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# Real-world long-term analysis of daclatasvir plus asunaprevir in patients with hepatitis C virus infection

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## Abstract

**Background and Aim:** This study aimed to evaluate the long-term clinical course of patients achieving a sustained virologic response (SVR) with daclatasvir plus asunaprevir (DCV/ASV) therapy.

**Methods:** A total of 911 patients who achieved SVR with DCV/ASV were assessed. To evaluate pretreatment factors contributing to hepatocellular carcinoma (HCC) after SVR, univariate and multivariate analyses were performed in all patients, in those with preexisting HCC, and in those without preexisting HCC. We selected a low-risk group of HCC cases after SVR. Finally, we evaluated liver function after achieving SVR.

**Results:** In multivariable analyses, male sex, older age, patients with a history of HCC treatment, excess alcohol use, lower albumin, and low platelet count remained significant in the overall group; male sex and low albumin remained significant in patients with a history of HCC treatment; and male sex, older age, excess alcohol use, low platelet count, high alpha-fetoprotein (AFP), and high des-γ-carboxy prothrombin (DCP) remained significant in those without a history of HCC treatment. Patients who had not received treatment for HCC, females, those under 70 years of age, and those with platelet count ≥13 ($\times 10^4$/μL), AFP <6 ng/mL, and DCP <23 mAU/mL were at low risk of HCC. The process of liver function improvement was different according to the factors.

**Conclusions:** The incidence rate of HCC, risk factors associated with HCC, group with very low risk of developing HCC, and the clinical course in a real-world long-term study were evaluated.

## Introduction

The number of hepatitis C virus (HCV)-infected people worldwide is estimated to be 71.1 million.[1,2] In Japan, an estimated 1.5–2.0 million people were previously infected with HCV, with approximately 60% also having genotype 1.[3,4] Development of direct-acting antivirals (DAAs) has been a turning point in chronic hepatitis C (CHC) treatment. With an extremely high efficacy rate for viral eradication and an excellent safety profile, DAAs have replaced interferon (IFN)-based treatments as the first-line therapy for HCV.

Combination therapy using two oral DAAs, daclatasvir (DCV)[5] plus asunaprevir (ASV),[6] was first approved in Japan for the treatment of carriers of genotype 1 who were HCV infected with CHC and compensation cirrhosis in 2014.[7,8] HCV reduction was rapid in both real-world therapy studies and mathematical models.[9]

HCV infection often causes CHC and leads to cirrhosis, decompensated cirrhosis, and hepatocellular carcinoma (HCC). As DAAs have greatly improved cure rates with low side-effect profiles, the Japan Society of Hepatology has published

guidelines recommending that HCV-infected patients must be treated using DAAs unless there are contraindications.[10] Patients achieving sustained virologic response (SVR) showed decreased rates of life-threatening complications, all-cause mortality, decompensated cirrhosis, and HCC.[11,12] HCC preservation is one of the most important treatment goals.[13–15] Until recently, DAA therapies were available for patients with fully preserved liver function until they were classified as Child–Pugh class A. Thus, HCC is one of the most frequent and important complications to monitor in patients who achieved SVR compared with other decompensated cirrhosis-related events. HCC prevention has been firmly established by many long-term studies in patients treated with IFN-based regimens.[16–18] Although similar effectiveness was expected for DAA therapies, the first studies triggered a debate on the early incidence of HCC.[19] Although affirmative articles subsequently increased,[20,21] the long-term incidence rate of HCC and characteristic features for developing HCC after SVR have not been fully described. The present study is specifically focused on outcomes occurring during a long follow-up evaluation according to SVR status after a DCV/ASV regimen. The incidence rate of HCC, risk factors associated with HCC, liver function improvement, and clinical events were evaluated.

## Methods

**Patients.** Patients infected with genotype 1 HCV diagnosed by board-certified hepatologists were enrolled in a Japanese Red Cross Liver Study Group from 2015 to 2020. Patients judged unsuitable by their principal physician were excluded. The patients received DCV (Daklinza; Bristol-Myers Squibb) plus ASV (Sunvepra; Bristol-Myers Squibb) according to the prescribing guidelines for 24 weeks. DCV/ASV therapy was started in patients who did not have obvious HCC, as revealed by imaging studies (abdominal ultrasonography and/or computed tomography and/or magnetic resonance imaging), or did not have life-threatening comorbidities. The period between the last HCC treatment and DCV/ASV therapy was not specified. The study protocols were approved by the ethics committees of each institution. This study was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

**Laboratory assessments.** Aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyltransferase (γ-GTP), albumin, bilirubin, platelet count, alpha-fetoprotein (AFP), and des-γ-carboxy prothrombin (DCP) were measured regularly. Patients with an undetectable level of HCV RNA 24 weeks after completing treatment were judged as achieving SVR24. Participants in this study achieving SVR24 were mainly selected from our earlier study,[22] as previously described. Patients were considered dropouts if they were not present for at least two consecutive scheduled clinical check-ups or if they were referred to their primary care physician. Fibrosis-4 (FIB-4) values were calculated before DCV/ASV therapy. For estimating the presence of advanced fibrosis, we selected a cutoff point of the FIB-4 index at 3.25.[23,24] Patients who consumed more than approximately 50 g/day of alcohol were classified as excess drinkers.[25] Fatty liver was judged according to abdominal echo image. Patients who were prescribed medicine for diabetes mellitus were judged diabetes patients.

