Current Status of Hepatocellular Carcinoma Occurrence after Achieving a Sustained Virologic Response with Direct-Acting Antiviral Drug Treatment for Type C Chronic Liver Disease: A Study at Kawasaki Municipal Tama Hospital

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Abstract
With the development of direct-acting antiviral (DAA) treatment for chronic hepatitis C including liver cirrhosis (type C chronic liver disease; type C CLD), almost 100% virus elimination can be achieved. DAA treatment is also being expanded for elderly patients and/or patients with advanced fibrosis. Therefore, there is a concern that the risk of hepatocellular carcinoma (HCC) after DAA treatment may increase. We examined the current status of HCC occurrence in 241 patients who achieved a sustained virologic response (SVR) upon DAA treatment for type C CLD with no history of liver cancer. HCC was developed in 12 patients (5.0%), and the cumulative incidence rate was 1.3% for 1 year, 3.1% for 2 years, 3.6% for 3 years, 4.7% for 4 years, and 6.4% for 5 years, respectively. Moreover, the rate of HCC incidence was significantly higher in elderly patients (≧70 years old; P=0.0488). Nine cases were diagnosed as HCC stages I and II, and the prognosis was favorable with proper treatment. The remaining three cases were suspected to rapidly progress toward advanced HCC, diagnosed as stage III and IV at the time of diagnosis; all the patients died within a year. Consequently, post-SVR HCC occurrence after DAA treatment should be carefully followed on a case-by-case basis, especially in the elderly and in patients with advanced fibrosis.

Key word
Hepatitis C virus, direct-acting antivirals, sustained virologic response, hepatocellular carcinoma, hepatocarcinogenesis

I. Introduction
In Japan, the treatment of chronic hepatitis C including liver cirrhosis (type C chronic liver disease; type C CLD) with interferon (IFN)-free all oral direct acting antivirals (DAA) was initiated in 20141; since then, the treatment outcomes have been improving remarkably. Using the currently approved DAA regimens, the sustained virologic response (SVR) reaches almost 100%2-4. Furthermore, DAA treatment is well tolerated and widely used in the elderly and in pa-

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II. Patients and methods

We targeted 241 patients who achieved SVR among type C CLD with no history of liver cancer and were treated with IFN-free all oral DAA from September 2014 to March 2020 at our hospital. SVR is defined as the persistent absence of detectable serum hepatitis C virus (HCV) RNA 12 weeks after the end of treatment. The HCV- RNA levels were assessed using the COBAS® Amplicor HCV Test version 2.0 (Roche Diagnostics, Branchburg, NJ, USA)(10). The dynamic range of this assay is 1.2–7.8 Log IU/mL. The average age of the patients at the initiation of DAA treatment was 65.2±13 years (99 males and 142 females). HCV serotype was determined using Imcheck F-HCV Gr “Kokusai”® kit (Sysmex Corporation, Hyogo, Japan). There were 174 cases in Serogroup 1, 65 cases in Serogroup 2, and 2 cases were undetermined(11). The DAA treatments that resulted in SVR were as follows: daclatasvir + asunaprevir (DCV+ASV), 68 cases; ledipasvir +sofosbuvir (LDV/SOF), 44 cases; sofosbuvir+ribavirin (SOF+RBV), 45 cases; ombitasvir+paritaprevir (OBV/PTV), 28 cases; elbasvir+grazoprevir (EBR +GZR), 2 cases; and glecaprevir/pibrentasvir (GLE/PIB), 54 cases. LDV/SOF, OBV/PTV and GLE/PIB mean a mixture of two drugs. Administration of DAA was carried out according to the manufacturer’s instructions. The median observation period from the initiation of DAA treatment was 52.4 months, and the longest observation period was 77.6 months. The cumulative HCC incidence rate during this observation period, characteristics of HCC cases, and clinical course were examined retrospectively. The stage diagnosis of HCC was determined according to the general rules for clinical and pathological study of primary liver cancer(12,13). The fibrosis-4 (FIB-4) index was calculated using the following equation: (age [years]) × (aspartate aminotransferase [U/L]/(platelet counts ×10^9/μL)) × (alanine aminotransferase [U/L]). In this study, it was determined that a platelet count of less than 16 × 10^9/μL reflects fibrosis, and a FIB-4 index of 3.25 or higher reflects advanced fibrosis(14). We also determined that Mac-2 binding protein glycosylation isomers (M2BPGi) ≥1 reflected fibrosis and ≥2 reflected advanced fibrosis. The level of M2BPGi was evaluated using the lectin antibody sandwich immunoassay method (Sysmex Corporation, Kobe, Japan)(15). This study was approved by the Ethics Committee of St. Marianna University School of Medicine (Approval No. 4917).

