

ORIGINAL ARTICLE

Hepatocellular carcinoma after direct-acting antiviral drug treatment in patients with hepatitis C virus

Tomomi Kogiso,*¹ Takaomi Sagawa,* Kazuhisa Kodama,* Makiko Taniai,*² Satoshi Katagiri,^{†,‡} Hiroto Egawa,[‡] Masakazu Yamamoto[‡] and Katsutoshi Tokushige*

Departments of *Internal Medicine, Institute of Gastroenterology, [‡]Surgery, Institute of Gastroenterology, Tokyo Women's Medical University, Tokyo and [†]Department of Surgery, Tokyo Women's Medical University, Yachiyo Medical Center, Yachiyo, Japan

Key words

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Correspondence

Tomomi Kogiso, 8-1, Kawada-cho, Shinjuku-ku, Tokyo, 162-8666, Japan.
Email: kogiso.tomomi@twmu.ac.jp

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Introduction

Hepatitis C virus (HCV) infection is a major cause of chronic hepatitis, which leads to cirrhosis and hepatocellular carcinoma (HCC).¹ Hepatocarcinogenesis due to HCV infection is thought to be initiated via direct and indirect mechanisms.² It is mainly triggered by indirect immune-mediated chronic inflammation. During HCV infection, liver-infiltrating lymphocytes fail to clear the virus, and chronic inflammation occurs. A high CD8+ T cell count is correlated with HCC occurrence and is considered a predictor of recurrence after surgical resection.³ Many inflammatory cytokines have been implicated in chronic liver inflammation and HCC progression in HCV-infected patients, including tumor necrosis factor- α , interleukin (IL)-1, IL-23, IL-6, and lymphotaxins.^{4,5} In terms of a direct effect of HCV on HCC development, HCV proteins are associated with an increased epithelial/mesenchymal transition and reactive oxygen species expression, which lead to chronic inflammation and hepatocarcinogenesis. Host tumor suppressors and proto-oncogenes are the direct targets of HCV. The HCV core, E2, NS3, and NS5 proteins stimulate

Abstract

Background and Aim: Given the use of direct-acting antivirals (DAAs) to treat hepatitis C virus (HCV), their effects on hepatocarcinogenesis should be determined.

Methods: This study enrolled 349 patients with HCV who underwent DAA treatment at our hospital between 2014 and 2018. Their median age was 65 years, and 184 were male; 301 cases were of HCV serotype 1, and 48 were of serotype 2. The DAA treatment was daclatasvir/asunaprevir in 107 cases, sofosbuvir (SOF)/ledipasvir in 147 cases, ritonavir-boosted ombitasvir/paritaprevir in 28 cases, elbasvir/grazoprevir in 19 cases, and SOF/ribavirin in 48 cases. The patients' histories included hepatocellular carcinoma (HCC) in 45 cases, liver transplant (LT) in 10 cases, and kidney transplant (KT) in 17 cases.

Results: Sustained virological responses occurred in 335 cases (96%). DAA treatment was initiated a median of 16.3 months after HCC treatment. After DAA treatment, 15 cases (33%) had recurrence of HCC after a median of 11.6 months, and 3 cases (1%) developed *de novo* HCC. Six LT patients and one KT patient had HCC; however, no HCC was observed after DAA. The incidence of HCC was significantly higher in patients with multiple HCC treatments in the Cox hazard model (hazard ratio 1.664, 95% confidence interval 1.134–2.441, $P < 0.01$). Surgical resection or LT reduced the risk of HCC.

Conclusions: DAA did not increase the rate of HCC, even in immunosuppressed patients. However, careful follow-up for HCC recurrence is required in previously treated cases.

cellular proliferation by interfering with the rapidly accelerated fibrosarcoma/mitogen-activated protein kinase/extracellular signal-regulated kinase pathway.^{6,7}

The eradication of HCV was expected to reduce HCC development because HCC develops frequently in HCV-based liver disease.¹ Antiviral therapy and interferon (IFN) reduce hepatocarcinogenesis by decreasing inflammation and fibrosis.^{8,9}

Direct-acting antivirals (DAA) were developed to treat HCV infection and can achieve high sustained virological response (SVR) rates of >90%, similar to conventional IFN therapy.¹⁰ Regimens approved by the US Food and Drug Administration are available for genotype 1, including daclatasvir (DCV)/asunaprevir (ASV), sofosbuvir (SOF)/ledipasvir (LDV), SOF/simeprevir (not in Japan), ritonavir (r)-boosted ombitasvir (OBV)/paritaprevir (PTV), DCV/ASV/beclabuvir, and elbasvir/grazoprevir (EBR/GZR); in Japan, SOF/ribavirin (RBV), SOF/LDV, and OBV/PTV/r have been approved for genotype 2.^{11–13} Recently, a DAA with pan-genotypic activity, glecaprevir/pibrentasvir, was introduced, and HCV eradication is being achieved more frequently.¹⁴

Although IFN therapy reduces HCC,^{15,16} it is still unclear whether DAA reduces the risk of *de novo* or recurrent HCC. Here, we examined hepatocarcinogenesis in DAA-treated patients and considered the risk of HCC and the timing of DAA therapy following HCC treatment.

