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Predictive Factors Differ between Hepatocellular Carcinoma Occurrence and Recurrence after Sustained Virological Responses by Direct-Acting Antivirals in Patients with Hepatitis C

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Abstract

Background: Interferon-free direct-acting antivirals (DAA) have markedly increased the sustained virological response (SVR) rate among patients with hepatitis C. Although DAA inhibit the development of hepatocellular carcinoma (HCC), predictive factors remain unclear. The aims of the present study were to investigate predictive factors for HCC occurrence and recurrence after SVR by DAA in prospectively followed patients with hepatitis C (HCV). **Methods:** One hundred and eighty-three HCV-infected patients treated with DAA and achieving SVR were prospectively followed up for more than one year. Among these patients, 166 had no history of HCC before DAA therapy, while 17 had a history of being treated for HCC by radiofrequency ablation or resection before the initiation of DAA. Liver stiffness (LS) measurements were conducted using transient elastography, and LS was assessed at the initiation of DAA (LS0), 24 weeks after the initiation of DAA (LS24), 48 weeks after (LS48), and every year after that. **Results:** HCC occurred in 7 out of 166 patients without a history of HCC (4.2%), and recurred in 9 out of 17 with a history of HCC (52.9%). Patients with a history of HCC were significantly older, mainly males, had higher alpha-fetoprotein (AFP) levels before DAA and at SVR24, higher Fib-4 levels, and higher LS0, 24, and 48 than those without a history of HCC. Age ($p = 0.013$) and AFP at SVR24 ($p = 0.036$) correlated with occurrence. LS48 ($p = 0.043$) correlated with recurrence.

Conclusions: Predictive factors differed between HCC occurrence and recurrence after SVR by DAA in HCV patients. High recurrence rates were due to fibrosis in the liver being more advanced in patients with than in those without a history of HCC. Age and AFP at SVR24 were identified as predictive factors of HCC occurrence and LS48 of HCC recurrence.

Keywords

Predictive Factor, HCC Occurrence and Recurrence, SVR, DAA

1. Introduction

Chronic hepatitis C (HCV) is an important risk factor for the development of hepatocellular carcinoma (HCC). Interferon (IFN)-free direct-acting antivirals (DAA) have markedly increased the sustained virological response (SVR) rate to more than 90% in patients with HCV [1] [2] [3] [4] [5]. Previous studies reported that SVR by DAA effectively reduced the risk of HCC [6] [7] [8]. On the other hand, DAA has been reported to increase the risk of HCC in patients with HCV, particularly in those receiving DAA after the treatment of HCC, namely, HCC recurrence [9]. HCC may develop after SVR by DAA because it has been reported to occur even after SVR by IFN-based therapy. Risk factors for the development of HCC need to be identified in recent DAA therapies. Some risk factors after SVR by IFN-based therapy in patients without HCC, namely, HCC occurrence, include an older age, male gender, severe fibrosis, diabetes mellitus, and elevated alpha-fetoprotein levels (AFP) [10]-[17]. While AFP [18], Wisteria floribunda agglutinin-positive Mac-2 binding protein [19], and TLL1 [20] are known prognostic factors of HCC occurrence and recurrence in HCV-positive patients after SVR by DAA, there may be other prognostic factors that are yet to be identified. The aims of the present study were to investigate predictive factors for HCC occurrence and recurrence after SVR by DAA in prospectively followed patients with HCV.

2. Materials and Methods

In the present study, 201 HCV-infected patients treated with DAA between September 2014 and July 2018 and achieving SVR were prospectively followed up for more than one year at Department of Gastroenterology, National Hospital Organization Nagoya Medical Center, Nagoya, Japan. These patients were treated with daclatasvir plus asunaprevir 39, sofosbuvir plus ledipasvir 55, sofosbuvir plus ribavirin 42, ombitasvir plus paritaprevir with ritonavir 6, elbasvir plus grazoprevir 9, and pibrentasvir plus glecaprevir 32. Patients with decompensated cirrhosis, autoimmune hepatitis, primary biliary cirrhosis, and co-infection with hepatitis B virus were not included in the present study. Patients co-infected with human immunodeficiency virus (HIV) were also excluded because HIV co-infection increases the severity of hepatitis C [21]. Therefore, 183 patients were enrolled

and prospectively followed up for more than one year (Figure 1). Among the 183 patients, 166 had no history of HCC before DAA therapy and 17 had a history of being treated for HCC by radiofrequency ablation (RFA) or resection and achieved complete responses before the initiation of DAA.

