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ORIGINAL ARTICLE

Liver stiffness measurement predicts hepatocellular carcinoma development in patients treated with direct-acting antivirals

Yoshihiko Tachi,* Takanori Hirai,* Yuko Kojima,* Yoji Ishizu,† Takashi Honda,† Teiji Kuzuya,† Kazuhiko Hayashi,† Masatoshi Ishigami† and Hidemi Goto†

*Department of Gastroenterology, Komaki City Hospital, Komaki and †Department of Gastroenterology and Hepatology, Nagoya University School of Medicine, Nagoya, Japan

Key words
acoustic radiation force impulse elastography, chronic hepatitis C, hepatocellular carcinoma, liver stiffness, sustained virological response.

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Correspondence
Yoshihiko Tachi, Department of Gastroenterology, Komaki City Hospital, 1-20, Joubushi, Komaki, Aichi 485-8520, Japan. Email: ytachi@komakihp.gr.jp

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Abstract

Background and Aim: Predictive factors for hepatocarcinogenesis following eradication of hepatitis C virus by direct-acting antivirals (DAAs) are unknown. The aim of the study was to investigate the relationships between liver stiffness (LS) using acoustic radiation force impulse (ARFI) erastograghy and the development of hepatocellular carcinoma (HCC) in patients who achieved sustained virological response (SVR) treated with DAA.
Methods: In this prospective study, we enrolled 263 hepatitis C patients with SVR who underwent ARFI before DAA treatment. Thirty patients had previous HCC.
Results: The median LS value according to ARFI measurements was 1.34 m/s (range: 0.67–4.35). During the follow-up period (median: 18.1 months), development of HCC occurred in 19 patients (7.2%; HCC occurrence in 7 patients and HCC recurrence in 12 patients). By multivariate Cox regression analysis, HCC history (hazard ratio [HR]: 10.634; 95% confidence interval [CI]: 4.13–27.37; $P = 0.001$), older age (HR: 4.638; 95% CI: 1.63–13.61; $P = 0.004$) and higher total bilirubin levels (HR: 4.189; 95% CI: 1.66–10.60; $P = 0.002$) were independent predictors for the development of HCC, and higher LS value (≥1.73 m/s) at baseline was an independent predictor for HCC occurrence (HR: 8.350; 95% CI: 1.62–43.09; $P = 0.011$). The cumulative recurrence of HCC was statistically similar according to the degree of LS in patients who were previously treated for HCC.
Conclusion: The LS value at baseline is useful for predicting HCC occurrence. Thus, even if SVR is achieved, patients with higher LS at baseline must be followed carefully for HCC occurrence.

Introduction

Hepatitis C virus (HCV) infection is a leading cause of chronic hepatitis, cirrhosis, and hepatocellular carcinoma (HCC), and is a major global public health issue.[1,2] The emergence of novel direct-acting antivirals (DAAs) against HCV has dramatically increased the number of patients who achieve a sustained virological response (SVR).[3,4] The elimination of HCV will prevent the progression of chronic hepatitis and associated complications.[5] Several studies have reported that achievement of SVR results in the resolution of liver fibrosis[6,7] and a decreased incidence of HCC.[8] However, development of HCC is sometimes seen even in patients who achieve SVR after DAA treatment, indicating the need for continuous surveillance for HCC after the eradication of HCV.[9–12] Several previous studies have reported that the degree of liver fibrosis is closely associated with the risk of the development of HCC in patients with chronic hepatitis C.[13] Pretreatment staging is important in order to plan initial management of the patient post-SVR. As liver fibrosis persists even after the elimination of HCV despite its gradual resolution after SVR is achieved, accurate estimation of liver fibrosis is desirable.

Previous studies have reported that advanced fibrosis, advanced age, lower albumin levels, lower platelet counts, and higher α-fetoprotein (AFP) levels before and after treatment are important predictors of HCC in patients who achieve SVR with interferon-based treatment.[14–16] However, the risk factors for the development of HCC in patients who achieve SVR after DAA treatment have not been adequately clarified in a prospective study.

