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Early recurrence of hepatocellular carcinoma in patients with HCV infection followed by transient elastography for two years after successful treatment with daclatasvir plus asunaprevir

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Abstract

Aim and background: Recent advances have been achieved in HCV treatments because direct-acting antivirals (DAA) result in a sustained viral response (SVR) in more than 90% of HCV-infected patients, even those with severe fibrosis. However, an unexpected high rate of HCC recurrence following DAA has been reported. The aim of this study was to prospectively evaluate the relationship between the occurrence or recurrence of HCC and regression of fibrosis followed by transient elastography for two years after successful treatment with daclatasvir plus asunaprevir (DCV plus ASV) in patients with HCV infection.

Method: Forty-four patients who were treated with DCV plus ASV for 24 weeks and achieved SVR were followed-up for two years and analyzed. Eight patients had a history of being treated for HCC by radiofrequency ablation (RFA) or resection. Transient elastography (Fibroscan®) with liver stiffness measurements (LSM) was performed at the initiation of DCV plus ASV, at the end of the treatment (EOT), and 24 and 72 weeks after the treatment.

Results: The occurrence of HCC was not detected during the follow-up. Three out of eight patients with a history of HCC treatment subsequently developed radiological HCC recurrence a few months after the treatment with DCV plus ASV. LSM values measured by Fibroscan at the initiation of DCV plus ASV increased to 19.1, 13.1, and 26.0 kPa in Cases 1, 2, and 3, respectively, while those at EOT were 12.8, 12.0, and 26.6 kPa, respectively. The five other patients with LSM values less than 12 kPa at the last Fibroscan have shown no recurrence.

Conclusion: Chronic hepatitis C patients previously treated for HCC with high LSM values before and after DAA may be at a high risk of HCC recurrence, suggesting that strict HCC surveillance is required even after the achievement of SVR by DAA.

Introduction

Since 2013, IFN-free, direct-acting antiviral agents (DAA) represent an important opportunity for improving the treatment of HCV in patients with hepatitis C, and have shown superior efficacy and the amelioration of adverse events [1-6]. In Japan, daclatasvir (DCV) plus asunaprevir (ASV) was the first-generation treatment option approved for chronic HCV genotype 1b patients in September 2014 [5]. Without a previous history of simeprevir therapy and pre-existing NS5A Y93H, DCV plus ASV was reported to be approximately 90% effective for patients with HCV genotype 1b [7]. DAA were recently shown to achieve sustained virological responses (SVR) in more than 90% of HCV-infected patients, even those with severe fibrosis. However, an unexpected high rate of HCC recurrence following DAA has been reported [8]. In previous studies on interferon (IFN)-based antiviral treatments for chronic hepatitis C, SVR decreased the long-term risk of HCC in patients with advanced fibrosis [9]. On the other hand, SVR patients following interferon-based treatments showed the significant regression of liver stiffness measurements (LSM) [10]. In the present study, among eight patients infected with HCV genotype 1b who achieved SVR by DCV plus ASV for 24 weeks after the treatment of HCC by radiofrequency ablation (RFA) or resection, three with high LSM values subsequently developed radiological HCC recurrence a few months after the achievement of SVR.

Patients and methods

This study included 44 patients infected with HCV genotype 1b who were treated with DCV plus ASV for 24 weeks, achieved SVR, and was followed-up for two years. The baseline characteristics of the patients included in this study are shown in Table 1. There were 28 IFN-ineligible patients: 5 with diabetes mellitus, 6 with depression, 4 with idiopathic thrombocytopenia, 1 with paroxysmal nocturnal hemoglobinuria, 2 with myocardial infarction, 1 with non-tuberculosis mycobacteria 1, 4 with collagen disease, and 5 older than 75 years of age. There were 3 IFN-intolerant patients: 2 with retinal hemorrhage and 1 with vertebritis. Eight patients achieved complete responses after the previous treatment of HCC by RFA or resection before the initiation of