**Clinical events.** The primary outcome of the study was the incidence of HCC. Other clinical events were also collected, for example, liver failure, malignant tumors except HCC, and occurrence of severe comorbidities or death. Death was classified as liver-related death or non-liver-related death. Liver-related death is considered as due to HCC or to deteriorating liver function.

**Follow-up.** Patients who achieved SVR24 were followed up by board-certified hepatologists at least every 6–12 months for as long as possible. Because treatment for HCC can influence the clinical and biological data, data before HCC occurrence were used for statistics analysis. HCC was confirmed by imaging by a hepatologist. Other clinical events were collected when they were life-threatening or required medical intervention and admission to a hospital, and were categorized as severe comorbidities.

**Statistical analysis.** We used the date before DCV/ASV therapy as the index and followed up patients to the incidence of HCC, death, or September 30, 2020, whichever was earlier. We calculated the incidence rate with 95% confidence interval (CI) as the number of HCC events divided by total person-years (PY) of follow-up.

We used a univariate and multivariable Cox proportional hazards model to compare the risk of HCC in all patients, in those who had undergone treatment for HCC, and in those who did not undergo treatment for HCC. Independent variables were age, sex, previous IFN-based therapy, HCC treatment before DCV/ASV therapy, FIB-4 index >3.25, alcohol consumption ≥50 g/day,[25] comorbidity with fatty liver and diabetes mellitus, as well as AST, ALT, γ-GTP, albumin, bilirubin, platelet count, AFP, and DCP levels before treatment. To assess the distribution between patients with or without a history of HCC treatment before DCV/ASV therapy, continuous data were assessed using the Shapiro–Wilk test, and these data were tested using the Mann–Whitney test. Power analysis was conducted as follows: from previous study or pilot study,[22] we estimated the ratio of patients without and with a history of HCC treatment as 5, and the median follow-up time was 5 years. We roughly estimated the median incidence time in the groups without and with HCC treatment as 20 years and 5 years, respectively. The power calculations showed that 20 experimental subjects and 100 control subjects would be needed to reject the null hypothesis. Based on these results, we judged our sample size to be sufficient in this study. We generated Kaplan–Meier curves to illustrate and compare the cumulative incidence rates of HCC. A log-rank test was used to evaluate the differences between these curves.

We selected significant factors that predicted the development of HCC after treatment in patients who had not received HCC treatment. We categorized continuous variables according to the area under the receiver operating characteristic (ROC) curve to predict the development of HCC.

To assess how blood test parameters changed over time, we calculated repeated measures one-way ANOVA. The distribution of each factor was confirmed as following a normal distribution using histograms. The change in platelet count was compared between the advanced fibrosis and non-advanced fibrosis groups according to the cutoff point of the FIB-4 index, as described previously. We calculated the ratio as the value at each observation time divided by the value of each parameter before

treatment. Repeated measures two-way mixed ANOVA was used to compare each ratio at each year point, and Greenhouse–Geisser correction was adopted when the data did not satisfy spherical symmetry. $P < 0.05$ was regarded as statistically significant. We analyzed all statistical results using SPSS version 25 or STATA version 15.

## Results

**Patient characteristics.** This analysis included 911 patients who achieved SVR. Follow-up started on 10 March 2016, and was completed on 30 September 2020. The median duration of follow-up after SVR was 4.50 years (interquartile range [IQR] 2.50–5.00 years) with a total of 3393.71 PY. Table 1 summarizes the characteristics of the patients. During the follow-up period, 273 patients dropped out.

**Overall HCC incidence rate and risk factors in patients with DCV/ASV therapy-induced SVR.** There were 194 incidences of HCC diagnosed after starting treatment, which resulted in an annual HCC incidence rate of 5.72 per 100 PY (95% CI 5.00–6.60; Fig. 1). The time interval between starting DCV/ASV therapy and the diagnosis of HCC was a median of 1.83 years (IQR, 1.00–3.02). Table 2 presents the results of the Cox regression analyses regarding HCC occurrence among the entire group of patients. In the multivariable

analyses, male sex, older age, patients with a history of HCC treatment, excess alcohol use, low albumin, and low platelet count remained independent predictors of HCC (Table 2). The hazard ratio of patients with a history of HCC treatment was most significantly associated with the cumulative incidence rate of HCC.