Preliminary study results indicate that the acoustic radiation force impulse (ARFI) elastography is a noninvasive method for measuring liver stiffness (LS) and can be used for the

diagnosis of liver fibrosis and cirrhosis in chronic liver disease.[17–23] However, the relationship between LS and its impact on the development of HCC remains unclear in chronic hepatitis C patients who achieved SVR following DAA therapy.

To understand this relationship, we enrolled 263 patients who had achieved an SVR following DAA therapy and clinical follow-up study in which LS measurements were obtained from patients before treatment. The aim of the study was to investigate the relationships between LS using ARFI erastograghy and the development of HCC in this patient population.

Methods

Patients. Between November 2014 and December 2016, a total of 268 patients were treated with oral direct-acting anti-HCV drugs (daclatasvir/asunaprevir combination therapy, sofosbuvir/ledipasvir combination therapy, ombitasvir/paritaprevir/ritonavir combination therapy, and sofosbuvir/ribavirin combination therapy) at Komaki City Hospital, Japan. A total of 263 patients achieved an SVR after antiviral therapy (98.1%). This prospective study thus included 263 patients who underwent ARFI elastography before antiviral treatment. Thirty patients had history of curative treatment for HCC and 233 had no history of previous HCC. HCV infection was defined by a real-time polymerase chain reaction (COBAS TaqMan HCV test; Roche Molecular Systems, Pleasanton, CA, USA; lower limit of detection: 1.2 $\log_{10}$ IU/mL). SVR was determined as undetectable serum HCV RNA, 24 weeks after the completion of antiviral therapy by a real-time polymerase chain reaction. Clinical data were collected at the time of measurement of ARFI elastography and blood samples were collected no more than 7 days before the DAA treatment. Patients who had antibodies against human immunodeficiency virus or hepatitis B virus surface antigen, excessive active alcohol consumption (daily intake >40 g of ethanol) or drug abuse, or other forms of liver disease (e.g. autoimmune hepatitis, alcoholic liver disease, or hemochromatosis) were excluded. The study was approved by the institutional Review Board of Komaki City Hospital and conducted in accordance with the ethical principles of the Declaration of Helsinki. Written informed consent for use of clinical data was obtained from all patients at the time of the measurement of ARFI elastography.

Antiviral therapy. For chronic HCV genotype 1 infection, 67 patients received fixed doses of daclatasvir (60 mg once daily) and asunaprevir (100 mg twice daily) for 24 weeks. One hundred and twenty-nine patients received fixed doses combination of sofosbuvir/ledipasvir (400/90 mg once daily) for 12 weeks. Two patients received the fixed doses combination of ombitasvir/paritaprevir/ritonavir (25/150/100 mg twice daily) for 12 weeks. For chronic HCV genotype 2 infection, 65 patients received fixed doses of sofosbuvir (400 mg once daily) and ribavirin (600–1000 mg daily based on bodyweight, orally) for 12 weeks. We treated patients based on a standard treatment protocol for Japanese patients.

Diagnosis of HCC and follow-up. Before starting antiviral therapy, all patients without history of previous HCC underwent abdomen ultrasound to exclude the presence of HCC. On the other hand, all patients with a history of HCC underwent magnetic resonance imaging (MRI) and dynamic computed tomography (CT), besides ultrasound, to exclude recurrent HCC. After the end of antiviral therapy, patients attended medical consultations at the Komaki City Hospital outpatient clinic every 3–6 months. Biochemical measurements, including AFP and tumor marker levels, were assessed from whole blood samples every 3–6 months; ultrasonography, MRI, and dynamic CT were performed every 6 months. Typical imaging findings for HCC included a high-density mass in the arterial phase and a low-density mass in the portal phase of dynamic CT or MRI studies. To investigate the incidence of HCC after SVR, the start date of follow-up was defined as the date of the start of DAA treatment and the endpoint was the development of HCC or the latest medical follow-up visit prior to May 2017. The factors associated with the development of HCC were prospectively analyzed.