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DCV plus ASV. The median LSM value was 11.1 (3.8-28.8) kPa, with the number of patients with LSM<12.0 and ≥12.0 kPa being 26 and 18, respectively. There were 36 patients with a Child Pugh score of 5 and 8 with a score of 6; none of the patients exhibited decompensated cirrhosis. Table 2 showed the previous history of HCC treatment before DCV plus ASV. Only one HCC nodule was present in each patient. Previous studies reported that the existence of pre-treatment variants in the NS3A and NS5A regions affected the outcomes of DCV plus ASV combination therapy [5,11,12]. Therefore, variants associated with L31 and Y93 in the NS5A region of the HCV genome in pre-treatment patient serum were tested using a direct sequencing method [12,13] because the variants associated with Y93 and L31 in the NS5A region are known to be the most strongly associated with treatment outcomes [5,11,12]. NS5A variants were confirmed to be absent in all patients before the initiation of DCV plus ASV. All patients were administered 60 mg DCV (Daklinza®; Bristol-Myers KK, Tokyo, Japan) once daily and 100 mg ASV (Sunvepra®; Bristol-Myers KK, Tokyo, Japan) twice daily for 24 weeks between December 2014 and August 2015. Blood chemistry examinations and patient interviews were conducted every two weeks after the initiation of the treatment in order to detect adverse events. Virological responses were assessed 24 weeks after the completion of the treatment. SVR24 was defined as undetectable serum HCV RNA 24 weeks after the completion of the treatment. All patients were confirmed to have no HCC recurrence using abdominal computed tomography (CT) or magnetic resonance (MR) and ultrasound (US) before the initiation of DCV plus ASV.

Follow-up of patients

HCC was diagnosed based on the pattern of nodules on contrast-enhanced CT and gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA)-enhanced MR. CT or MR was performed every 6 months during the follow-up. Transient elastography (Fibroscan®) with LSM was prospectively performed at the initiation of DCV plus ASV, at the end of the treatment (EOT), and 24 and 72 weeks after the treatment. **Statistical analysis**

Quantitative variables were shown as medians (minimum-maximum). LSM values were compared using the *t*-test (parametric data). P<0.05 was defined as significant.

Results

Development of HCC (Table 2)

The occurrence of HCC was not detected during the follow-up. Three out of eight patients developed radiological HCC recurrence a few months after the treatment with DCV plus ASV. Case 1 is a 56-year-old male. Six months before DCV plus ASV, a 13-mm HCC nodule at segment 8 was detected on CT and he was treated with RFA. Three months after the HCV treatment, recurrence of the 13-mm HCC nodule at segment 7 was detected on CT and he was treated with Proton therapy. LSM values at SVR24 and 72 were 11.8 and 14.4 kPa, respectively, which were still high. Case 2 is a 68-year-old female. Fourteen months before DCV plus ASV, a 15-mm HCC nodule at segment 8 was detected on CT and she underwent liver resection. Her LSM value at EOT was 12.0 kPa, which was high. Three months after the HCV treatment, recurrence of the 15-mm HCC nodule at segment 6 was detected on MR and she was treated with RFA. LSM values at SVR24 and 72 were 10.1 and 6.8 kPa, respectively. Case 3 is a 65-year-old male. Four months before DCV plus ASV, a 20-mm HCC nodule at segment 6 was detected on MR and he was treated with RFA. His LSM value at EOT was 20.4 kPa, which was high. Five months after the HCV treatment, recurrence of the 15-mm HCC nodule at segment 3 was detected on MR and he was treated with RFA. His LSM value at SVR24 was 26.0 kPa, which was still high. LSM values measured by Fibroscan at the initiation of DCV plus ASV increased to 19.1, 13.1, and 26.0 kPa in Cases 1, 2, and 3, respectively, showing the development of liver cirrhosis [14]. LSM values at EOT were 12.8, 12.0, and 26.6 kPa, respectively, while the five other patients with LSM values less than 12 kPa at the last Fibroscan have shown no recurrence.