Blood chemistry examinations for all patients were conducted every two weeks after the initiation of DAA to detect adverse events and every 6 months after DAA therapy. Virological responses were assessed 24 weeks after the completion of treatment. SVR24 was defined as undetectable serum HCV RNA 24 weeks after the completion of treatment. All patients were confirmed to have no HCC tumor using abdominal contrast-enhanced computed tomography (CT) or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced magnetic resonance imaging (MR) and ultrasound (US) before the initiation of DAA. CT or MR was performed every 6 months before DAA and during the follow-up. HCC was diagnosed by a positive result for vascular patterns, as shown in CT or MR. Transient elastography (Fibroscan®) with liver stiffness (LS) measurements was performed using the M probe at a skin-liver capsule distance of less than 20 mm and XL probe of more than 20 mm [22]. Ten validated measurements were performed on each patient and a success rate of least 60% was considered to be reliable. LS were measured at the initiation of DAA (LS0), 24 weeks after the start of DAA (LS24), 48 weeks after (LS48), and every year after that (LS2y, LS3y, and LS4y).

Statistical analysis

Quantitative variables were shown as medians (minimum-maximum). Laboratory data were compared using the Student's *t*-test (parametric data) and

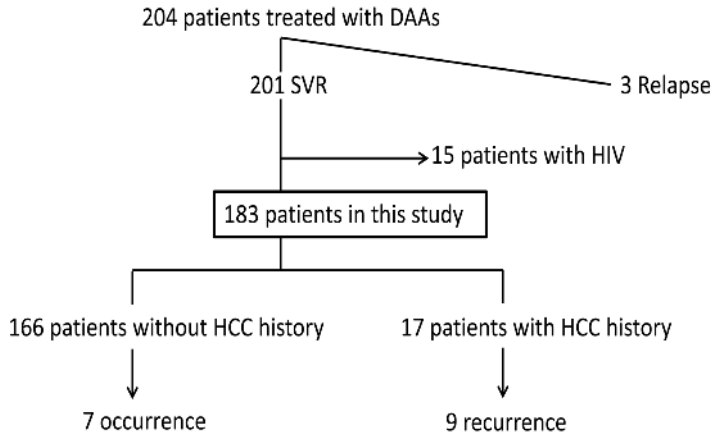


Figure 1. Flowchart showing the analytical process. Two hundred and one HCV-infected patients treated with DAA and achieving SVR were prospectively followed up. Patients co-infected with human immunodeficiency virus (HIV) were also excluded. Therefore, 183 patients were enrolled and prospectively followed up for more than one year. Among these patients, 166 had no history of HCC before DAA therapy, while 17 had a history of being treated for HCC before the initiation of DAA. HCC occurred in 7 out of 166 patients without a history of HCC, and recurred in 9 out of 17 with a history of HCC. DAA: direct-acting antivirals, SVR: sustained virological response, HIV: human immunodeficiency virus, HCC: hepatocellular carcinoma.

chi-squared test. $p < 0.05$ was considered to be significant. The HCC development rate was calculated using the Kaplan-Meier technique. LS0, LS24, LS48, LS2y, and LS3y were compared using the Mann-Whitney U test. To identify factors associated with HCC occurrence and recurrence, gender was compared by the chi-squared test and age, AFP before DAA, AFP at VR24, Fib-4, LM0, LS24, and LS48 by a logistic regression analysis. According to a Diagnostic performance plot (DP-plot) analysis [23], an optical common cut-off value was selected by Youden's index of receiver operating characteristic (ROC) curve analysis. Sensitivity, specificity, and accuracy were calculated at the common cut-off point for each factor. Statistical analyses were performed using the Statistical Package for Social Science software v.22 (SPSS, Chicago, IL) in the Sugimoto Data Analysis Service.