Methods

Patients and study design. This retrospective study enrolled 349 patients with HCV infection, including 10 liver transplant (LT) recipients and 17 kidney transplant (KT) recipients. The treatment of HCV serotype 1 included a 24-week combined regimen of NS5A and NS3 protease-targeted DAAs (DCV; Daklinza) at 60 mg once a day and ASV (Sunvepra) at 100 mg twice a day (Bristol-Myers Squibb, New York, NY, USA) in 107 patients and a 12-week treatment with NS5A protease- and NS5B polymerase-targeted DAAs (400 mg SOF/90 mg LDV [Harvoni] once a day [Gilead Sciences, Foster City, CA, USA]) in 147 patients; a 12-week combined regimen of NS5A and NS3 protease-targeted DAAs (Viekirax, OBV 25 mg/PTV 150 mg/r 100 mg once a day [AbbVie, North Chicago, IL, USA]) in 28 cases; and EBR/GZR, Erelsa 50 mg/Grazina 100 mg once a day (Merck Sharp & Dohme, Kenilworth, NJ, USA) in 19 cases. HCV serotype 2 was treated with SOF + RBV (Sovaldi, 400 mg once a day [Gilead Sciences] and Rebetol at 800–1400 mg orally according to patient body weight [Merck Sharp & Dohme, Kenilworth, NJ, USA]) for 12 weeks.

Patients with severe liver dysfunction were excluded, such as those with decompensated cirrhosis or residual HCC. Patients with severe renal impairment were not given the SOF-based regimen (estimated glomerular filtration rate [eGFR] < 30 mL/min/1.73 m²) or RBV (eGFR < 50 mL/min/1.73 m²).

Detailed clinical and demographic information was collected, including patient age, gender, and body mass index (BMI), and complications due to lifestyle-related diseases. Laboratory data were evaluated at the time of DAA treatment, including the tumor markers alpha fetoprotein (AFP) and des-gamma-carboxy pro-thrombin (DCP). The Child–Turcotte–Pugh score and fibrosis-4 index were evaluated.

The diagnosis of fatty liver was based on fat accumulation as seen on abdominal ultrasound or computed tomography (CT). Liver cirrhosis (LC) was defined as stage 4 fibrosis on a liver biopsy or typical clinical findings on liver imaging, esophageal and gastric varices formation, and splenomegaly. HCC was diagnosed histologically or based on imaging findings consistent with the diagnosis, using at least two of the following methods: abdominal ultrasound, CT, magnetic resonance imaging, and selective hepatic arteriography.¹⁷ The macroscopic classification, histological differentiation, and architectural pattern were evaluated according to the classification based on the general rules for the clinical and pathological studies of primary liver cancer.^{18–20} For HCC therapy, surgical resection, radiofrequency ablation (RFA), transcatheter arterial chemoembolization (TACE), and LT were conducted according to guidelines,²¹ and treatment selection was dependent on each doctor in charge. Before the DAA treatment, the HCC was completely treated and was diagnosed as healing HCC by imaging.

This study was conducted according to the principles of the 1964 Declaration of Helsinki and the ethical guidelines of the Tokyo Women's Medical University Hospital (TWMU, Tokyo, Japan). The TWMU Institutional Review Board approved the study protocol.

Statistical analysis. We compared the clinical features of patients with and without HCC before the DAA treatment. Data are presented as medians and range for continuous variables and as proportions for categorical variables. Significant differences between the two groups were assessed using the Mann–Whitney *U* test and χ^2 test. A *P* value < 0.05 was considered to indicate statistical significance. The occurrence of HCC after DAA treatment was evaluated by constructing Kaplan–Meier curves in patients with and without a history of HCC, as well as according to the HCC treatment regimen, number of treatments, and transplantation. Univariate and multivariate Cox regression analyses were used to evaluate the occurrence of HCC after DAA treatment in patients with and without a history of HCC, as well as according to the HCC treatment regimen, number of treatments, and transplantation. Hazard ratios (HR) and 95% confidence intervals (CI) were estimated. The SPSS software package (SPSS Inc., Chicago, IL, USA) was used for the statistical analyses.