ARFI elastography measurement. In this prospective study, the baseline ARFI elastography measurements of all patients were performed less than 3 months before HCV treatment initiation. The Siemens Acuson S2000TM ultrasound system (Siemens Medical Solutions, Mountain View, CA, USA) was used for the measurement of ARFI elastography using a curved linear 6C1 transducer in all the patients.[24] The measurement of ARFI elastography was performed in the right liver lobe through the 7th to 10th intercostal space while the patient was in a supine position with the right arm in abduction. The patients were asked to hold their breath for a moment at the end of expiration to minimize breathing motion during the examination. ARFI measurements were obtained at a depth of 1–2 cm from the liver capsule, avoiding large vessels and fissures in the liver. A total of 10 valid measurements were performed in every patient and a median value was calculated; the result is expressed in m/s.

Statistical analysis. Categorical data are presented as numbers (percentages). Continuous data are presented as means ± SD) and medians (ranges). Normally distributed variables were compared using Student's *t*-test and non-normally distributed variables were compared using the Mann–Whitney *U*-test between the two groups of patients who did and did not develop HCC. Frequency data were compared using a chi-square test or Fisher's exact test, as appropriate. The cumulative incidence of HCC was calculated using the Kaplan–Meier method. Differences among patients with mild LS, moderate LS, and severe LS were assessed using by the log-rank test. The time frame for HCC incidence was defined as the time from start of DAA treatment to diagnosis of HCC. The Cox proportional hazard model was used for multivariate analyses of factors associated with the incidence of HCC. We determined the cut-off values of the factors associated with the incidence of HCC using receiver operating characteristic analyses. Statistical analyses were performed using SPSS Statistics 21.0 (IBM SPSS, Chicago, IL, USA); $P < 0.05$ was considered statistically significant using a two-tailed test.

Results

Baseline characteristics of patients with antiviral treatment. The overall study population of 263 patients consisted of 122 males (46.4%) and 141 females (53.6%). The mean

Table 1 Baseline characteristics of patients from the entire study population

Characteristics	Patients
Age (years)	71.4 (16–88)
Sex (female/male)	141 (53.6%)/122 (46.4%)
HCV genotype (1b/2a or 2b)	198/65
HCC history (absent/present)	233/30
Body mass index (kg/m^2)	22.6 (15.0–32.9)
Serum albumin (g/dL)	4.0 (2.2–4.9)
Total bilirubin (mg/dL)	0.74 (0.3–2.6)
Alanine aminotransferase (IU/L)	34.0 (10–299)
Aspartate aminotransferase (IU/L)	38.7 (16–224)
Gamma-glutamyl transpeptidase (IU/L)	31.2 (8–521)
Platelet count (×10^3/μL)	149 (14–351)
α-Fetoprotein (ng/mL)	4.5 (0.9–172.3)
Liver stiffness (m/s)	1.41 (0.67–4.35)
Antiviral regimens	
Daclatasvir + asunaprevir	67 (25.5%)
Ledipasvir/sofosbuvir	129 (49.1%)
Ombitasvir paritaprevir/ritonavir	2 (0.7%)
Sofosbuvir + ribavirin	65 (24.7%)

HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

age was 70.7 years (range: 20–88). Detailed demographic data are shown in Table 1. Liver cirrhosis was identified in 49 patients (18.6%), and all patients had well-preserved liver function of Child–Pugh class A. HCV genotype 1 was present in 198 patients (75.6%). The median V_s value according to ARFI measurements was 1.34 m/s (range: 0.67–4.35; Table 1).

Development of HCC in patients with SVR following DAA therapy. During the follow-up period (median: 18.1 months; range: 5.6–31.2 months), HCC was identified in 19 patients (7.2%). The median time between the start of DAA treatment and the development of HCC was 8.6 months (range: 6.0–21.3 months). The cumulative incidence of HCC at 1 and 2 years after the start of DAA treatment was 5.4% and 9.7%, respectively. HCC patients included 7 males and 12 females; 12 patients had history of previous HCC and 7 had no history of previous HCC.