3. Results

Profiles of patients with and without a history of HCC

The baseline characteristics of patients included in the present study are shown in Table 1.

Patients with a history of HCC were significantly older (median age, 71 vs. 65 years, $p < 0.001$), mainly males ($p < 0.001$), had higher AFP levels before DAA and at SVR24 (9.0 vs. 4.0 ng/ml and 6.0 vs. 3.0 ng/ml, $p < 0.001$), higher Fib-4 levels (4.35 vs. 2.52, $p < 0.001$), and higher LS0, 24, and 48 (16.1 vs. 7.5 kPa, 14.8 vs. 5.8 kPa, 12.0 vs. 5.0 kPa, $p < 0.001$) than those without a history of HCC. Thus, fibrosis in the liver was more advanced in patients with than in those without a history of HCC.

HCC was detected in 7 out of 166 patients without a history of HCC (4.2%), and recurred in 9 out of 17 with a history of HCC (52.9%).

HCC development

One-, 2-, 3-, and 4-year HCC development rates were 35%, 53%, 53%, and 53%, respectively, for recurrence and 1.4%, 3.0%, 5.4%, and 6.8%, respectively, for occurrence. Figure 2(a) shows the clinical course of HCC occurrence in 7 patients without a history of HCC before the DAA treatment. HCC was detected 12 - 36 months between DAA therapy and occurrence. HCC nodules were diagnosed by CT or MR and were 23 - 20 mm in diameter. Figure 2(b) shows a patient with a history of HCC before DAA, but without recurrence for 29 - 57 months. The period between previous HCC therapy and DAA therapy was 3 - 12 months. Only one HCC nodule was present in each patient at previous HCC and was diagnosed by CT or MR. Previous HCC ranged between 12 and 30 mm in diameter. HCC therapies were RFA for five patients and hepatic resection for three. Figure 2(c) shows a patient with a history of HCC before DAA and recurrence was detected 4 - 16 months after DAA therapy. The period between previous HCC therapy and DAA therapy was 3 - 87 months. Only one HCC nodule was present in each patient at previous HCC and was diagnosed by CT or MR. Previous HCC ranged between 13 and 25 mm in diameter. HCC therapies were

Table 1. Baseline characteristics of patients (n = 183).

Characteristics	All (183)	without a history of HCC (166) a	with a history of HCC (17) b	p a vs. b
Age (year)*	68 (18 - 91)	65 (18 - 91)	71 (49 - 84)	**<0.001**
Male: Female	89:94	75:91	14:3	**<0.001**
BMI (kg/m^2)	22.6 (14.4 - 37.4)	22.7 (14.4 - 37.4)	22.0 (18.6 - 34.9)	NS
DCV/ASV:SOF/LDV: SOF/RBV:OBV/PTV/rit: EBR/GZR:PIB/GLE	39:55:42:6:9:32	32:52:38:5:8:30	7:3:4:0:1:2	NS
Duration of the follow-up (months)*	33.4 (12 - 58.2)	32.8 (12 - 58.2)	40.7 (12 - 57.0)	NS
Previous IFN treatment, Non-responder: Relapse	15:38	15:6	0:2	NS
DM	8	5	3	NS
Daily alcohol intake ≥ 40 g/day	2	1	1	NS
HCV genotype 1:2:3	123:58:2	85:53:2	12:5:0	NS
HCV RNA (Log IU/ml)*	6.2 (2.3 - 7.4)	6.2 (2.3 - 7.4)	5.8 (4.5 - 7.3)	NS
White cell count (/μl)*	5000 (2100 - 18,000)	5000 (360 - 10,600)	4400 (2600 - 18,000)	NS
Hemoglobin (g/dl)*	13.5 (7.3 - 17.2)	13.5 (7.3 - 17.2)	13.1 (11.0 - 15.4)	NS
Platelets (10^4/μl)*	17.0 (5.2 - 80.9)	17.3 (5.2 - 68.0)	11.7 (8.3 - 80.9)	NS
Aspartate aminotransferase (IU/L)*	41 (4 - 361)	39 (4 - 361)	50 (27 - 213)	NS
Alanine aminotransferase (IU/L)*	40 (7 - 472)	39 (7 - 472)	51 (21 - 212)	NS
Total bilirubin (mg/dl)*	0.73 (0.34 - 5.00)	0.73 (0.34 - 5.00)	0.71 (0.5 - 1.84)	NS
Albumin (g/dl)*	4.1 (2.2 - 5.2)	4.1 (2.4 - 5.2)	3.7 (2.6 - 4.6)	NS
Creatinine (mg/dl)*	0.72 (0.45 - 8.69)	0.70 (0.45 - 8.69)	0.82 (0.56 - 1.30)	NS
Alpha-fetoprotein (AFP) (ng/ml)*				
Before	5.0 (1 - 560)	4.0 (1 - 450)	9.0 (1 - 560)	**<0.001**
SVR24	4.0 (1 - 133)	3.0 (1.0 - 19)	6.0 (10 - 133)	**<0.001**
Fib-4*	2.82 (0.09 - 5.72)	2.52 (0.16 - 3.99)	4.35 (1.52 - 5.06)	**<0.001**
LS0 (kPa)*	8.0 (2.5 - 40.9)	7.5 (2.5 - 40.9)	16.1 (7.3 - 28.0)	**<0.05**
LS24 (kPa)*	6.0 (2.6 - 31.6)	5.8 (2.6 - 31.6)	14.8 (9.9 - 29.1)	**<0.01**
LS48 (kPa)*	5.3 (2.4 - 29.5)	5.0 (2.4 - 25.1)	12.0 (5.3 - 29.5)	**<0.001**
HCC		Occurrence 7	Recurrence 9	

