/L	40 (13-86)	35 (10 -175)	0.931
GGT, g/dL	29 (14-132)	29 (12 -110)	0.768
Albumin, g/dL	3.9 (3.3-4.6)	4.0 (2.7 -4.6)	0.876
Platelet count, ×10^4/μL	9.7 (4.5-29.9)	10.5 (4.6 -21.9)	0.614
AFP, ng/mL	8.2 (2.4-90.0)	5.7 (1.8-55.9)	0.377
FIB-4 index	5.00 (1.50-14.21)	4.67 (1.88-32.52)	0.876
Liver volume/body weight, mL	24.0 (20.7-35.7)	21.1 (14.5-37.9)	0.037
Spleen volume/body weight, mL	5.35 (1.1-11.3)	3.88 (1.1-14.4)	0.433
ΔLiver volume/body weight, %	87 (73-98)	97 (82-114)	<0.001
ΔSpleen volume/body weight, %	89 (58-117)	85 (53-144)	0.543

Results are presented as n (%) for qualitative data or as median (range) for quantitative data.
SVR: sustained virological response, BMI: body mass index, HCC: hepatocellular carcinoma, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transferase, AFP: alpha-fetoprotein

Table 3. Factors Associated with Improvement in Platelet Count at 48 Weeks after SVR from Baseline Evaluated by Multivariate Analysis.

	Category	Multivariate analysis	
		OR (95% CI) [a]	p value
Platelet count	1:<10×10^4/μL		0.122
	2: ≥10×10^4/μL		
Albumin	1:<3.8 g/dL		0.413
	2: ≥3.8 g/dL		
Total bilirubin	1: ≥1.0 mg/dL		0.225
	2:<1.0 mg/dL		
Δ Liver volume/body weight	1:<95%	1	0.005
	2: ≥95 %	76.9 (3.78-100)	

OR: odds ratio, CI: confidence interval

factor (TGF)-β, which is derived from the spleen and hepatic stellate cells, was found to inhibit liver regeneration (17). Asanoma et al. reported that TGF-β1 and cytokines such as IL-6, which are produced by macrophages in the spleen, might affect the progression of liver fibrosis and regeneration in patients with liver cirrhosis (15).

Several limitations associated with the present study warrant mention. First, this was a retrospective single-center study with an insufficient number of participants in order to exclude the effect of antitumor therapy. This small sample size may over- or underestimate the relationship between changes in the platelet count and changes in the LV/BW. The wide 95% confidential interval was also affected by the small sample size. Second, we did not evaluate the change in TPO or other liver regenerative factors in the follow-up period. Additional studies evaluating the hematopoietic ability in relation to the liver volume and platelet count are needed. Investigations are also needed to evaluate the asso-
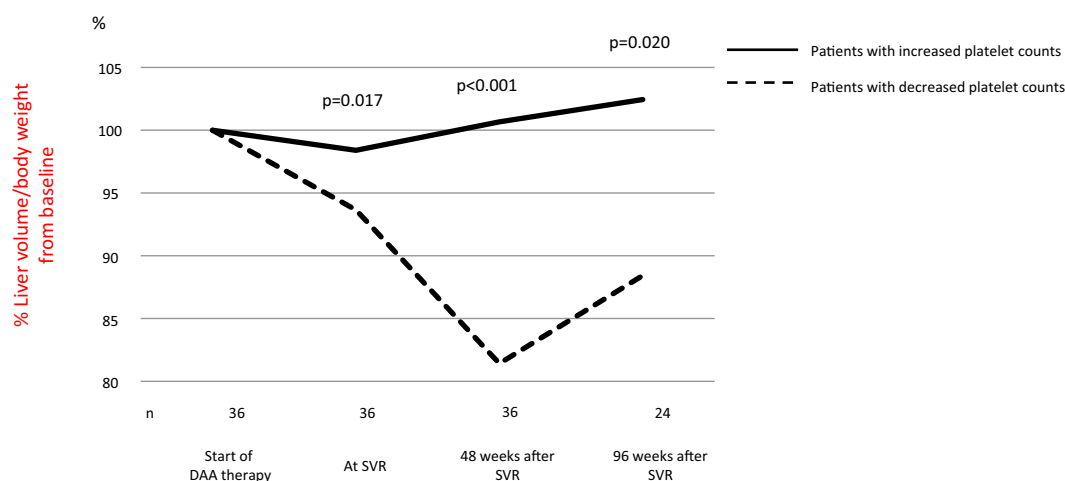
Figure 4. The percent liver volume/body weight according to the change in the platelet counts from baseline to 48 weeks after sustained virological response from baseline.

ciation between the platelet count and portal vein pressure or hepatic venous pressure gradient (HVPG). We were unable to estimate the change in HVPG and the impact of HVPG on the platelet count. According to these limitations, the exact relationship among such factors as the SVR, liver volume and platelet count still remains unclear.

Conclusion

In conclusion, the change in the platelet count after SVR achieved through DAA therapy is related to the change in the liver volume. Although most patients experienced an increase in their platelet count and liver volume as a result of DAA therapy, those showing a slow improvement in the platelet count might also show a reduced regenerative ability of the liver. The platelet count after SVR obtained by DAA therapy should be considered a noninvasive liver marker of regeneration and should therefore be monitored over time.

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

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