**HCC incidence rate and risk factors in patients with and without previous treatment for HCC.** In the Cox regression analyses of HCC occurrence among the entire group of patients, those with and without a history of treatment for HCC had a great influence on developing HCC. We assessed the distribution between patients with or without a history of HCC treatment before DCV/ASV therapy, and the continuous data did not follow a normal distribution by the Shapiro–Wilk test. Age, AST, ALT, γ-GTP, bilirubin levels, AFP, and DCP levels in patients with a history of HCC treatment were significantly higher than in patients without a history of HCC treatment (Mann–Whitney test). Pretreatment albumin and platelet count in the HCC treatment group were significantly lower than those without a history of HCC treatment (Mann–Whitney test). The HCC incidence rate was markedly higher among patients with a history of HCC treatment (22.58 per 100 PY [95% CI 18.71–27.24]) than that among patients without a history of HCC treatment (2.92 per 100 PY [95% CI 2.36–3.61]), and the incidence rate ratio of HCC was 7.73-fold (95% CI. 5.77–10.39) (Fig. 1) Stratified analyses were conducted to determine whether the risk factors were different in patients with and without a history of treatment for HCC. In patients with a history of HCC,

**Table 1** Overall baseline characteristics of 911 patients achieving sustained virologic response treated with daclatasvir plus asunaprevir

| Parameters | Number or median All patients 911 | IQR or % |
| --- | --- | --- |
| Sex, male/female, *n* (%) | 414/497 | (45.4%/54.6%) |
| Age, years, median | 70.0 | (63.0–76.0) |
| Previous interferon based therapy, *n* (%) | 449 | (49.3%) |
| HCC treatment experience, *n* (%) | 170 | (18.7%) |
| History of malignant tumor except HCC | 56 | (4.8%) |
| Excess alcohol use, *n* (%) | 61 | (6.7%) |
| Fatty change of the liver, *n* (%) | 87 | (9.5%) |
| Diabetes mellitus, *n* (%) | 213 | (23.4%) |
| AST (IU/L) | 45.0 | (32.0–66.0) |
| ALT (IU/L) | 41.0 | (27.0–64.0) |
| γ-GTP (IU/L) | 35.0 | (22.0–58.0) |
| Albumin (g/dL) | 4.0 | (3.6–4.2) |
| Bilirubin (mg/dL) | 0.7 | (0.6–1.0) |
| Platelet count ($\times 10^4/\mu$L) | 12.9 | (9.3–17.3) |
| FIB-4 index | 3.96 | (2.53–6.39) |
| AFP (ng/mL) | 6.5 | (3.9–12.4) |
| DCP (mAU/mL) | 18.0 | (14.0–24.0) |

Data are presented as numbers with percentages or median with IQR in parentheses.
γ-GTP, gamma-glutamyltransferase; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis-4; HCC, hepatocellular carcinoma; IQR, interquartile range.
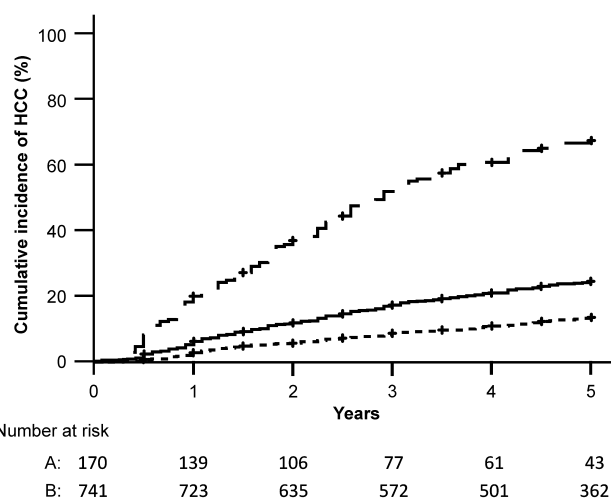


**Figure 1** Cumulative incidence of hepatocellular carcinoma (HCC). Overall cumulative rate of HCC in patients with a history of HCC treatment and in those without a history of HCC treatment status. Continuous line, overall cumulative rate of HCC; dashed line, patients with a history of HCC treatment; dotted line, those without a history of HCC treatment. The log-rank test between those with a history of HCC treatment versus patients without a history of HCC treatment revealed a significant difference ($P < 0.001$). (——), Overall HCC incidence; (– – –), A: with HCC treatment experience; (-----), B: without HCC treatment experience.