*Median (range), NS: not significant, LS0: LS at the initiation of DAA, LS24, 48: LS 24, 48 months after DAA, DCV/ASV: daclatasvir plus asunaprevir, SOF/RBV: sofosbuvir plus ribavirin, SOF/LDV: sofosbuvir plus ledipasvir, OBV/PTV/rit: ombitasvir plus paritaprevir with ritonavir, EBR/GZR: elbasvir plus grazoprevir, PIB/GLE: pibrentasvir plus glecaprevir, RFA: radiofrequency ablation.

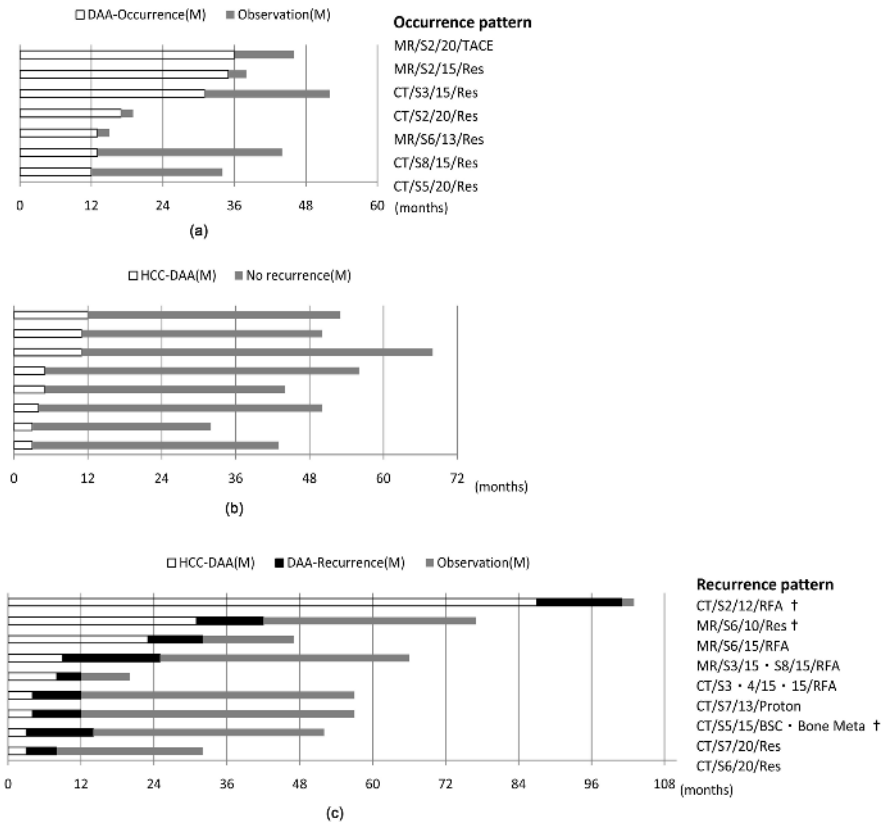


Figure 2. Clinical course of HCC. (a) Shows the clinical course of HCC occurrence in 7 patients without a history of HCC before the DAA treatment. The white boxes show the time between the start of DAA and HCC occurrence. The grey boxes show the duration of the follow-up after HCC occurrence. Right side comments show the pattern of HCC occurrence (Imaging technique/Location/Size (mm)/Treatment); (b) Shows 8 patients who had a history of HCC before DAA and recurrence were not detected during the follow-up. The white boxes show the time between the previous HCC treatment and start of DAA. The grey boxes show the duration of the follow-up after DAA; (c) Shows 9 patients who had a history of HCC before DAA and recurrence were detected during the follow-up. The white boxes show the time between the previous HCC treatment and start of DAA. The black box shows the duration from the start of DAA and HCC recurrence. The grey boxes show the duration of the follow-up after HCC recurrence. Right side comments show the pattern of HCC recurrence (Imaging technique/Location/Size (mm)/Treatment). CT: contrast-enhanced computed tomography, MR: magnetic resonance imaging, TACE: Transarterial chemoembolization, Res: Resection, Proton: proton therapy, BSC: best supportive care, Meta: metastasis. (a) Occurrence 7; (b) Recurrence (-) 8; (c) Recurrence 9.

RFA for four patients and hepatic resection for five. Three patients died of HCC.

Analysis to identify factors associated with HCC occurrence and recurrence

We examined factors associated with HCC occurrence and recurrence. Gender was not significant for occurrence or recurrence (Table 2). Age ($p = 0.013$) and AFP at SVR24 ($p = 0.036$) correlated with occurrence, whereas AFP before DAA, Fib-4, LM0, LS24, and LS48 did not. LS48 ($p = 0.043$) correlated with recurrence, whereas age, AFP before DAA, AFP at SVR24, Fib-4, LM0, and LS24 did not (Table 3).

Since the univariate analysis identified factors associated with HCC occurrence and recurrence, we assessed their cut-off values for predicting HCC occurrence and recurrence using a DP-plot analysis. Regarding HCC occurrence, cut-off values for age and AFP at SVR24 were 70.1 years and 4.6 ng/ml, respectively (**Figure 3(a)**). Concerning HCC recurrence, LS0, LS24, and LS48 cut-off values were 16.2, 14.8, and 11.4 kPa, respectively (**Figure 3(b)**).

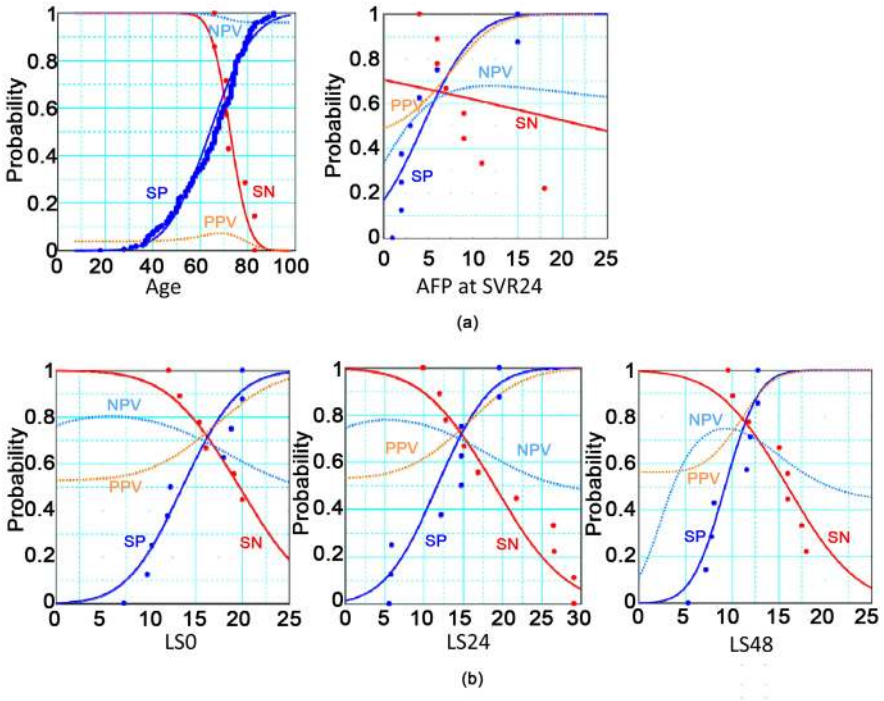
Table 2. Analysis to identify factors associated with HCC occurrence and recurrence (the chi-squared test).