Results

Patient characteristics and comparison of those with and without a history of HCC. Of the 349 DAA-treated cases, 45 had a history of HCC (Fig. 1, Table 1). Comparing patients with and without a history of HCC, the HCC cases were older (median age, 65 years [range: 22–86 years]) and more likely to be male (both *P* < 0.01). Advanced fibrosis was more frequent in the HCC cases (*P* < 0.01). IFN therapy was administered more often in HCC cases; however, the difference was not significant after adjusting for age and fibrosis. BMI

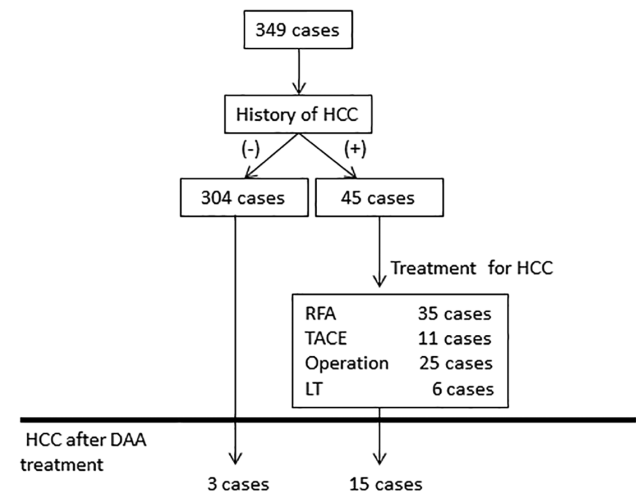


Figure 1 Flow chart of the study. In total, 349 cases were analyzed, and 45 cases had a history of hepatocellular carcinoma (HCC) before direct-acting antiviral drug (DAA) treatment. After DAA treatment, 18 cases were complicated with HCC.

Table 1 Patient characteristics

	Total (<i>n</i> = 349)	HCC (-) (<i>n</i> = 304)	Previous HCC (+) (<i>n</i> = 45)	HCC (+) Post DAA (<i>n</i> = 15)	HCC (-) vs HCC (+) <i>P</i> value	
					HCC (-) vs Previous HCC (+)	Post-DAA among previous HCC (+)
Age (years)	65 (22–86)	65 (22–86)	69 (48–82)	70 (52–81)	<0.01	0.78
Males (% male)	184 (53)	152 (50)	32 (71)	11 (73)	<0.01	0.82
HCV serotype 1/2	301/48	262/42	39/6	13/2	0.93	1.00
HCV-RNA (logIU/mL)	6.2 (2.5–8.0)	6.2 (2.5–7.5)	6.0 (3.8–8.0)	6.2 (4.1–6.7)	0.30	0.47
Body mass index (kg/m ²)	22.6 (13.9–40.4)	22.5 (13.9–40.4)	22.8 (17.4–28.0)	23.0 (17.4–25.3)	0.71	0.76
Complications						
Diabetes (%)	72 (21)	60 (20)	12 (27)	4 (27)	0.29	1.00
Dyslipidemia (%)	61 (17)	55 (18)	6 (13)	0 (0)	0.42	0.06
Fatty liver (%)	35 (10)	32 (11)	3 (7)	1 (7)	0.44	0.98
Liver cirrhosis (%)	56 (16)	41 (13)	15 (33)	6 (40)	<0.01	0.50
Hemoglobin A1c (%)	5.8 (4.5–9.7)	5.8 (4.6–9.7)	5.9 (4.5–7.6)	6.0 (5.2–7.6)	0.41	0.40
CTP score	5 (5–8)	5 (5–8)	5 (5–8)	5 (5–6)	0.20	0.35
FIB-4 index	2.63 (0.28–23.91)	2.50 (0.28–23.91)	5.33 (1.64–15.40)	6.71 (2.10–15.40)	<0.01	0.20
AFP (ng/mL)	5 (1–230)	5 (1–230)	8 (2–140)	11.5 (2–48)	0.37	0.98
DCP (mAU/mL)	17 (6–74)	17 (7–74)	15 (6–55)	17 (14–55)	0.93	0.34
Previous IFN therapy (%)	126 (36)	102 (34)	24 (53)	6 (40)	<0.01	0.09
Previous HCC treatment		—			—	<0.05
Surgical resection	25		25	8		
RFA	35		35	20		
TACE	11		11	3		
LT	6		6	0		
Number of previous HCC treatments	1 (1–6)	—	1 (1–6)	2 (1–6)	—	0.13
DAA treatment					0.88	0.30
DCV/ASV	107	91	16	7		
SOF/LDV	147	129	18	6		
OBV/PTV/r	28	25	3	0		
EBR/GZR	19	17	2	0		
SOF + RBV	48	42	6	2		
SVR (%)	335 (96)	292 (96)	43 (96)	14 (93)	0.85	0.87
Initiation of DAA after HCC treatment (months)	16.3 (2.1–242.5)	—	16.3 (2.1–242.5)	22.0 (2.9–46.3)	—	0.25
Observation period (months)	25.5 (0.3–41.3)	25.5 (0.3–41.1)	25.9 (2.7–41.3)	25.9 (13.0–41.3)	0.32	0.07
Posttransplantation					<0.05	0.10
Liver	10	4	6	0		
Kidney	17	16	1	0		
HCC occurrence following DAA treatment	18	3	15	15	<0.01	—
Time of HCC occurrence after DAA (months)	15.6 (2.2–34.2)	20.5 (18.8–23.5)	11.6 (2.2–34.2)	11.6(2.2–34.2)	<0.05	—

