Prognosis and incidence of immunological and oncological complications after direct-acting antiviral therapy for chronic hepatitis C

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Abstract

Background and study aims: **The long-term comprehensive prognosis of chronic hepatitis C after direct-acting antiviral (DAA) therapy is unclear. This study aimed to investigate the prognosis and incidence of immunological and oncological complications after DAA therapy.**

Patients and methods: **The study included a total of 1461 patients who received DAA therapy in our university hospital and affiliated hospitals between September 3, 2014 and September 30, 2018.**

Results: **The incidence rates of total malignancies in overall or female patients after DAA therapy were significantly greater than expected in the corresponding general population. The same was true for lung malignancies. Predictive risk factors associated with the occurrence and recurrence of hepatic malignancies after DAA therapy in patients with sustained virological response were cirrhosis and insulin use, protein induced by vitamin K absence or antagonist-II level, and albumin-bilirubin score, respectively. Eight (0.5%) patients were diagnosed with autoimmune diseases after starting DAA therapy. Importantly, the attending physician considered a possible causal relationship between DAA therapy and these autoimmune diseases in five cases (four rheumatoid arthritis and one membranoproliferative glomerulonephritis). The 5-year overall survival rate was 91.6%. The most frequent primary cause of death was malignancy in 41 (60.2%) patients, including 25 with hepatic malignancies. Lung and colorectal cancers were the next most common.**

Conclusions: **Given that the incidence of total and lung cancers might increase and DAA-related autoimmune diseases might emerge after DAA therapy, we should be alert for the development of these diseases as well as hepatic malignancies.** (Acta gastroenterol. belg., **2022,** 85, **601-609**).

Keywords: malignancy, autoimmune disease, survival.

Introduction

Hepatitis C virus (HCV) is a main cause of chronic liver disease that has the potential to progress to a more advanced stage, such as liver cirrhosis and hepatocellular carcinoma (HCC). Direct-acting antiviral (DAA) therapy has shown remarkable efficacy and safety for treating HCV infection. With the advent of such effective therapy, chronic HCV infection can now be cured.

The potential long-term benefits of DAA therapy are classified as hepatic and extrahepatic benefits (1). Hepatic benefits include (decompensated) cirrhosis/fibrosis regression, cirrhosis complications (portal hypertension, ascites, encephalopathy, and varices) reduction, removal from the liver transplant waitlist, reduction of HCC incidence/recurrence, and reduction of liver-related mortality (1). In contrast, extrahepatic benefits include the amelioration of insulin resistance and diabetes, cardiovascular diseases, cryoglobulinemic vasculitis, and lymphoma, improvement of patient-reported outcomes, and a reduction in all-cause mortality (1).

However, the incidence of extrahepatic malignancies remains unclear because there have been few reports outside Japan regarding the development of extrahepatic malignancies after DAA therapy (2-4). We therefore comprehensively investigated extrahepatic as well as hepatic malignancies after DAA therapy. We experienced a valuable case of chronic hepatitis C complicated by Vogt-Koyanagi-Harada (VKH) disease. After DAA therapy, VKH disease was exacerbated, and rheumatoid arthritis (RA) newly developed. Based on this experience, we considered a potentially causal relationship between DAA therapy and the "deterioration of VKH disease and emergence of RA" (5). Indeed, DAA therapy has been shown to affect the immune system (6-8). We therefore investigated complications, primarily immune-related complications, after DAA therapy. We also investigated the long-term prognosis and causes of death that were considered potentially related to the development of malignances after DAA therapy.

Methods

Study outline and ethics

This study was a multicenter retrospective observation cohort study. The study was approved by the ethics committee of each institution. We complied with the ethical guidelines of the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research

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Involving Human Subjects. Informed consent was obtained by the opt-out method on the websites and at the study sites.

Patient evaluations

The study included patients with HCV infection who received DAA therapy in our university hospital or affiliated hospitals between September 3, 2014 and September 30, 2018. The inclusion criteria were patients ≥ 20 years old with serum HCV RNA levels assessed before the most recent session of DAA therapy. The study subjects were limited to those who could be followed up for ≥ 24 weeks after completion of DAA therapy. In addition, if the subjects had a history of malignancy, the disease had to be considered cured by the attending physician at the start of DAA therapy. Patients with chronic hepatitis B, primary biliary cirrhosis, or autoimmune hepatitis and those infected with human immunodeficiency virus infection were excluded from the analysis based on a survey form completed by the attending physician.