Characteristics	Occurrence p	Recurrence p
Gender	0.415	0.299

Table 3. Analysis to identify factors associated with HCC occurrence and recurrence (a logistic regression analysis).

Characteristics	Occurrence	Recurrence
	p	p
	partial regression coefficient (95% CI)	partial regression coefficient (95% CI)
Age	**0.013** 0.050 (0.050 - 10.039)	0.177 −0.077 (−0.189 - 0.828)
AFP before DAA	0.760 −0.005 (−0.041 - 0.960)	0.442 0.034 (−0.038 - 0.963)
AFP at SVR24	**0.036** 0.126 (0.008 - 1.008)	0.105 0.267 (−0.056 - 0.945)
Fib-4	0.795 −0.049 (−0.421 - 0.657)	0.173 0.339 (−0.149 - 0.862)
LS0	0.287 0.050 (−0.041 - 0.960)	0.067 0.224 (−0.014 - 0.987)
LS24	0.413 0.053 (−0.074 - 0.929)	0.068 0.200 (−0.014 - 0.986)
LS48	0.092 0.104 (−0.017 - 0.983)	**0.043** 0.519 (0.016 - 1.016)

LS0: LS at the initiation of DAA, LS24, 48: LS 24, 48 months after DAA.



Variables	Cut-off	Sensitivity	Specificity	accuracy
Occurrence Age	**70.3**	0.71	0.58	0.59
Occurrence AFP at SVR24	**4.6**	0.43	0.74	0.73
Recurrence LS0	16.2	0.88	0.63	0.76
Recurrence LS24	14.8	0.66	0.85	0.76
Recurrence LS48	**11.4**	0.67	1.00	0.81

Figure 3. DP-plot analysis for the prediction of HCC. Since the univariate analysis identified factors affecting HCC occurrence and recurrence, we assessed their cut-off values for predicting HCC occurrence and recurrence using a DP-plot analysis. (a) Regarding HCC occurrence, age and AFP at SVR24 cut-off values were 70.1 years and 4.6 ng/ml, respectively. (b) Concerning HCC recurrence, LS0, LS24 and LS48 cut-off values were 16.2, 14.8, and 11.4 kPa, respectively. (a) Occurrence; (b) Recurrence.

Variations in LSM during the follow-up

Figure 3 shows the courses of LSM in all patients.

The course of LS in 159 patients without a history of HCC before DAA and who did not develop HCC is shown in Figure 4(a). LS0, LS24, LS48, LS2y, LS3y, and LS4y median values were 7.1, 5.8, 4.8, 4.7, 4.3, and 4.6 kPa, respectively, showing significant improvements. Seven out of 166 patients subsequently developed HCC, and their LS0, LS24, LS48, and LS2y median values were 10.4, 8.7, 6.8, and 5.8 kPa respectively (closed squares), as shown in Figure 4(b). The course of LS in eight patients with a previous history of HCC treatments and no recurrence is shown in Figure 4(c). Their LS median values also significantly improved: LS0, LS24, LS48, LS2y, and LS3y values were 12.3, 14.8, 8.1, 8.0, and 6.1 kPa, respectively. Nine out of 17 patients subsequently developed HCC, and LS0, LS24, LS48, and LS2y median values were 19.1, 16.9, 16.0, and 14.4 kPa, respectively (closed circle), as shown in Figure 4(d).