AFP, alpha fetoprotein; CTP, Child–Turcotte–Pugh; DAA, direct-acting antiviral; DCP, des-gamma-carboxy pro-thrombin; DCV/ASV, daclatasvir/asunaprevir; EBR/GZR, elbasvir/grazoprevir; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; LT, liver transplantation; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; RBV, ribavirin; RFA, radiofrequency ablation; SOF/LDV, sofosbuvir/ledipasvir; SVR, sustained virological response; TACE, transcatheter arterial chemo-embolization.

and the rates of complications of lifestyle-related diseases did not differ significantly between patients with and without a history of HCC. After DAA treatment, the SVR rate was ≥93%, and 18 cases developed HCC, including 15 cases of recurrent HCC and 3 of *de novo* HCC. Recurrent HCC (*n* = 15) (Table 2) occurred after a median of 22.0 (2.9–46.3) months of DAA treatment, whereas those with no HCC (*n* = 30) had 16.1 (2.1–242.5) months of DAA treatment. No significant difference was observed in the starting time of DAA between HCC-recurrent and nonrecurrent cases. However, the patients with multiple

HCC treatments were administered DAA soon after HCC therapy (cases 5 and 13).

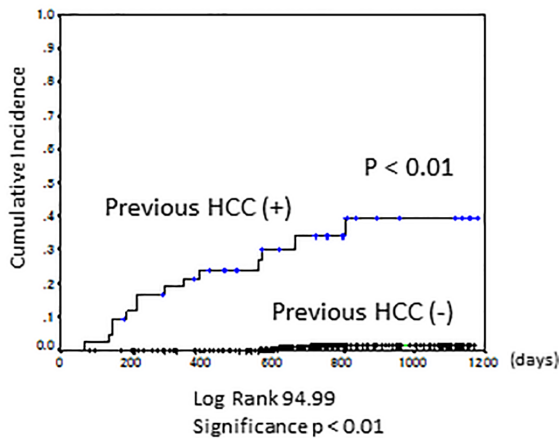
Previous treatment of HCC and HCC recurrence after DAA. The rate of HCC occurrence following DAA treatment was significantly increased in patients with a history of HCC on Kaplan–Meier analysis (Fig. 2, *P* < 0.01). The median interval until HCC occurrence after DAA therapy was 11.6 months (range: 2.2–34.2 months) in recurrent HCC and

Table 2 Hepatocellular carcinoma (HCC) cases after direct-acting antiviral (DAA) treatment

Case	Age (y)	Sex	fibrosis	IFN treatment	DAA regimen	HCC treatment pre-DAA	DAA after last HCC treatment (months)	First HCC occurrence after DAA (months)	The number of HCC	HCC treatment post-DAA	Prognosis and HCC control
1	72	M	CH	—	SOF + RBV	Resection RFA	40.7	7.1	1	RFA	Alive
2	70	M	CH	PEG-IFN + RBV+SMV	SOF/LDV	Resection	44.7	11.6	1	RFA twice	Alive
3	52	M	LC	IFN + RBV	SOF/LDV	TACE twice	3.1	4.8	1	TACE twice	Death
4	74	M	CH	—	SOF/LDV	RFA	12.4	19.1	2	Resection	Alive
5	58	M	CH	PEG-IFN + RBV	SOF/LDV	RFA 6 times	6.7	6.3	Multiple	TACE twice RFA	Alive and uncontrolled
6	69	M	LC	—	SOF/LDV	Resection TACE	22.2	9.8	2	TACE	Alive
7	70	F	LC	—	SOF/LDV	RFA twice	26.9	7.2	1	RFA	Alive
8	72	M	LC	PEG-IFN + RBV	DCV/ASV	Resection RFA	13.3	34.2	1	RFA	Alive
9	75	F	CH	PEG-IFN + RBV+SMV	DCV/ASV	Resection	10.1	2.2	Multiple	TACE	Alive
10	70	M	LC	IFN-beta	DCV/ASV	RFA	46.3	26.9	unknown	RFA	Alive
11	68	M	CH	unknown	DCV/ASV	Resection twice	43.8	19.0	1	TACE	Alive
12	65	F	CH	PEG-IFN + RBV	DCV/ASV	RFA twice	14.4	4.8	1	RFA	Alive
13	74	M	LC	PEG-IFN	DCV/ASV	RFA 4 times Resection	2.9	27.7	Multiple	RFA TACE 6 times	Alive and uncontrolled
14	74	F	CH	PEG-IFN + RBV	DCV/ASV	RFA	22.0	22.1	1	Resection	Alive
15	81	M	CH	—	SOF + RBV	RFA	27.5	13.1	Multiple	TACE 4 times Sorafenib	Alive and uncontrolled
16	74	F	CH	PEG-IFN + RBV	SOF/LDV	—	—	20.5	1	RFA	Alive
17	76	M	LC	PEG-IFN + RBV	DCV/ASV	—	—	18.8	1	RFA	Alive
18	71	M	CH	PEG-IFN + RBV	DCV/ASV	—	—	23.5	1	Resection	Alive