Statistical analysis

The cumulative incidence of HCC was computed using the Kaplan–Meier methods and was calculated using the log-rank test. We performed by the statistical software Graph Pad Prism (Graph Pad Software, San Diego, CA, USA). Values of P <0.05 were considered indicative of a significant difference.

III. Results

1) Cumulative incidence of HCC following SVR

During the observation period, HCC was developed in 12 of 241 cases (5.0%). The cumulative incidence rate was 1.3% for 1 year, 3.1% for 2 years, 3.6% for 3 years, 4.7% for 4 years, and 6.4% for 5 years, respectively (Fig. 1). In this study, we divided the patients into elderly (≧70 years old) and non-elderly (<70 years old) patients; the incidence rate of HCC was significantly higher in the elderly patients (P=0.0488) (Fig. 2).

2) Characteristics of HCC cases

Table 1 shows the characteristics of 12 cases in which HCC occurred. There were eight males and four females; their ages at the start of DAA treatment were 56 to 85 years; transaminase showed abnormal values except in Case 1. Nine cases showed a predominance of aspartate aminotransferase (AST). Platelet levels decreased below 16×10^9/μL in 8 cases. The median Fib-4 index was 4.53 (1.76–7.95), and 10 cases exhibited advanced fibrosis (≧3.25). In addition, non-insulin-dependent diabetes was recognized in Case 1, and Case 12 was recognized as an addicted drinker.

The median time from the completion of DAA treatment to the onset of HCC and the longest observation period were 19.5 and 54 months, respectively. The mean age at HCC diagnosis was 77.6 ± 8.9, and seven cases were over 80 years old. Ten HCC cases were diagnosed via contrast-enhanced CT, and the remaining two cases were diagnosed via contrast-enhanced ultrasound (contrast-enhanced US), and via gadolinium-ethoxybenzyl-diethylene-triamine-penta-acetic acid magnetic resonance images (EOB-MRI). Nine cases were diagnosed with HCC stage I or II, and were treated with proper treatment (operation (OPE), radiofrequency ablation (RFA), or transarterial chemoembolization (TACE)). Eight of these nine proper treatment cases survived 10 to 46 months after HCC treatment, except for Case 8 (death due to interstitial pneumonia). However, Case 1, Case 5, and Case 7 were diagnosed at advanced HCC stages (III,
Cumulative incidence of HCC (%)

0 1 2 3 4 5 6 7

0
2
4
6
8
10
Years

Fig. 1. Cumulative incidence of HCC in patients with after SVR by DAA treatment (analyzed by Kaplan-Meier Method).
The cumulative carcinogenicity rate was 1.3% for 1 year, 3.1% for 2 years, 3.6% for 3 years, 4.7% for 4 years, and 6.4% for 5 years, respectively.
HCC, hepatocellular carcinoma; SVR, sustained virologic response; DAA, direct acting antivirals.

Cumulative incidence of HCC (%)

0 1 2 3 4 5 6 7

0
5
10
15
P = 0.0488 ※
※ Log-rank test

≧ 70
< 70

Fig. 2. Cumulative incidence of HCC in patients with after SVR cases by DAA treatment according to age: ≧70 (solid line) vs. <70 (broken line) (analyzed by Kaplan-Meier Method).
The carcinogenicity rate was significantly higher in the elderly patients (P=0.0488). Abbreviations are already described.

IVa, and IVb); hence, the prognosis was poor, and all the three died of cancer within 12 months.

3) Changes in tumor markers and the fibrosis marker in HCC cases
   
   Table 2 shows the changes in the tumor markers
### Table 1. Characteristics of HCC Cases