Variations in LSM during the 2-year follow-up

Twenty-four patients underwent LSM at the time of starting DCV plus ASV <12.0 kPa without HCC (Figure a). Their LSM values at the initiation of DCV plus ASV, at EOT, and 24 and 72 weeks after the treatment were 7.2 (3.8-11.8), 6.1 (2.6-14.0), 5.4 (3.2-7.8), and 4.7 (3.3-8.1) kPa, respectively, showing significant improvements (Figure a). Twelve patients underwent LSM at the time of starting DCV plus ASV

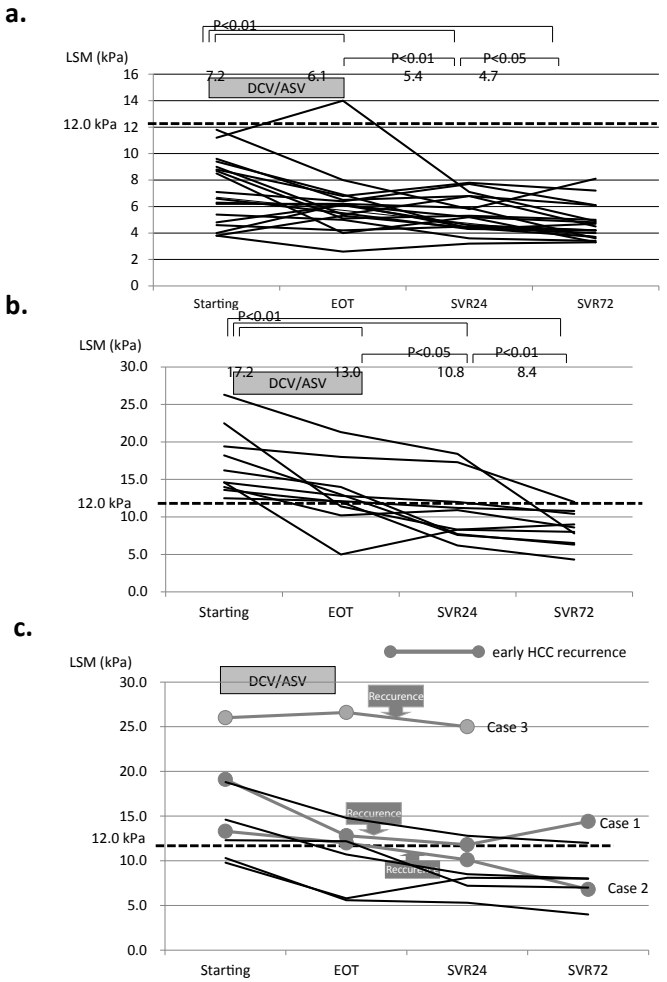

Figure 1. a. Twenty-four patients underwent LSM at the time of starting DCV/ASV <12.0 kPa without HCC. Their LSM values at the initiation of DCV plus ASV, at the end of the treatment (EOT), and 24 and 72 weeks after the treatment significantly improved: 7.2 (3.8-11.8), 6.1 (2.6-14.0), 5.4 (3.2-7.8), and 4.7 (3.3-8.1) kPa, respectively. DCV: daclatasvir, ASV: asunaprevir. **b.** Twelve patients underwent LSM at the time of starting DCV plus ASV ≥12.0 kPa without HCC. Their LSM values also significantly improved: 17.2 (12.5-28.8), 13.0 (5.0-21.3), 10.8 (6.2-18.4) and 8.4 (4.3-12.0) kPa, respectively, ultimately reaching less than 12 kPa. DCV: daclatasvir, ASV: asunaprevir. **c.** LSM values of three recurrent HCC patientsat the initiation of DCV plus ASV increased to 19.1, 13.1, and 26.0 kPa, respectively, and LSM values at EOTwere 12.8, 12.0, and 26.6 kPa, respectively, while five other patients with LSM values less than 12 kPa at the last Fibroscan have shown no recurrence. DCV: daclatasvir, ASV: asunaprevir.

≥12.0 kPa without HCC (Figure b). Their LSM values also significantly improved: 17.2 (12.5-28.8), 13.0 (5.0-21.3), 10.8 (6.2-18.4) and 8.4 (4.3-12.0) kPa at the initiation of DCV plus ASV, at EOT, and 24 and 72 weeks after the treatment, respectively, ultimately reaching less than 12 kPa (Figure b). LSM values in the 3 patients with recurrent HCC at the initiation of DCV plus ASV increased to 19.1, 13.1, and 26.0 kPa, respectively, while LSM values at EOT were 12.8, 12.0, and 26.6 kPa, respectively. The five other patients with LSM values less than 12 kPa at the last Fibroscan have shown no recurrence (Figure c).