Factors associated with the development of HCC in patients with SVR following DAA therapy. The clinical characteristics according to development of HCC are shown in Table 2. LS measurements were significantly higher in patients with the development of HCC than in those without (median: 2.06 m/s *vs* 1.33 m/s, $P < 0.001$). Univariate analysis revealed that the presence of HCC history, older age, lower platelet counts, higher total bilirubin levels, and higher AFP levels before treatment were significantly associated with the development of HCC after SVR. The Cox proportional hazards regression analysis confirmed that the presence of HCC history (hazard ratio [HR]: 10.634; 95% confidence interval [CI]: 4.13–27.37; $P = 0.001$), higher total bilirubin levels (HR: 4.189; 95% CI: 1.66–10.60; $P = 0.002$), and older age (HR: 4.638; 95% CI: 1.63–13.61; $P = 0.004$) were significant independent factors associated with the development of HCC in patients who had achieved an SVR following DAA therapy. The cumulative occurrence of HCC in patients with history of HCC was significantly higher than that in patients without history of HCC, based on the Kaplan–Meier analysis and log-rank test ($P < 0.001$) (Fig. 1).

Factors associated with the occurrence of HCC in patients with SVR following DAA therapy. In patients without history of HCC, occurrence of HCC was identified in seven patients (3.0%). The clinical characteristics according to the occurrence of HCC are shown in Table 3. The cumulative incidence of HCC at 1 and 2 years after the start of DAA treatment was 2.3% and 4.3%, respectively. Univariate analysis revealed that higher LS measurement and higher total bilirubin levels before treatment were significantly associated with the occurrence of HCC after SVR. The Cox proportional hazards regression analysis confirmed that higher LS measurement (HR: 8.350; 95% CI: 1.62–43.09; $P = 0.011$) was significant independent factor associated with the occurrence of HCC in patients who had achieved an SVR following DAA therapy (Table 4).

Correlation between LS values and development of HCC. Patients without history of HCC were divided into two groups based on measurement of ARFI elastography levels, with the value as the cutoff (1.73 m/s) using receiver operating

Table 2 Baseline characteristics of patients according to the development of HCC after DAA therapy

Characteristics	Patients without HCC after DAA	Patients with HCC after DAA	*P*-value
Age (years)	70.8 (16–88)	77.0 (65–85)	<0.001
Sex (female/male)	129 (52.9%)/115 (47.1%)	12 (63.2%)/7 (36.8%)	0.477
HCV genotype (1b/2a or 2b)	181/63	17/2	0.173
HCC history (absent/present)	226/18	7/12	<0.001
Body mass index (kg/m^2)	22.5 (15.0–32.9)	23.3 (16.4–31.5)	0.563
Serum albumin (g/dL)	4.0 (2.2–4.9)	3.8 (2.4–4.5)	0.097
Total bilirubin (mg/dL)	0.72 (0.3–2.5)	0.98 (0.6–2.6)	0.001
Alanine aminotransferase (IU/L)	34.5 (10–299)	24.5 (14–106)	0.321
Aspartate aminotransferase (IU/L)	38.4 (16–224)	43.0 (27–106)	0.155
Gamma-glutamyl transpeptidase (IU/L)	31.7 (8–521)	21.5 (13–521)	0.519
Platelet count (×10^3/μL)	151 (14–351)	118 (38–221)	0.005
α-Fetoprotein (ng/mL)	4.4 (0.9–172.3)	6.3 (2.5–132.5)	0.011
Liver stiffness (m/s)	1.39 (0.67–4.35)	2.04 (1.12–4.00)	<0.001

DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

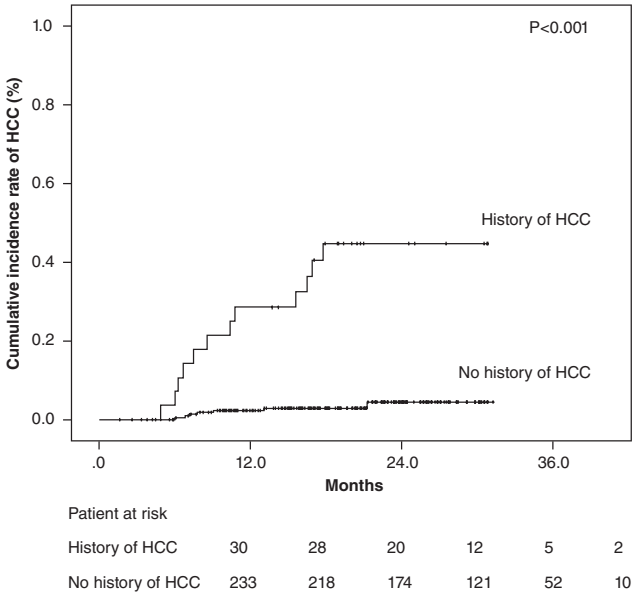

Figure 1 Cumulative incidence rates of hepatocellular carcinoma (HCC) development after sustained virological response following direct-acting antiviral treatment according to the history of hepatocellular carcinoma. The cumulative incidence rates of HCC development increased significantly in patients with previous HCC treatment (log-rank test, $P < 0.001$).

characteristic analyses. The cumulative occurrences of HCC in patients with lower LS and higher LS were 1.2% and 6.1%, respectively, at 1 year after the start of DAA treatment and 1.2% and 13.4%, respectively, at 2 years after the start of DAA treatment. The cumulative occurrence of HCC in patients with higher LS was significantly higher than that in patients with lower LS, based on the Kaplan–Meier analysis and log-rank test ($P = 0.003$; Fig. 2). The cumulative recurrence of HCC was statistically similar according to the degree of LS in patients who were previously treated for HCC ($P = 0.099$) (Fig. 3).

Discussion

To the best of our knowledge, this is the first study that evaluates the correlation between development of HCC and LS assessed by ARFI elastography in patients who achieved SVR following DAA therapies. The strengths of this study include the information it provides about the impact of LS measurement on development of HCC after SVR following DAA treatment, as assessed by ARFI elastography from 263 patients with HCV. Although patients who achieve SVR have little risk of developing HCC, given the marked increase in the number of patients who achieve SVR, there will also be an increase in the number of patients who develop HCC after SVR in the near future. Indeed, seven of our SVR patients without history of HCC after DAA treatment did develop HCC, demonstrating the need for continued screening of this population. In the present study, we identified the LS measurement before treatment as an independent predictor of HCC occurrence in patients who achieved an SVR following DAA treatment. Because patients with LS values of 1.73m/s have higher risk for HCC development, HCC surveillance strategies might be optimized according to LS values at baseline, even with complete viral eradication.

In the present study, a total of 32 patients with a history of previous HCC were treated with oral direct-acting anti-HCV drugs. In these patients, 30 patients achieved an SVR after antiviral therapy (93.8%). Eleven of our SVR patients with history of HCC after DAA treatment did develop HCC. We found 1- and 2-year HCC recurrence rates of 28.6% and 44.7% in a cohort of patients with a history of previous HCC who achieved a complete radiological response after tentatively curative resection or ablation. The risk of recurrence of HCC was statistically similar according to the degree of LS in patients who were previously treated for HCC after SVR following DAA treatment. Several previous studies have reported that HCC recurrence after surgical resection or radiofrequency ablation is not uncommon.[25] We found that patients previously treated for HCC have still a high risk of recurrence in the short term, despite DAA treatment, irrespective of the degree of LS.