| Case | Age/sex | Sero- group | DAA regimen | AST(U/L)/ALT(U/L)/Fib index | Time to HCC (mo) | HCC age/ diagnosis | HCC number/ size(cm) | HCC stage | HCC treatment | Prognosis (mo) |
|------|---------|-------------|--------------|-----------------------------|-----------------|---------------------|----------------------|-----------|---------------|----------------|
| 1    | 78/M   | 1           | GLE/PIB      | 25/25/22.1                 | 14              | 80/CT              | Multiple/2.5         | IVb       | -             | 12 Death       |
| 2    | 84/M   | 1           | OBV/PTV      | 45/41/12.9                 | 6               | 84/EUS             | Single/1.3           | I         | RFA           | 49 Alive       |
| 3    | 75/F   | 1           | GLE/PIB      | 52/43/22.4                 | 20              | 77/CT              | Single/2.6           | II TACE   | 28 Alive       |
| 4    | 81/M   | 1           | DSA+ASV      | 102/74/13.4                | 39              | 80/CT              | Single/1.6           | I         | RFA           | 25 Alive       |
| 5    | 64/F   | 1           | OBV/PTV      | 55/47//11.1                | 39              | 85/CT              | Single/1.3           | II TACE   | 28 Alive       |
| 6    | 80/M   | 1           | DSA+ASV      | 91/75/10.7                 | 54              | 85/CT              | Single/1.5           | II TACE   | 13 Alive       |
| 7    | 85/M   | 1           | GLE/PIB      | 42/61/11.1                 | 16              | 88/US/CT           | Single/4.8           | III TACE  | 9 Death        |
| 8    | 77/M   | 1           | DSA+ASV      | 49/26/15.6                 | 19              | 80/CT              | Single/2.0.1         | II TACE   | 49 Alive       |
| 9    | 64/F   | 2           | SOF+RBV      | 64/41/17.7                 | 17              | 80/CT              | Single/1.4           | I OPE     | 25 Alive       |
| 10   | 72/F   | 1           | LDV/SOF      | 81/54/17.7                 | 10              | 74/CT              | Single/1.5           | I TACE    | 48 Alive       |
| 11   | 67/M   | 1           | DSA+ASV      | 49/33/14.2                 | 12              | 60/CT              | Single/2.4           | II TACE   | 46 Alive       |
| 12   | 56/M   | 1           | DSA+ASV      | 73/75/7.6                  | 6.21            | 60/CT              | Single/2.0           | I OPE     | 13 Alive       |

*complicated diabetes; **addicted drinker; †complicated cryoglobulinemia, death caused by interstitial pneumonia; ‡time to HCC after the end of DAA treatment; DAA, direct acting antivirals; mo, months; HCC, hepatocellular carcinoma; cm, centimeter; GLE/PIB, Glecaprevir/Pibrentasvir; OBV/PTV, Ombitasvir/Paritaprevir; DCV+ASV, Daclatasvir+Asunaprevir; SOF+RBV, Sofosbuvir+Ribavirin; LDV/SOF, Ledipasvir+Sofosbuvir; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PL, platelet counts; CT, computed tomography; EUS, enhanced ultrasound; US, ultrasound; EOB-MRI, gadolinium-ethoxybenzyl-diethylene-triamine-pentaacetic acid-magnetic resonance imaging; Vp, portal trunk thrombus; RFA, radiofrequency ablation, TACE, transcatheter arterial chemoembolization; OPE, operation.

### Table 2. Changes in Tumor Markers and Fibrosis Markers in HCC Cases

| Case | At time of DAA | At time of SVR | At time of HCC |
|------|----------------|----------------|---------------|
| 1    | 4.4            | 7.4            | 284.1         |
| 2    | 5              | 3              | 3.2           |
| 3    | 5.5            | 4.5            | 4.1           |
| 4    | 8.7            | 3.2            | 2.5           |
| 5    | 13.7           | 9686.4         | 9686.4        |
| 6    | 17.3           | 5.4            | 3.7           |
| 7    | 7.6            | 3.2            | 11049.5       |
| 8    | 4.3            | 4.4            | 153.7         |
| 9    | 5.8            | 4.2            | 39.7          |
| 10   | 5.7            | 14.2           | 19.2          |
| 11   | 6.5            | 8.3            | 6.5           |
| 12   | 18.8           | 15.7           | 7.8           |