Discussion

In the present study, among eight patients infected with HCV genotype 1b who achieved SVR by DCV plus ASV for 24 weeks after complete responses to the treatment of HCC by ablation or resection (only one HCC nodule was present in each patient), three subsequently developed radiological HCC recurrence after a few months. In the study by Reig M [8], some patients were treated with TACE, which is potentially non-curative because of its characteristic of a high early recurrence rate. Although all our patients were treated with potentially curative treatments, resection and RFA, HCC recurrence occurred a few months after DCV plus ASV.

Fibroscan with LSM was prospectively performed at the initiation of DCV plus ASV, at EOT, and 24 and 72 weeks after the treatment. LSM values measured by Fibroscan increased to 19.1, 13.1, and 26.0 kPa in Cases 1, 2, and 3, respectively, showing the development of liver cirrhosis. Since an LSM value of more than 12.0 kPa was previously reported to indicate severe fibrosis [14], we considered it important to start the treatment of HCV as soon as possible. Conti F, *et al.* showed that, in an analysis of 59 patients with a previous history of HCC, patients with LSM >21.5 kPa before DAA were significantly more likely to show HCC recurrence [15]. LSM >21.5 kPa is assumed to be liver cirrhosis, and that of Case 3 only was >21.5 kPa. Cheung M C M, *et al.* found that DAA therapy in patients with decompensated cirrhosis did not decrease the occurrence of HCC [16]. However, Conti F, *et al.* did not examine changes in LSM during the prospective follow-up. Therefore, we analyzed changes in LSM in eight patients with a previous history of HCC during a follow-up of two years. LSM values at the initiation of DCV plus ASV increased to 19.1, 13.1, and 26.0 kPa in Cases 1, 2, and 3, respectively, showing the development of liver cirrhosis. Moreover, LSM values at EOT were 12.8, 12.0, and 26.6 kPa, respectively, which were high before the recurrence of HCC. On the other hand, the LSM values of the other 5 cases without recurrence gradually decreased to less than 12.0 kPa between EOT and 72 weeks after DCV plus ASV. In HCV patients previously treated for HCC with high LSM values not only before, but also after DAA, the risk of HCC recurrence may still be high.

SVR patients following interferon-based treatments showed the significant regression of LSM [10]. Furthermore, in SVR patients treated with DAA, LSM values were significantly reduced during follow-ups [17,18]; this early improvement was attributed to a decrease in inflammation. LSM values showed further significant decreases during the subsequent follow-up, even in the other 5 cases without recurrence (Table 1). Since artifacts due to liver inflammation were no longer implicated, decreases in LSM values at EOT and SVR24 and 72 may reflect a genuine and progressive reduction in fibrosis (Figure a, b). However, LSM values at EOT were 12.8, 12.0, and 26.6 kPa in Cases 1, 2, and 3, respectively, which were high without the regression of fibrosis before HCC recurrence. In patients previously treated for HCC with high LSM values before and after DAA, the risk of HCC recurrence may be high. The effects of DAA on the occurrence and recurrence of HCC currently remain unknown because of a lack of evidence [19]. A large prospective study with properly selected populations and a long-term follow-up needs to be conducted. Furthermore, the extent to which the regression of LSM decreases the risk of HCC recurrence after DAA achieving SVR needs to be estimated in a large number of cases.

Table 1. Baseline characteristics of patients included in this study (n=44).