CH, chronic hepatitis; DCV/ASV, daclatasvir/asunaprevir; EBR/GZR, elbasvir/grazoprevir; F, female; LC, liver cirrhosis; M, male; OBV/PTV/r, ombitasvir/paritaprevir/ritonavir; PEG-IFN, pegylated interferon; RBV, ribavirin, RFA, radiofrequency ablation; SMV, simeprevir; SOF/LDV, sofosbuvir/ledipasvir; TACE, transcatheter arterial chemo-embolization.

HCC occurrence in patients with and without a previous history of HCC.



	Total	Number of events	Number of censored	Percent of censored
Previous HCC (-)	304	3	301	99.01
Previous HCC (+)	45	15	30	66.67
Overall	349	18	331	94.81

Figure 2 Hepatocellular carcinoma (HCC) occurrence and recurrence in patients with and without a previous history of HCC. HCC occurred significantly more often in patients with a history of previous HCC by Kaplan–Meier analysis ($P < 0.01$).

20.5 months (range: 18.8–23.5 months) in *de novo* HCC ($P < 0.05$, Table 1).

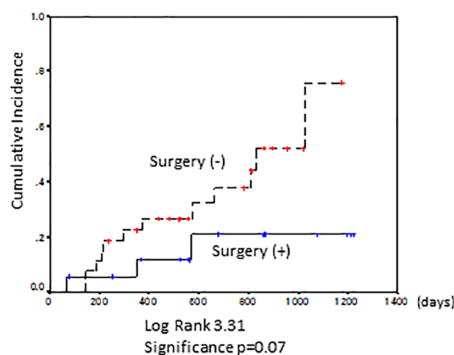
The treatments of previous HCC ($n = 45$) included surgical resection in 25 cases, RFA in 35 cases, TACE in 11 cases, and LT in 6 cases. The previous HCC treatment differed

significantly between recurrence ($n = 15$) and nonrecurrence ($n = 30$, $P < 0.05$). The patients treated with surgical resection or LT had a lower rate of recurrence than those administered the other treatment regimens ($P = 0.07$, Fig. 3a). Ten LT recipients received DAA, of whom six had HCC at the time of transplantation. All patients were started on DAAs with immunosuppression using tacrolimus and mycophenolate mofetil (MMF) \pm steroid at 18.5 months (range: 5.9–242.5 months) after transplantation. Nine patients achieved SVR (90%), and following DAA therapy, no patient developed HCC after follow-up for 32.4 months (range: 12.3–40.7 months), even those with a history of HCC. In comparison, the 17 KT recipients were treated with DAA with azathioprine or tacrolimus and MMF \pm methyl prednisone or everolimus. All patients achieved SVR, and one had a history of HCC; however, there was no recurrent or *de novo* HCC following DAA treatment after follow-up for 27.9 months (range: 5.2–40.4 months). Compared with nonrecipients ($n = 322$), LT/KT recipients ($n = 27$) did not show significantly increased HCC occurrence, even under immunosuppression, during 29.0 (range: 5.2–40.7) months of follow-up (Fig. 4).

The median number of HCC treatments before DAA was two (range: 1–6) in recurrent HCC and one (range: 1–5) in the nonrecurrent HCC cases (Table 1, $P = 0.13$). The patients who were treated for HCC multiple times had significantly more recurrence after DAA (Fig. 3b, $P < 0.01$). They especially required multiple HCC treatments after DAA, delivered over a short period. Three patients had five or more treatments before DAA, and two of these developed multiple HCC and required several treatments after DAA (cases 5 and 13, Table 2).