**AFP**, alpha-fetoprotein; **DCP**, des-gamma-carboxy prothrombin (DCP); **M2BPGi**, Mac-2 binding protein glycosylation isoform; **DAA**, direct acting antivirals; **SVR**, sustained virologic response; **HCC**, hepatocellular carcinoma; **HCC diagnosis** was the same time. Case 1, Case 5, and Case 7 were diagnosed at advanced stages, and AFP and DCP were obviously increased at the time of HCC. Case 1, Case 5, and Case 7 were diagnosed at advanced stages, and AFP and DCP were obviously increased at the time of HCC. AFP showed a slight increase in three cases at the time of DAA. An abnormal AFP values was observed in three cases at the time of SVR and in six cases at the time of HCC, including Case 5, in which the SVR determination and HCC diagnosis were conducted simultaneously. By contrast, DCP did not display abnormal values at the time of DAA. An abnormal DCP values were observed in Case 1 and Case 5 at the time of SVR and showed a noticeable increase in seven cases at the time of HCC. At the time of DAA, M2BPGi showed abnormal values (1.0 or more) in eight cases except in Case 1, and six of these showed values of 2.0 or
4) Clinical course of rapidly progressing cases (Case 1, Case 5, and Case 7)

Figure 3 shows the clinical course of Case 1. An 80-year-old man with non-insulin-dependent diabetes and a pacemaker as well as chronic hepatitis with normal liver function had been treated with DAA (DSA+ASV, LDV/SOF, DSA/ASV/beclabuvir, GLE/PIB) four times for about 3 years, and finally achieved SVR with 12 weeks of GLE/PIB treatment. No apparent tumor lesions were observed in the abdominal US at 6 months after DAA treatment. Back pain appeared at 12 months, and the patient was admitted in our hospital at 14 months after DAA. Multiple HCC and multiple bone metastasis (stage IVb) were diagnosed by contrast-enhanced CT and tumor biopsy near fourth lumber vertebra (L4).

Figure 4 shows the clinical course of Case 5. A 64-year-old woman with cirrhosis presented with a slight fluctuating of AFP levels since more than 1 year before DAA treatment. AFP increased at the time of HCC diagnosis. AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; CT, contrast-enhanced computed tomography; US, ultrasound; EOB-MRI, gadolinium-ethoxybenzyl-diethylene-triamine-pentaacetic acid- magnetic resonance imaging.

HCC occurrence after SVR by DAA for type C chronic liver disease

![Clinical course in case 1](image)

**Fig. 3.** Clinical course in case 1

Contrast-enhanced CT 14 months after DAA treatment revealed multiple liver tumors and multiple bone tumors. DCP showed a gradual increase from the end of DAA treatment. AFP increased at the time of HCC diagnosis.

AFP, alpha-fetoprotein; DCP, des-gamma-carboxy prothrombin; CT, contrast-enhanced computed tomography; US, ultrasound; EOB-MRI, gadolinium-ethoxybenzyl-diethylene-triamine-pentaacetic acid- magnetic resonance imaging.
Fig. 4. Clinical course in case 5

Contrast-enhanced CT revealed an invasive hyper vascular tumor lesion in S6 at the time of SVR, 3 months after DAA treatment, which was accompanied by portal vein tumor thrombus. In the hepatocyte phase of EOB-MRI suggested that tumor lesions developed in the S6 widely, although there was no tumor lesion in EOB-MRI at 9 months before DAA treatment. Slight fluctuating of AFP continued before DAA treatment and increased significantly after DAA treatment. DPC also increased after DAA treatment.

Abbreviations are already described.

regular follow-up. For up to 33 months after DAA treatment, the AFP and DCP levels remained in the normal range, and no obvious tumor lesions were observed upon ultrasound or contrast-enhanced CT. However, 6 months later, 39 months after DAA treatment, the AFP and DPC levels increased remarkably. Contrast-enhanced CT revealed a hyper-vascular tumor with central necrosis of 48 × 38 mm in size; the patient was then diagnosed with HCC (stage III).

IV. Discussion

Since 2014, when the IFN-free DAA treatment strategy was initiated, treatment for the patients with type C CLD has been continuing to improve, and the SVR rate has reached almost 100%\(^\text{1-4}\), even in our hospital. However, following the elimination of the virus, the main concern in type C CLD treatment is the exact manner in which the post-SVR carcinogenesis is to be dealt with. Recent DAA treatment is well tolerated; therefore, it is widely used in the elderly and in patients with advanced fibrosis, in whom conventional IFN treatment is difficult\(^\text{16}\). Advanced age and the progression of hepatic fibrosis are closely related to carcinogenesis\(^\text{17}\), and it has been assumed that the risk of HCC after achieving SVR with DAA treatment is higher than that after achieving SVR with IFN treatment according to speculation or clinical experience. However, in a large cohort study carried out in the US, there was no significant difference in HCC risk between IFN and IFN-free DAA treatments\(^\text{18}\). Furthermore, a study from Japan revealed that there was no difference in the risk of HCC development between IFN-free DAA treatment and IFN combination therapy\(^\text{19,20}\).