Liver biopsy had long remained the gold standard for staging fibrosis. However, liver biopsy is no more considered as a perfect methodology because of the invasive nature of the procedure, sampling error, and interobserver variability. In contrast to liver biopsy, ARFI elastography is noninvasive and can be repeated multiple times in the same patient. This is clinically very helpful. Assessment of residual liver fibrosis in patients who achieve SVR is of strategic importance not only for

Table 3 Baseline characteristics of patients with no history of previous HCC according to HCC occurrence after DAA therapy

Characteristics	Patients without occurrence of HCC	Patients with occurrence of HCC	*P*-value
Age (years)	70.5 (16–88)	76.0 (65–84)	0.051
Sex (female/male)	119 (51.1%)/107 (48.9%)	6 (85.7%)/1 (14.3%)	0.126
HCV genotype (1b/2a or 2b)	165/61	6/1	0.678
Body mass index (kg/m^2)	22.4 (15.4–32.9)	23.5 (18.1–31.5)	0.171
Serum albumin (g/dL)	4.1 (2.2–4.9)	3.8 (2.8–4.4)	0.307
Total bilirubin (mg/dL)	0.72 (0.3–2.5)	0.97 (0.6–1.8)	0.031
Alanine aminotransferase (IU/L)	34.1 (10–299)	25.0 (17–106)	0.616
Aspartate aminotransferase (IU/L)	37.7 (16–224)	43.0 (28–106)	0.194
Gamma-glutamyl transpeptidase (IU/L)	30.7 (8–521)	22.0 (13–146)	0.315
Platelet count (×10^3/μL)	156 (14–351)	118 (48–204)	0.107
α-Fetoprotein (ng/mL)	4.2 (0.9–172.3)	6.4 (2.5–50.5)	0.099
Liver stiffness (m/s)	1.33 (0.67–4.35)	2.06 (1.40–4.00)	0.005

DAA, direct-acting antiviral; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

Table 4 Predictive factors related to the development of HCC after DAA therapy on Cox proportional hazard analysis

Characteristics	Category	Hazard ratio	95% Confidence interval	*P*-value
Development of HCC				
History of previous HCC	1: No 2: Yes	10.634	4.132–27.369	0.001
Total bilirubin (mg/dL)	1: <1.0 2: ≥1.0	4.189	1.656–10.597	0.002
Age (years)	1: <75 2: ≥75	4.638	1.634–13.161	0.004
Occurrence of HCC				
Liver stiffness (m/s)	1: <1.73 2: ≥1.73	8.350	1.618–43.09	0.011

DAA, direct-acting antiviral; HCC, hepatocellular carcinoma.

prognostication but also for defining cost-effective programs of surveillance or liver-related complications. We previously reported that ARFI elastography is an acceptable method for predicting the severity of fibrosis in HCV patients with SVR.[26] Therefore, ARFI elastography will be useful not only during the persistent HCV infection but also after the eradication of HCV.

A recent study has reported that the value of ARFI elastography is closely associated with the risk of the development of HCC in patients with chronic liver diseases.[27] In the present study, we also identified that the value of ARFI elastography before treatment correlated with the development of HCC in patients who achieved an SVR following DAA treatment. However, LS measurements may also be affected by factors other than fibrosis stage, for example, inflammatory activity,[28,29] and intrahepatic pressure. There are a few studies demonstrating a decrease in LS in patients on eradication of HCV where both pre- and post-treatment ARFI elastography assessments were available.[30] It is probable that such reduction in ARFI elastography values indicates not only the improvement in fibrosis but also inflammation in the liver because of treatment. This is because ARFI elastography reductions are significantly correlated with the grade of activity in the liver.[31] Moreover, it is not clear whether the change in LS after treatment, is useful in predicting the development of HCC in patients who achieved an SVR following DAA treatment. Therefore, prospective and more long-term studies are necessary to confirm whether a change in LS after achieving SVR is correlated with the risk of developing HCC.