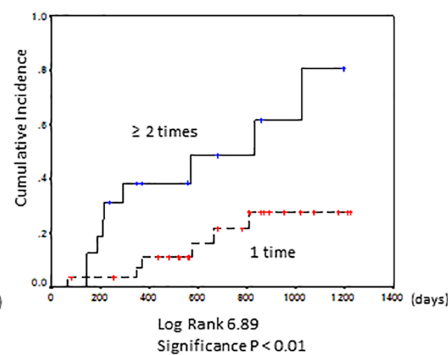
The incidence of HCC after DAA was significantly higher in patients with multiple episodes of HCC treatment; this was a risk factor for developing HCC in the Cox hazard analysis (HR 1.664, 95% CI 1.134–2.441, $P < 0.01$). Treatment with surgical resection or LT was not a significant risk factor for developing HCC (HR 0.274, 95% CI 0.060–1.262, $P = 0.097$).

(a) HCC treatment regimen



	Total	Number of events	Number of censored	Percent of censored
Surgery (-)	27	12	15	55.56
Surgery (+)	18	3	15	83.33
Overall	45	15	30	66.67

(b) The number of HCC treatment



	Total	Number of events	Number of censored	Percent of censored
< 1 time	29	6	23	79.31
≥ 2 times	16	9	7	43.75
Overall	45	15	30	66.67

Figure 3 Hepatocellular carcinoma (HCC) recurrence according to type of previous HCC treatment. (a) HCC treatment regimen, (b) number of treatments. Kaplan–Meier analysis showed that the rate of HCC tended to be reduced by surgery (surgical resection or LT [a, $P = 0.07$]) and significantly increased with multiple treatments (b, $P < 0.01$).

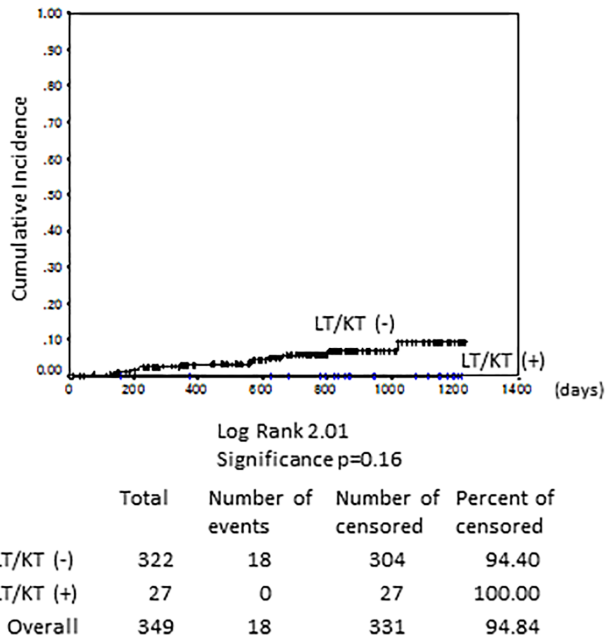


Figure 4 Hepatocellular carcinoma (HCC) recurrence according to liver/kidney transplant (LT/KT). Compared with nonrecipients, LT/KT recipients did not show significantly increased HCC occurrence, even under immunosuppression, during 29.0 (range: 5.2–40.7) months of follow-up (Kaplan–Meier analysis, $P = 0.16$).

Factors affecting the incidence of HCC included age, gender, LC, a history of HCC, number of HCC treatments, HCC and DAA treatment regimens, and HCV genotype.

Characteristics of HCC following DAA treatment and prognosis.

Regarding the characteristics of HCC, the diameter of the HCC before DAA was 18 mm (range: 3–70 mm, Table 3). The macroscopic classification included 33 cases of the simple nodular type, 5 cases of the small nodular type with indistinct margins, 5 cases of the simple nodular type with extranodular growth, and 3 cases of the multinodular type. Histologically, 32 HCC cases were moderately differentiated, 34 had a trabecular pattern, and 2 had a tubular pattern. The diameter of the HCC that occurred after beginning DAA treatment was 18 mm (range: 4–25 mm), and five cases were of the simple nodular type, with moderate differentiation and a trabecular pattern. Overall, 9 of 31 (29%) nodules were recurrent cases. HCC after DAA was predominantly of the multiple nodule type ($P < 0.05$).

One patient died (case 3) of HCC, and all of the other patients are alive. HCC was uncontrolled in three cases despite several treatments (cases 5, 13, and 15).

A case of massive HCC recurrence after DAA treatment.