**Table 2** Risk factors for hepatocellular carcinoma (HCC) occurrence by Cox multivariate model in all patients

| Parameters | Univariate analysis | | | | Multivariate analysis | |
| --- | --- | --- | --- | --- | --- | --- |
| | No HCC | HCC | HR (95% CI) | *P*-value | Adjusted HR (95% CI) | *P*-value |
| Total number, *n* | 717 | 194 | | | | |
| Sex, male/female | 289/428 | 125/96 | 2.408 (1.795–3.232) | <0.001 | 2.264 (1.627–3.152) | <0.001 |
| Age, years | 69.0 (62.0–75.0) | 72.0 (65.8–78.0) | 1.038 (1.020–1.065) | <0.001 | 1.029 (1.011–1.047) | 0.001 |
| Previous interferon-based therapy | 370 (51.6) | 95 (49.0) | 0.861 (0.650–1.142) | 0.30 | | |
| HCC treatment experience | 61 (8.5%) | 109 (56.2%) | 7.721 (5.806–10.269) | <0.001 | 5.493 (3.973–7.595) | <0.001 |
| Excess alcohol use | 33 (4.6%) | 28 (14.4%) | 2.624 (1.845–3.723) | <0.001 | 1.716 (1.115–2.641) | <0.001 |
| Fatty change of the liver | 76 (10.6%) | 11 (5.7%) | 0.510 (0.277–0.937) | 0.030 | | |
| Diabetes mellitus | 142 (19.8%) | 71 (36.6%) | 1.973 (1.473–2.642) | <0.001 | | |
| AST (IU/L) | 44.0 (31.0–65.0) | 47.0 (35.0–69.0) | 1.002 (0.998–1.006) | 0.289 | | |
| ALT (IU/L) | 41.0 (27.0–67.0) | 42.0 (27.0–58.3) | 0.998 (0.994–1.002) | 0.405 | | |
| γ-GTP (IU/L) | 34.0 (22.0–56.0) | 44.0 (26.8–62.0) | 1.000 (1.003–1.008) | 0.017 | | |
| Albumin (g/dL) | 4.0 (3.7–4.2) | 3.8 (3.4–4.1) | 0.375 (0.227–0.507) | <0.001 | 0.575 (0.400–0.825) | 0.003 |
| Bilirubin (mg/dL) | 0.7 (0.6–0.9) | 0.8 (0.6–1.1) | 1.504 (1.081–2.092) | 0.015 | | |
| Platelet count (×$10^4$/μL) | 13.7 (10.0–17.9) | 10.6 (7.4–14.2) | 0.921 (0.895–0.947) | <0.001 | 0.956 (0.930–0.990) | 0.009 |
| FIB-4 index > 3.25 | 405 (56.5%) | 155 (79.9%) | 2.678 (1.845–3.723) | <0.001 | | |
| AFP (ng/mL) | 6.0 (3.6–11.3) | 10.0 (5.3–19.3) | 1.003 (1.001–1.004) | <0.001 | | |
| DCP (mAU/mL) | 18.0 (14.0–23.0) | 19.0 (14.0–26.0) | 1.002 (1.001–1.004) | 0.001 | | |

Data are presented as numbers with percentages or median with IQR in parentheses.
γ-GTP, gamma-glutamyltransferase; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; CI, confidence interval; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis-4; HR, hazard ratio; IQR, interquartile range.

**Table 3** Risk factors for hepatocellular carcinoma (HCC) occurrence by Cox multivariate model in patients with experience of HCC treatment

| Parameters | Univariate analysis | | | | Multivariate analysis | |
| --- | --- | --- | --- | --- | --- | --- |
| | No HCC | HCC | HR (95% CI) | *P*-value | Adjusted HR (95% CI) | *P*-value |
| Total number, *n* | 61 | 109 | | | | |
| Sex, male/female | 25/36 | 72/37 | 2.046 (1.372–3.584) | <0.001 | 2.042 (1.375–3.033) | <0.001 |
| Age, years | 73.0 (65.5–77.0) | 72.0 (66.0–78.5) | 1.008 (0.985–1.032) | 0.495 | | |
| Previous interferon-based therapy | 32 (52.5) | 51 (46.8) | 0.830 (0.569–1.210) | 0.333 | | |
| Number of previous HCC treatments | 2.33 (1–9) | 2.93 (1–17) | 1.127 (1.050–1.210) | 0.001 | 1.117 (1.036–1.203) | 0.004 |
| Excess alcohol use | 2 (3.3%) | 19 (17.4%) | 1.936 (1.178–3.182) | 0.009 | | |
| Fatty change of the liver | 4 (6.6%) | 5 (4.6%) | 0.818 (0.333–2.007) | 0.651 | | |
| Diabetes mellitus | 16 (26.2%) | 44 (40.4%) | 1.372 (0.935–2.015) | 0.106 | | |
| AST (IU/L) | 52.0 (33.5–69.5) | 48.0 (35.0–72.0) | 1.002 (0.996–1.008) | 0.489 | | |
| ALT (IU/L) | 47.0 (29.0–69.5) | 43.0 (27.5–59.5) | 0.930 (0.994–1.007) | 0.930 | | |
| γ-GTP (IU/L) | 28.0 (21.0–41.0) | 44.0 (26.5–64.0) | 1.002 (1.000–1.004) | 0.125 | | |
| Albumin (g/dL) | 3.9 (3.7–4.1) | 3.7 (3.4–4.0) | 0.486 (0.311–0.762) | 0.002 | 0.504 (0.321–0.790) | 0.003 |
| Bilirubin (mg/dL) | 0.7 (0.6–1.1) | 0.8 (0.6–1.0) | 0.984 (0.604–1.605) | 0.949 | | |
| Platelet count (×$10^4$/μL) | 10.0 (7.7–13.9) | 10.9 (7.6–14.4) | 1.007 (0.972–1.041) | 0.694 | | |
| FIB-4 index > 3.25 | 46 (75.4%) | 87 (79.8%) | 1.231 (0.771–1.966) | 0.384 | | |
| AFP (ng/mL) | 8.6 (4.9–28.5) | 10.0 (5.0–23.1) | 1.000 (0.999–1.002) | 0.626 | | |
| DCP (mAU/mL) | 19.0 (14.0–26.5) | 19.0 (13.0–26.0) | 1.000 (0.999–1.002) | 0.573 | | |