Age (year)	70 (39-84)
Male : Female	15 : 29
Duration of the follow-up (months)	22 (17-28)
IFN ineligible : intolerant	28*: 3**
Previous IFN treatment Non-responder : Relapse	11 : 3
History of HCC treatment with a complete response 8	
Resection : Ablation	2 : 6
HCV RNA (Log IU/ml)	6.2 (4.0-7.4)
White cell count (/μl)	4800 (1500-10600)
Hemoglobin (g/dl)	13.6 (7.3-17.2)
Platelets (10^4/μl)	15.5 (6.9-28.4)
Aspartate aminotransferase (IU/L)	42 (20-234)
Alanine aminotransferase (IU/L)	44 (18-389)
γ-Glutamyltransferase (IU/L)	36 (15-55)
Total Bilirubin (mg/dl)	0.70 (0.34-1.94)
Albumin (g/dl)	4.1 (2.8-5.2)
Creatinine (mg/dl)	0.73 (0.46-1.63)
Alpha-fetoprotein (ng/ml)	5 (1-450)
Fib-4	3.21 (0.87-9.66)
<3.25 : ≥3.25	23 : 21
Liver stiffness (kPa)	11.1 (3.8-28.8)
<12.0 : ≥12.0	26 : 18
Child Pugh score 5 : 6	36 : 8

*Diabetes mellitus 5, Depression 6, Idiopathic thrombocytopenia 4, Paroxysmal nocturnal hemoglobinuria 1, Myocardial infarction 2, Non-tuberculosis mycobacteria 1, Collagen disease 4, older than 75 years 5. **Retinal hemorrhage 2, Vertebritis 1

Table 2. Courses of HCCbefore and LSM after the treatment with DCV/ASV in 8 cases.

3 Cases with recurrence	**Age**	**Gender**		**Before or after DCV/ASV (months)**	**Pattern of HCC* (Imaging technique/Location/Size/ Treatment)**		**LSM (kPa)**		
						At the initiation of DCV/ASV	EOT	SVR24	SVR72
1	56	M	At the occurrence of HCC	-6	CT/S8/13 mm/RFA	**19.1**	**12.8**	11.8	14.4
			At the recurrence of HCC	+3	CT/S7/13 mm/Proton therapy				
2	65	F	At the occurrence of HCC	-14	CT/S8/15 mm/Resection	**13.3**	**12.0**	10.1	6.8
			At the recurrence of HCC	+3	MR/S6/15 mm/RFA				
3	65	M	At the occurrence of HCC	-4	MR/S6/20 mm/RFA	**26.0**	**26.6**	**26.0**	
			At the recurrence of HCC	+5	MR/S3/15 mm/RFA				

5 other caseswithout recurrence	**Age**	**Gender**		**Before DCV/ASV (months)**	**Pattern of HCC* (Imaging technique/Location/Size/ Treatment)**		**LSM (kPa)**		
						At the initiation of DCV/ASV	EOT	SVR24	SVR72
4	80	M	At the occurrence of HCC	-19	CT/S4/18 mm/RFA	**18.8**	**14.8**	**12.8**	12.0
5	74	M	At the occurrence of HCC	-3	MR/S4/10 mm/RFA	**14.6**	10.7	8.5	8.0
6	84	M	At the occurrence of HCC	-8	MR/S4/10 mm/RFA	12.3	12.2	7.2	7.0
7	78	M	At the occurrence of HCC	-12	CT/S8/30 mm/Resection	10.3	5.6	5.3	4.0
8	71	M	At the occurrence of HCC	-6	CT/S3/12 mm/RFA	9.8	8.8	8.1	8.0

*one nodule in each patient
DCV/ASV: daclatasvir plus asunaprevir, LSM: liver stiffness measurement (≥12.5 kPa indicates liver cirrhosis), ETO: end of the treatment, SVR: sustained viral response, RFA: radiofrequency ablation.

Conclusion

In conclusion, HCV patients previously treated for HCC with high LSM values before and after DAA are still at a high risk of HCC recurrence, and need to be closely monitored while LSM values are high, even after the achievement of SVR.

Conflict of interest

Noboru Hirashima, Hiroaki Iwase, Masaaki Shimada, Nobumitsu Ryuge, Noboru Urata, and Etsuko Iio have no conflict of interest. Yasuhito Tanaka received lecture fees from Bristol-Myers Squibb Company, MSD K.K., Chugai Pharmaceutical Co., Ltd., Janssen Pharmaceutical K.K., and Gilead Sciences. Yasuhito Tanaka received Commercial research funding from Bristol-Myers Squibb Company, Chugai Pharmaceutical Co., Ltd., and AbbVie Inc.

Human rights

All procedures followed have been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments.

Informed consent

Informed consent was obtained from all patients before their inclusion in this study.

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