The results of our study were for a sufficient observation period (median 52.4 months) in a single institution, and images evaluation and tumor marker monitoring were performed at intervals of 3 to 6
months in almost all cases. Twelve cases (5.0%) of patients developed HCC were observed throughout the whole clinical course, and the cumulative incidence rate was higher than that with Pegylated-IFN + ribavirin therapy over an almost similar observation period with other patients in Japan. This could be attributed to the fact that there were a large number (44%) of elderly patients who were over 70 years old at the time of DAA administration; 9 of 12 patients who developed HCC were these elderly individuals. Furthermore, in the HCC cases, high FIB-4 index and M2BPGi values were observed at the time of DAA administration; hence, it can be speculated that the high proportion of cases with advanced fibrosis affected the incidence rate. Other factors, such as being male, drinking, having diabetes and a fatty liver, and presenting with high AFP levels after treatment, have been reported to be associated with post-SVR carcinogenesis, in addition to being elderly and exhibiting progression of hepatic fibrosis. In this study as well, eight males developed HCC, and the incidence rate was 8.1% (8/99), which was higher than that of females (2.8%, 4/142). However, there was only one diabetic and one addicted drinker. Regarding the AFP values after treatment, compared with the values observed at the time of DAA, some cases displayed an increase at the time of SVR; however, 7 cases showed low levels of AFP (<6 ng). Consequently, it can be suggested that long-term HCC surveillance is necessary in elderly patients and patients with advanced fibrosis, even among patients with low levels of AFP due to a better control of hepatitis owing to DAA treatment.

Nine out of 12 HCC patients had small lesions with tumor diameters of 2 cm or less. These patients were treated with conventional therapy and are alive. This result indicates that early diagnosis of HCC may lead to improved prognosis, even after SVR.
HCC either develops in a stepwise carcinogenesis from dysplastic nodule to early HCC, and advanced HCC\textsuperscript{5,26} or develops de novo. Carcinogenic patterns observed immediately after DAA treatment include cases diagnosed with the characteristics of advanced HCC, and de novo carcinogenesis is assumed. Here, some direct effects of DAA treatment are suggested\textsuperscript{9,27,28}. In this study, three cases of HCC suspected of rapid progression were diagnosed with advanced HCC and represented multiple distant metastasis, major portal invasion, and central tumor necrosis. In any of these cases, the image findings showed no apparent hepatic nodular lesion until just before the diagnosis of HCC, suggesting de novo carcinogenesis. In Case 1, the actual onset time of carcinogenesis was estimated to be within 12 months after DAA treatment, which was several months before the onset of low back pain. In addition, Case 5 was considered to be carcinogenic during DAA treatment. It cannot be ruled out that DAA administration itself may have promoted hepatocarcinogenesis. Interestingly, it was reported that the expression of vascular endothelial growth factor (VEGF)\textsuperscript{29}, which is associated with angiogenesis and proliferation during liver carcinogenesis, increased 4-fold during DAA treatment\textsuperscript{30}. Case 7 is a case of advanced liver cancer that occurred suddenly after a stable liver condition of about 3 years after DAA treatment; it is an example that can be considered de novo HCC. These data suggest that there are various modes of carcinogenesis and cancer progression after DAA treatment, which require further studies.

Regarding HCC surveillance in SVR patients after DAA treatment, APASL guidelines recommend follow-up at 6-month to 12-month intervals in SVR patients without a history of liver cancer\textsuperscript{31}. In many cases of HCC in our study, it was possible to appropriately treat patients based on a 6-month follow-up. However, in some cases, HCC development was observed immediately after DAA treatment, and it appears necessary to continue to monitor the elderly and patients with advanced fibrosis, closely. Furthermore, a rapid progressive HCC was observed even in long-term stable follow-up after SVR. Therefore, the development of new biomarkers related to the risk of carcinogenesis is desired.

V. Conclusion

Type C CLD became a disease that can be eliminated with DAA treatment. However, SVR should be recognized as a transit point in Japan, where the number of elderly people is consistently increasing. It is important to properly monitor post-SVR HCC occurrence and link it to curative treatment. To achieve this goal, it is necessary to establish some method to predict HCC outbreaks, such as new biomarkers. For now, careful follow-up is essential based on individual cases, mainly in the elderly and in patients with advanced fibrosis.

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Conflicts of Interest

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