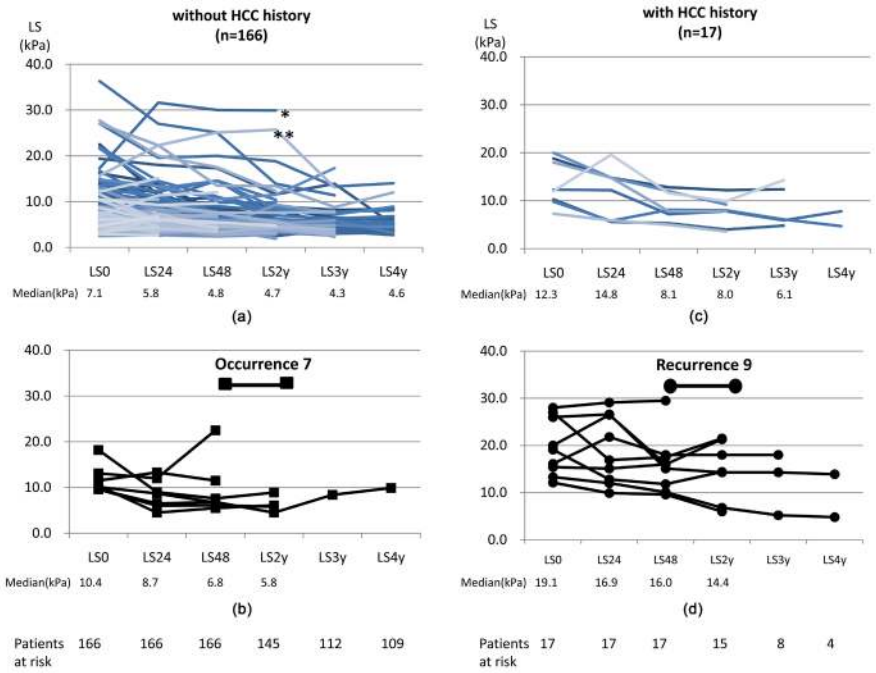


Figure 4. Course of LS. The course of LS in 159 patients without a history of HCC before DAA and no HCC occurrence are shown in (a). Their LS0, LS24, LS48, LS2y, LS3y, and LS4y median values were 7.1, 5.8, 4.8, 4.7, 4.3, and 4.6 kPa, respectively, showing significant improvements. Seven out of 166 patients subsequently developed HCC, and their LS0, LS24, LS48, and LS2y median values were 10.4, 8.7, 6.8, and 5.8 kPa, respectively (closed squares), shown in (b). The course of LS in eight patients with a previous history of HCC treatments and no HCC recurrence are shown in (c). Their LS median values also significantly improved: LS0, LS24, LS48, LS2y, and LS3y were 12.3, 14.8, 8.1, 8.0, and 6.1 kPa, respectively. Nine out of 17 patients subsequently developed HCC recurrence, and their LS0, LS24, LS48, and LS2y median values were 19.1, 16.9, 16.0, and 14.4 kPa respectively (closed circle), shown in (d). *One patient had a history of variceal bleeding. **The other patient had a history of heart failure after DAA therapy. LS: liver stiffness, LS0: LS at the initiation of DAA, LS24, 48: LS 24, 48 months after DAA, LS2y, 3y, and 4y: LS 2, 3, and 4 years after DAA. (a) Occurrence (-) 159; (b) Occurrence 7; (c) Recurrence (-) 8; (d) Recurrence 9.

4. Discussion

IFN-free DAA therapy has markedly increased the SVR rate among patients with HCV to more than 90%, even under unfavorable conditions, such as elderly patients and those with advanced fibrosis [1] [2] [3] [4] [5]. However, a high rate of HCC recurrence after DAA therapy in patients with a previous history of HCC treatments has been reported [9]. The effects of DAA therapy after SVR on HCC occurrence and recurrence have been unclear; however, Nagata et al. concluded in 2017 that post-SVR HCC occurrence and recurrence were similar between IFN-based and IFN-free treatments [8]. Some patients after SVR by DAA therapy develop HCC. We hypothesized that LS, as an indicator of liver fibrosis, may be involved in HCC occurrence and recurrence and investigated the predictive factors of HCC occurrence and recurrence after SVR by DAA in prospectively followed patients with HCV.