There are several limitations in this study. The number of patients with the development of HCC was not very large. Because the development of HCC is very rare in patients who achieve SVR after DAA treatment,[10–12] this report described a

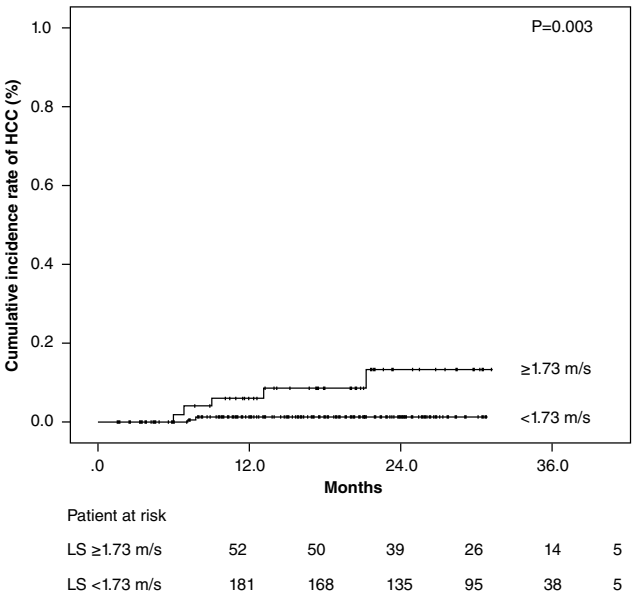

Figure 2 Cumulative incidence rates of hepatocellular carcinoma (HCC) occurrence after sustained virological response following direct-acting antiviral treatment based on stratified liver stiffness (LS) values. The cumulative incidence rates of HCC occurrence increased significantly in patients with higher LS values at baseline (log-rank test, $P = 0.003$).

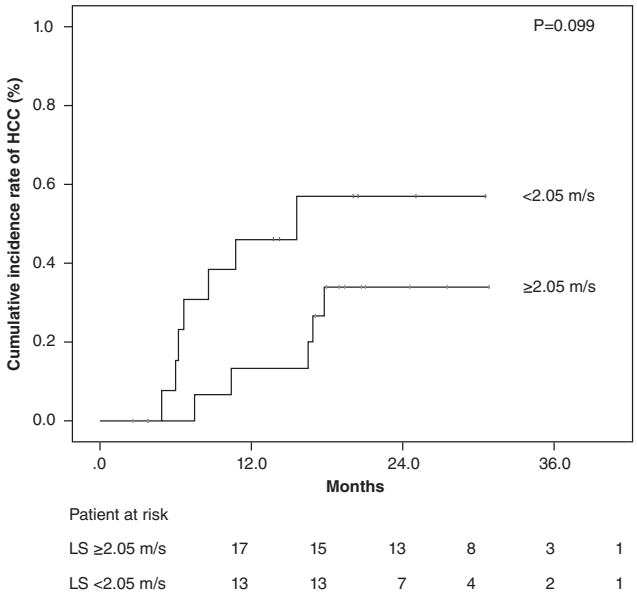

Figure 3 Cumulative incidence rates of hepatocellular carcinoma (HCC) recurrence after sustained virological response following direct-acting antiviral treatment based on stratified liver stiffness (LS) values. The cumulative incidence rates of HCC recurrence was statistically similar according to the degree of LS in patients who were previously treated for HCC (log-rank test, $P = 0.099$).

relatively small number of cases. Regardless, this information is useful because risk factors associated with the development of HCC in patients who achieve SVR after DAA treatment were identified. Second, among the enrolled patients, 190 patients underwent liver biopsy (72.2%). We acknowledge that our study could have been strengthened by pretreatment liver biopsies with all of enrolled patients, which, however, was not available. This is due to the difficulty in obtaining informed consent for the invasive liver biopsy before treatment. Finally, prospective data and long-term clinical follow-up are needed to assess the clinical course after achieving SVR and the impact of LS measurement after HCV elimination. Future large-scale studies with sufficient histological data and longer follow-up periods can resolve these issues.

In conclusion, the degree of LS assessed on ARFI elastography before antiviral treatment was related to the occurrence of HCC in patients who achieved an SVR following DAA treatment. Therefore, even if SVR is achieved, patients with higher LS at baseline must be followed carefully for the occurrence of HCC.

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