Number of previous HCC treatments is presented as the average, with range in parentheses.
γ-GTP, gamma-glutamyltransferase; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; CI, confidence interval; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis-4; HR, hazard ratio; IQR, interquartile range.

multivariable analyses revealed that male sex, number of previous HCC treatments, and low albumin remained independent predictors of HCC (Table 3). In patients without a history of HCC, multivariable analyses revealed that male sex, higher age, excess alcohol use, lower platelet count, high AFP, and high DCP remained independent predictors of HCC (Table 4). From the above analysis, we extracted low risk of occurrence of HCC and predicting factors in patients without a history of HCC. We used ROC curves to categorize the four continuous variables (age, platelet count, AFP, and DCP). This analysis confirmed that the age of 68.5 years (area under the curve [AUC] = 0.582; 95% CI, 0.518–0.645), a platelet count of 12.9 × $10^4$/μL (AUC = 0.695;

**Table 4** Risk factors for hepatocellular carcinoma (HCC) occurrence by Cox multivariate model in patients without experience of HCC treatment

| Parameters | Univariate analysis | | | | Multivariate analysis | |
| --- | --- | --- | --- | --- | --- | --- |
| | No HCC | HCC | HR (95% CI) | *P*-value | Adjusted HR (95% CI) | *P*-value |
| Total number, *n* | 656 | 85 | | | | |
| Sex, male/female | 264/392 | 53/32 | 2.311 (1.490–3.584) | <0.001 | 2.356 (1.439–3.859) | 0.001 |
| Age, years | 68.5 (62.0–74.0) | 72.0 (65.0–76.5) | 1.031 (1.006–1.057) | 0.015 | 1.039 (1.010–1.068) | 0.008 |
| Previous interferon-based therapy | 338 (51.5) | 44 (51.8) | 0.805 (0.619–1.451) | 0.805 | | |
| Excess alcohol use | 31 (4.7%) | 9 (10.6%) | 2.076 (1.040–4.143) | 0.038 | 3.091 (1.476–6.465) | 0.003 |
| Fatty change of the liver | 72 (11.0%) | 6 (7.1%) | 0.587 (0.256–1.347) | 0.209 | | |
| Diabetes mellitus | 126 (19.2%) | 27 (31.8%) | 1.768 (1.120–2.791) | 0.014 | | |
| AST (IU/L) | 44.0 (31.0–65.0) | 46.0 (37.0–65.0) | 1.001 (0.995–1.007) | 0.733 | | |
| ALT (IU/L) | 41.0 (27.0–67.0) | 40.0 (26.0–57.0) | 0.998 (0.992–1.004) | 0.532 | | |
| γ-GTP (IU/L) | 34.0 (22.0–57.0) | 41.0 (26.5–59.5) | 1.001 (0.999–1.003) | 0.169 | | |
| Albumin (g/dL) | 4.0 (3.7–4.3) | 3.9 (3.5–4.2) | 0.436 (0.276–0.687) | <0.001 | | |
| Bilirubin (mg/dL) | 0.7 (0.6–0.9) | 0.8 (0.6–1.2) | 1.835 (1.155–2.916) | 0.010 | | |
| Platelet count ($\times 10^4$/μL) | 14.0 (10.5–18.1) | 10.1 (7.1–13.1) | 0.886 (0.846–0.927) | <0.001 | 0.894 (0.849–0.940) | <0.001 |
| FIB-4 index > 3.25 | 359 (54.7%) | 68 (80.0%) | 2.945 (1.731–5.011) | <0.001 | | |
| AFP (ng/mL) | 6.0 (3.5–10.7) | 9.1 (6.0–17.5) | 1.007 (1.003–1.012) | 0.002 | 1.009 (1.004–1.015) | 0.001 |
| DCP (mAU/mL) | 17.5 (14.0–23.0) | 19.0 (15.0–26.0) | 1.014 (1.008–1.020) | <0.001 | 1.012 (1.005–1.019) | 0.001 |

γ-GTP, gamma-glutamyltransferase; AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, alanine aminotransferase; CI, confidence interval; DCP, des-γ-carboxy prothrombin; FIB-4, fibrosis-4; HR, hazard ratio; IQR, interquartile range.