Our patients with a history of HCC were significantly older, had higher AFP and Fib-4 levels, and higher LS values than those without a history of HCC. These results indicate fibrosis in the liver was more advanced in patients with than in those with a history of HCC. LS48 values correlated with recurrence. Although LS0 and LS24 values were not significantly correlated with recurrence, the association we observed ($p = 0.067$ and 0.067, respectively) may become significant with a larger sample size for patients with a history of HCC. Regarding HCC recurrence, LS0, LS24, and LS48 cut-off values were 16.2, 14.8, and 11.4 kPa, respectively, showing a poor regression. Patients who achieved SVR by IFN-based treatments showed the significant regression of LS [24]. LS values in patients who achieved SVR by DAA also significantly decreased during the follow-up period [25] [26]. This early improvement in LS (from LS0 to LS24) was attributed to a decrease in inflammation in liver tissue. Even in patients with and without a history of HCC, LS values significantly decreased during the follow-up. Since increases in LS due to artifacts associated with liver inflammation were no longer observed at LS24 and LS48, decreases at LS24 and LS48 were considered to reflect the true attenuation of liver fibrosis. However, LS0, 24, and 48 values were significantly higher in patients with than in those without a history of HCC, and the regression of LS in patients with HCC recurrence was lower. (**Figure 4**) Previous HCC treatments in the present study were limited to RFA and hepatic resection because of curative therapy (**Figure 2**), and recurrent HCC tumors were distinct from previous tumors. High recurrence rates may be due to fibrosis in the liver being more advanced in patients with than in those without a history of HCC.

On the other hand, LS values did not correlate with HCC occurrence in the present study. Therefore, fibrosis in the liver may have been less advanced in patients without than in those with a history of HCC; however, one patient (* in **Figure 4(a)**) had a history of variceal bleeding. Indeed, a previous study on liver cirrhosis demonstrated that patients with grade 2 - 3 esophageal varices had higher mean LS values than those without varices or with grade 1 varices (45 versus 26 kPa) [27]. The other patient (** in **Figure 4(a)**) had a history of heart failure after DAA therapy. LS values were previously reported to be high in patients with elevated central venous pressure [28]. Thus, it is not impossible to estimate the association between LS values and the initial occurrence of HCC in patients with portal and venous hypertension. Although LS values did not correlate with HCC occurrence, a relationship was observed for age and AFP at SVR24. An older age, male gender, severe fibrosis, and AFP were identified as risk factors after SVR by IFN-based therapy in patients without the treatment of HCC [10]-[17]. AFP was recently identified as a risk factor after SVR by DAA therapy [29]. Predictive factors differed between HCC occurrence and recurrence after SVR by DAA in patients with HCV.

This study has several limitations. Since the numbers of patients who developed HCC without a previous history of HCC and those with a previous a history of HCC were small, we were unable to perform a multivariate analysis to identify

the factors associated with HCC occurrence and recurrence in a logistic regression analysis. Furthermore, the observation period was only 33.4 months. HCC occurrence after achieving SVR on IFN-based therapy was previously reported to be less than 2.0% within three years [30]. Therefore, a longer follow-up is needed.

5. Conclusion

In conclusion, predictive factors differed between HCC occurrence and recurrence after SVR by DAA in patients with HCV. The high recurrence rate was attributed to fibrosis in the liver being more advanced in patients with than in those without a history of HCC. Age and AFP at SVR24 are predictive factors of HCC occurrence and LS48 of HCC recurrence.

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Ethics Approval and Consent to Participate

The present study was approved by the Ethics Committee of the National Hospital Organization Nagoya Medical Center in accordance with the Helsinki Declaration. Informed consent was obtained from all individual participants included in this study.

Availability of Data and Material

Our data are available at the National Hospital Organization Nagoya Medical Center.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

Authors' Contributions

All authors contributed to the conception, writing, and checking of this manuscript.

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