An 81-year-old man received RFA therapy 2.5 years before beginning DAA therapy (case 15). HCC was completely ablated (Fig. 5a,b). At the time of DAA treatment, no recurrence was observed on CT (Fig. 5c), and he was treated with SOF + RBV for 3 months. No adverse events were seen, and SVR was attained. Four months after DAA therapy, the

Table 3 Characteristics of hepatocellular carcinoma (HCC) before and after direct-acting antiviral (DAA) therapy

	HCC in pre-DAA ($n = 45$)	HCC in post-DAA ($n = 18$)	P value
Diameter of HCC (mm)	18 (3–70)	18 (4–25)	0.06
Number of HCCs			<0.05
Single	59	18	
Multiple	19	14	
Macroscopic classification			0.14
Small nodular type with indistinct margin	5	0	
Simple nodular	33	13	
Simple nodular type with extranodular growth	5	5	
Conflict multinodular type	3	0	
Histological differentiation			0.49
Well-differentiated	2	1	
Moderately differentiated	32	5	
Poorly differentiated	3	0	
Architectural pattern			0.55
Trabecular pattern	34	6	
Tubular pattern	2	0	

serum DCP level was slightly increased (to 55 mAU/mL). Eight months after the DAA treatment, the serum DCP level was further elevated (174 mAU/mL), and multiple tiny HCCs were seen on CT (Fig. 5d). Hepatic angiography detected multiple staining in both liver lobes (Fig. 5e). TACE was performed three times, and sorafenib was started at 19 months. However, the HCC was not controllable even after switching to sorafenib (Fig. 5f).

Discussion

We found 18 cases of HCC after DAA treatment, of which 15 (93%) were patients with a history of HCC before HCV eradication by DAA treatment. DAA did not increase HCC, even with an immunosuppressive regimen, and may not influence hepatocarcinogenesis directly. However, some patients developed faster-growing HCC with a more aggressive phenotype after DAA.

There is no DAA treatment that leads directly to HCC development, and there are also reports that it reduces, or at least does not increase, the risk of HCC.^{22–24} One meta-analysis found no evidence that HCC occurrence or recurrence differs between patients receiving DAA *versus* IFN therapy.²⁵ In the HEPATHER cohort, the 6-month recurrence rate was 10.6% in patients treated with DAA, whereas it was 18.7% in untreated patients.²⁶ Reports suggest that DAA does not increase the risk of HCC.²⁷ Clinically, as the transaminase level decreases sharply immediately after DAA initiation, HCV clearance may suppress the inflammation caused by DAA treatment. In addition, a study of HCV-infected patients treated with a SOF-based regimen reported a significant reduction in the rate of liver fibrosis.²⁸

Nevertheless, there are reports of DAA-associated hepatocarcinogenesis. Yang *et al.* reported that early HCC recurrence was more frequent in DAA-treated patients.²⁹ We experienced a patient who developed HCC after DAA. The HCC phenotype was obviously different from the previous HCC, and intrahepatic