**Table 5** Extracted low risk of occurrence hepatocellular carcinoma (HCC) and predicting factors in patients without history of HCC by Cox multivariate model

| Parameters | Adjusted HR (95% CI) | *P*-value |
| --- | --- | --- |
| Sex, male versus female | 2.710 (1.644–4.467) | <0.001 |
| Age, ≥70 versus <70 years | 1.618 (1.005–2.606) | 0.048 |
| Platelet count <13 versus ≥13 ($\times 10^4$/μL) | 2.776 (1.615–4.771) | 0.004 |
| AFP ≥6 versus <6 (ng/mL) | 2.340 (1.319–4.152) | 0.004 |
| DCP ≥23 versus <23 (mAU/mL) | 1.459 (0.897–2.371) | 0.128 |

AFP, alpha-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; HR, hazard ratio.

95% CI, 0.636–0.754), an AFP level of 5.95 ng/mL (AUC = 0.650; 95% CI, 0.591–0.710), and a DCP level of 22.5 mAU/mL (AUC = 0.589; 95% CI, 0.516–0.661) were the best diagnostic cutoff values for the incidence of HCC. Rounded cutoff values for the prediction of HCC were 70 years of age, a platelet count of $13 \times 10^4$/μL, an AFP of 6 ng/mL, and a DCP of 23 mAU/mL. Cox multivariate analysis was used to assess the crude rate of HCC at the end of follow-up among the risk groups (Table 5). Among patients without a history of HCC, 41 patients met all the five factors: female, <70 years of age, platelet count ≥13 × 10⁴/μL, AFP <6 ng/mL, and DCP <23 mAU/mL. None of these patients developed HCC during the observation period (Fig. 2).

***Improvement of liver function after achievement of SVR24.*** We assessed the variability of blood test parameters over time. AST, ALT, AFP, and γ-GTP significantly improved shortly after the treatment began. The albumin value increased
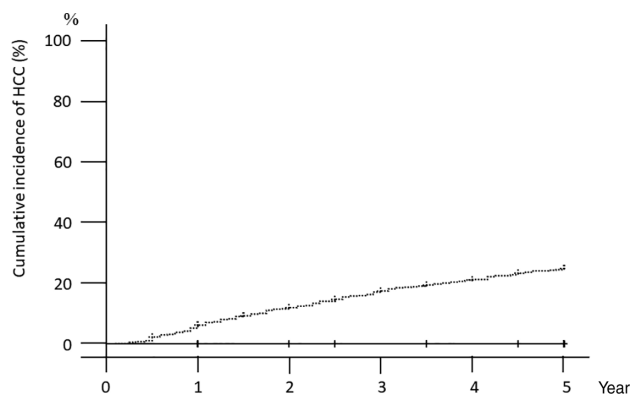


**Figure 2** Kaplan–Meier curve of patients who fulfilled the five factors and of the other patients. Continuous line, patients who fulfilled five factors; dotted line, other patients. The log-rank test revealed a significant difference (*P* = 0.023). HCC, hepatocellular carcinoma. (——), Patients with fulfilled of five factors; (┄┄┄), other patients.

and then reached a plateau at 3 years. Bilirubin was not significantly different. The change in DCP was small. The platelet count gradually increased. Because platelet count and liver fibrosis are related, we conducted two-way mixed ANOVA between the measurements. The main effect of the FIB-4 index at 3.25 was significant ($F(1361) = 17.192$, $P < 0.001$), as was the interaction between the time of the platelet count ratio and group of FIB-4 index 3.25 (Greenhouse–Geisser-corrected $F(2.188, 790.004) = 13.376$, $P < 0.001$). Groups with FIB-4 index had different behaviors over time. The high FIB-4 index group's inclination was greater than that of the low FIB-4 index group (Fig. 3). Because the baseline platelet count in high FIB-4 index
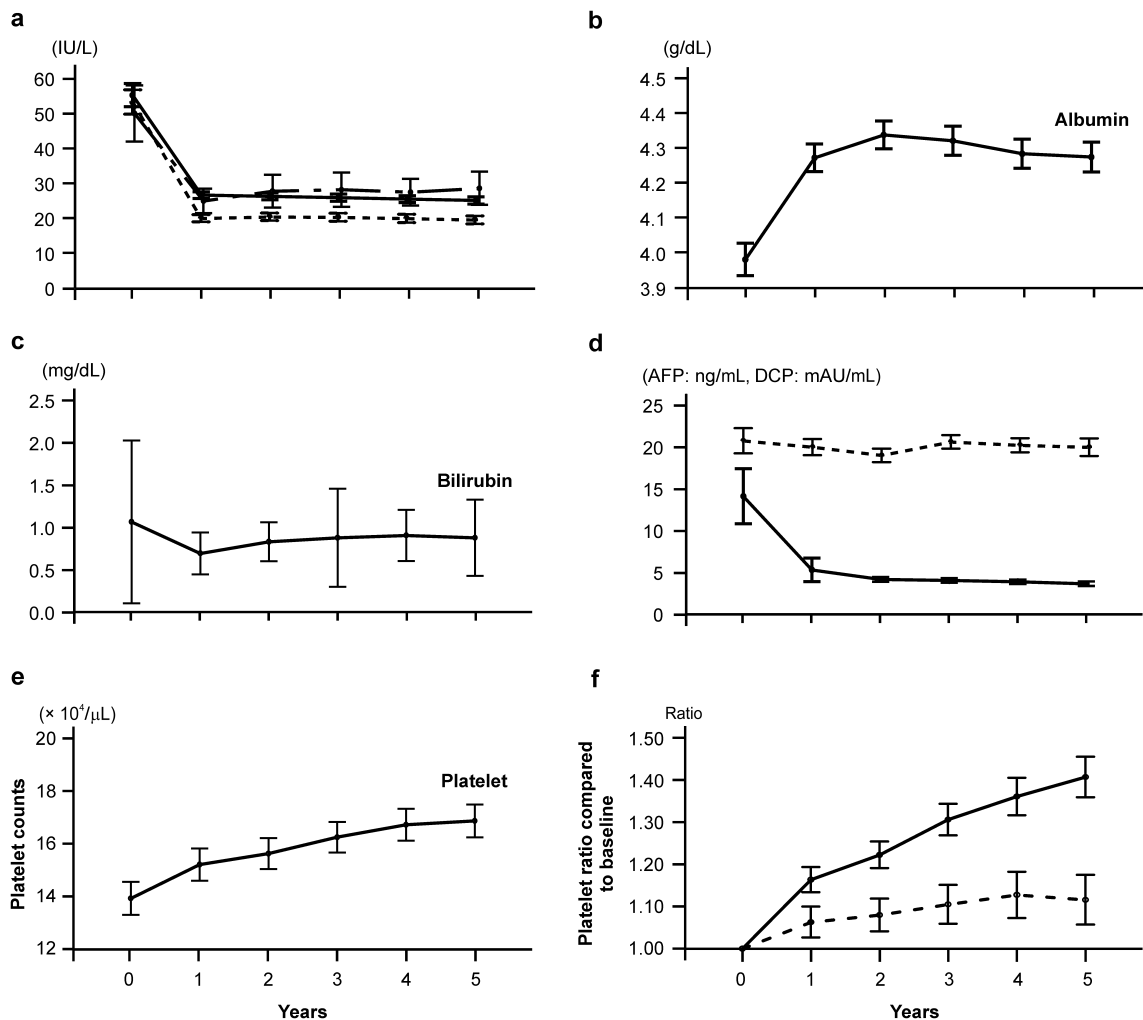
**Figure 3** Time course of liver function tests. Date show repeated measures ANOVA. Values represent mean ± SEM. (a) Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and gamma-glutamyltransferase (γ-GTP). (b) Albumin levels. (c) Bilirubin levels. (d) α-Fetoprotein (AFP), des-γ-carboxy prothrombin (DCP). (e) Platelet count. (f) Platelet ratios of patient observation times divided by the value of daclatasvir plus asunaprevir before therapy. Ratios vary between the fibrosis (FIB)-4 < 3.25 and FIB-4 ≥ 3.25 subgroups. a: (——), AST; (··········), ALT; (- - - -), GTP. d: (——), AFP; (··········), DCP. f: (——), FIB-4 ≥ 3.25; (··········), FIB-4 < 3.25.

was lower than that in low FIB-4 index, there is probably room for improvement. The benefit of DAA therapy for platelet count is probably more effective in the high FIB-4 index group.

***Clinical events during the observation period.***
During the observation period, 21 patients died of liver-related events, including HCC and liver failure. Four patients with no HCC treatment had HCC and liver-related death. Four patients died of liver failure without any HCC treatment before and after DCV/ASV therapy. Seventeen patents died of malignant tumors other than HCC. Among these 17 patients, 11 were affected before DCV/ASV therapy. Liver failure symptoms were observed in 40 patients. Twenty patients experienced malignant tumors other than HCC and survived (Table 6).

## Discussion

SVR achievement is a predictor of HCC-free survival in both IFN-based regimens and DAA therapy.[26,27] In SVR-achieving patients, male sex, older age, preexisting HCC treatment, overdose of alcohol, low albumin, and low platelet count were independent risk factors for developing HCC after DCV/ASV therapy. Among these, preexisting HCC treatment was the strongest contributing factor. These risk factors are similar to those of HCC development after previous IFN-based therapies.[28,29] Although many of these factors are unchangeable, alcohol use is modifiable and generally an important risk factor for clinical liver disease progression, so patients who achieved SVR should be advised to limit their alcohol intake.[30,31]

**Table 6** Clinical events during the observation period

| Clinical events | | | |
|---|---|---|---|
| Death-related comorbidities | | | |
| Liver-related death | 21 | With HCC treatment experience before DCV/ASV therapy | Varix rupture: 1 |
| | | | HCC related death: 12 |
| | | Without HCC treatment before DCV/ASC therapy, and HCC incidence after DCV/ASV therapy | Liver failure: 2 |
| | | | HCC related death: 2 |
| | | Liver failure without HCC treatment experience | 4 |
| Malignant tumor except HCC | 17 | The same malignant tumor before DAC/ASV therapy | 11 |
| | | Other malignant tumor | 6 |
| Other comorbidities | 29 | | |
| Life-threatening comorbidities except death cases | | | |
| Liver failure symptom | 40 | Gastroesophageal varices | 15 |
| | | Ascites | 17 |
| | | Hepatic encephalopathy | 4 |
| | | Others | 4 |
| Malignant tumor except HCC | 20 | | |
| Others | 62 | | |

DCV/ASV, daclatasvir plus asunaprevir; HCC, hepatocellular carcinoma.