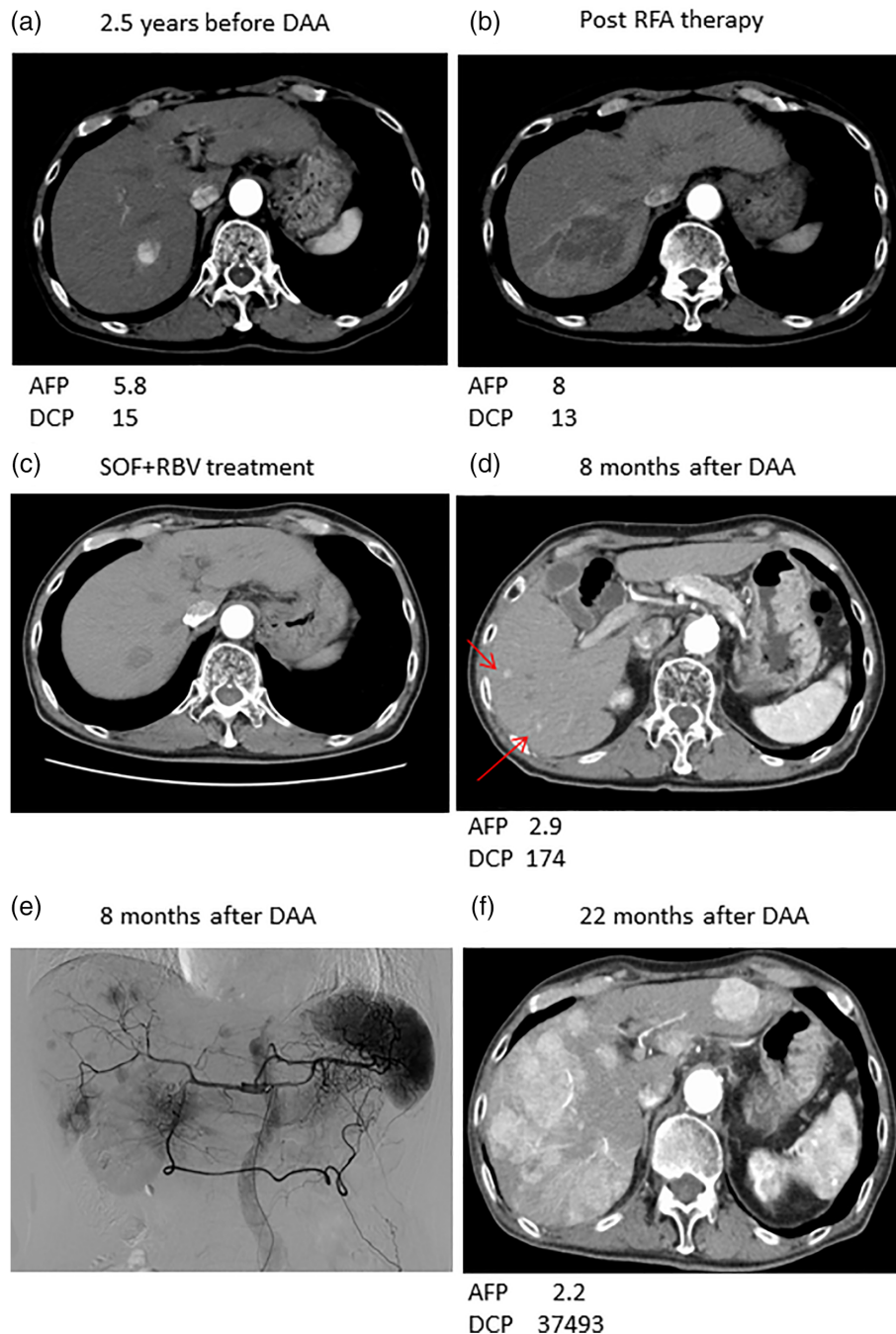


Figure 5 Representative computed tomography findings in case no. 15 after direct-acting antiviral (DAA) HCC recurrence: (a) 2.5 years before DAA treatment; (b) after RFA therapy; (c) at the time of sofosbuvir + ribavirin (SOF + RBV) treatment; and (d) 8 and (f) 19 months after DAA treatment. Red arrows, hepatocellular carcinoma (HCC). (e) Abdominal angiography 8 months after DAA treatment. HCC was observed 2.5 years before DAA treatment (a) and was completely cured by radiofrequency ablation (RFA) therapy (b). SOF + RBV was administered for 3 months (c). Tiny HCCs were observed 8 months after DAA treatment (d), and hepatic arteriography showed multiple tumors with des-gamma-carboxy pro-thrombin (DCP) elevation (e). HCC was uncontrollable despite many treatments (f). AFP, alpha fetoprotein.

metastasis was seen with DCP elevation (Fig. 5). An immunological mechanism is suspected to underlie the high rate of HCC occurrence with DAA. After HCV clearance, decreased levels of inflammation may lead to reduced tumor immunosurveillance by the host and promote HCC.^{30,31} Moreover, a reduction in natural

killer group 2 member D, which is associated with cancer immunity,³² and increased serum levels of vascular endothelial growth factor during the DAA treatment³³ have both been reported. Zanetto *et al.* reported that tumor growth in patients with HCC while on the waiting list for LT did not differ

significantly before *versus* after DAA treatment.³⁴ It is not clear whether DAA increases the malignant potential of HCC. We speculate that, while DAA may not directly promote HCC, it increased the incidence and number of HCCs at the time of DAA treatment via an indirect mechanism involving the suppression of inflammation.

In this study, HCC recurrence was observed in patients treated for HCC multiple times before DAA treatment. Patients with a history of HCC are already in a precarcinogenic state and thus develop HCC more quickly, and at high rates. However, even when immunosuppressed, such as in LT cases, hepatocarcinogenesis was suppressed when the HCC and fibrosed liver were removed completely.³⁵ Complete treatment by surgical resection or LT may reduce HCC recurrence. In the setting of KT, no report has analyzed HCC development after DAA treatment. We observed no HCC during follow-up for up to 40.4 months after KT. In these cases, although the DAA treatment was administered with the remaining native liver under immunosuppression, no HCC was observed. Therefore, HCC development may be influenced by past hepatocarcinogenesis more so than by DAA treatment or immunosuppression.

Our study had some limitations. It had a single-center, retrospective design, with an observation period of only 3.4 years, and we could not determine the levels of cytokines or immunological mediators. Further study of hepatocarcinogenesis after DAA treatment is required with a longer follow-up period.

In conclusion, DAA may not increase the incidence of HCC, and the timing of initiation of DAA did not influence the rate of HCC occurrence. However, the absolute risk of HCC remained high in patients with a history of HCC. Careful monitoring for HCC with liver imaging and AFP or DCP should be performed at least every 3 months.

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