Tumor recurrence after treatment of HCC is common even after treatments that are considered "curative." Approximately 60% of patients with preexisting HCC treatment developed HCC recurrence 5 years after starting DCV/ASV therapy. During HCC treatment, retaining liver function is important. Thus, the decision to start DCV/ASV therapy is urgent after HCC treatment. As a result, some patients received DCV/ASV therapy after a comparatively short time of responding to therapy for HCC (e.g. 2 or 3 months later). The modality used to treat previous HCC was not associated with the development of HCC after SVR (data not shown), but the number of previous HCC treatments was significantly associated with the recurrence of HCC. In any case, in patients who receive DAAs prior to HCC treatment, HCC sufficient surveillance is crucial before, during, and after therapy.

The incidence rate of HCC among patients without preexisting HCC treatment therapy was significantly lower than in those with preexisting HCC treatment, which is slightly higher than in another study.[32–34] This difference could be because patients at the highest risk for hepatic morbidity (e.g. older patients) received DCV/ASV in our study.

There are some HCC surveillance guidelines[35] that recommend regular check-ups for patients with cirrhosis. Meanwhile, eradication of HCV with SVR decreases the risk for HCC, and the American Association for the Study of Liver Diseases guidelines recommend that HCC surveillance is not recommend in patients with chronic hepatitis C without cirrhosis.[36] Moreover, there are some scoring systems of achieving SVR patients after DAA therapy.[37–39] Although the optimal screening tests remain controversial, we identified criteria that identified a set of low-risk patients, which could be incorporated into surveillance programs. However, these selection criteria, reasonable cutoff values, and the generalizability of the low-risk group should be supported by further validation studies.

Viral elimination brings various benefits. Albumin value was increased at the first and second year and roughly reached a plateau at 3 years. The differences are generally small, about 0.3 g/dL. Because DCV/ASV therapy was approved for CHC and liver cirrhosis until Child–Pugh class A, patients had well-preserved liver function. In contrast, platelet count took a long time to improve. Platelet count correlates with histologic fibrosis stages and is an independent predictor of the presence of cirrhosis.[40] Platelet count improvement has been interpreted as indicative of fibrosis/cirrhosis regression after HCV elimination.[41,42] The increase in platelet count was slightly different between the two groups. Patients in the high FIB-4 index group had lower platelet count at the beginning of treatment, had much room for improvement of their platelet count, and had a significant continuous positive change over time.

Several clinical events were observed. In liver-related deaths, almost all patients with HCC treatment experience before DCV/ASV therapy died of HCC-related events. HCC recurrence after achieving SVR can be thought to affect the clinical course. Meanwhile, four patients who did not receive treatment for HCC were thought to have died of HCC, and four patients were thought to have died of liver failure after DCV/ASV therapy. Although such cases were rare, it requires attention. Moreover, the coexistent malignancy except HCC is also a point that needs attention. Eleven patients died of affecting malignant tumor before DCV/ASV therapy, and 20 patients were affected by new malignant tumor after DCV/ASV therapy. Japanese CHC patients are generally older than patients in foreign countries,[43] and these patients are of susceptible age for malignant tumor.

There are some limitations to our study. One of the concerning results is the dropout rate during observation time after DAA treatment. At the end of the observation period, 30.0% patients changed hospital or dropped out. But this dropout rate is not very high compared with other studies.[18] Although many dropout patients changed to primary care physician and probably received annual check-up usually, a certain number of patients might be untraceable or stopped to see a doctor. One other limitation of this study is the potential heterogeneity caused by real-world clinical study. As described above, patients who had a

severe coexistent disease or recently received HCC treatment before DCV/ASV therapy were enrolled in this study. For example, some patients with a history of malignant tumor except HCC were enrolled in this study. The effect for malignant tumor except HCC could not be examined. Although this heterogeneity may affect the results, it is an intrinsic characteristic of all studies that include a large number of centers and reflect real-world practice.

In conclusion, considering preexisting HCC treatment before DCV/ASV therapy is important for the surveillance of HCC incidence. Self-managing of drinking is important even after achieving SVR. SVR patients who met all the following criteria, that is, received no treatment for HCC, were females, below 70 years of age, platelet count $\geq 13 \times 10^4/\mu L$, AFP under 6, and DCP under 23, were at a very low risk of developing HCC. The process of liver function improvement was different according to factors.

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