The characteristics of patients, hematological data, biochemical data [including tumor markers, such as protein induced by vitamin K absence or antagonist-II (PIVKA-II) and albumin-bilirubin (ALBI) score as assessment of liver function], virologic data, imaging data, treatment details, complications, date of death or last medical attendance or last survival date, and causes of death were evaluated. Malignancies were classified into two categories, namely, hepatic and extrahepatic malignancies, based on the International Classification of Diseases-10 classification. Hepatic malignancies included HCC and intrahepatic cholangiocarcinoma (ICC) among other entities. The causal relationship between not only HCC but also ICC and HCV infection has been established (9,10). Thus, ICC should be characterized as HCV-related hepatic malignancy, not extrahepatic malignancy.

DAA therapy and the definition of therapeutic efficacy

The type of DAA therapy and treatment duration were decided at the discretion of the attending physician based on the viral genotype, resistance-associated substitutions, and severity of liver disease. Therapeutic efficacy was judged by the achievement of a sustained virological response (SVR; undetectable serum HCV RNA at posttreatment week 12).

Follow-up and the diagnosis of death and hepatic malignancies

All subjects were followed up every 3 to 12 months. The start date of follow-up for the survival and the cumulative incidences for the occurrence/recurrence of hepatic malignancies was defined as the date of the start of DAA therapy. The end of follow-up was the date of the death and diagnosis of occurrence/recurrence of hepatic malignancies; the last medical attendance; or verification by telephone. Abdominal ultrasonography, computed tomography, or magnetic resonance imaging as diagnostic imaging was performed at intervals of 3 to 12 months. Hepatic malignancies were diagnosed mainly by diagnostic imaging combined with the values of tumor markers.

The diagnosis of immune-related complications and extrahepatic malignancies

Immune-related complications and extrahepatic malignancies were diagnosed from the start of DAA therapy to the date of death or the last medical attendance. The diagnosis of these diseases was based on symptoms associated with collagen disease, such as morning stiffness and arthralgia, or extrahepatic malignancies, such as pyrexia of unknown origin and/or diagnostic imaging and laboratory abnormalities during the attending or another physician's office visit or medical checkup. For the diagnosis of immune-related complications, the final diagnosis was performed by collagen disease, endocrinology, or kidney specialists based on the diagnostic criteria of each disease. For the diagnosis of extrahepatic malignancies, the final diagnosis was performed by experts in various fields based on the diagnostic criteria of each malignancy.

Statistical analyses

Quantitative variables are expressed as the median and interquartile range, whereas categorical variables are expressed as the count number and proportions. The incidence rates of extrahepatic malignancies were evaluated using the standardized incidence ratio (SIR), with the exception of 8 cases of recurrence. Independent predictive risk factors associated with the occurrence or recurrence of hepatic malignancies were analyzed using Cox proportional regression analyses. The cumulative incidence of the occurrence and recurrence of hepatic malignancies and the survival were analyzed using the Kaplan–Meier method and the log-rank test. If the predictive risk factor was a continuous variable, we identified the cutoff value by receiver operating characteristic analysis. The Kaplan–Meier method and a log-rank test were then performed using the identified cutoff value. The mortality rate was evaluated using the standardized mortality ratio (SMR). All p values were calculated by two-tailed tests, and p values < 0.05 were considered statistically significant.

Results

Patient characteristics

The demographic and clinical characteristics of a total of 1461 patients immediately before the most recent DAA therapy session are summarized in Table 1.

Table 1. — **Characteristics of the study population**

Number of patients	1461
Age (years) median (IQR)	68 (60-74)
Gender (Male/Female)	675/786
BMI (kg/m^2) median (IQR)†	22.7 (20.6-25.3)
HCV genotype 1a/1b/2a/2b/1b+2b/3/ sero 1/sero 2	9/863/229/144/3/1/144/68
SVR n (%)	1446 (98.9)
History of hepatic malignancies n (%)‡	195 (13.3)
Initial therapy (Surgery/Liver transplantation/RFA/Particle therapy/ TACE/NA)	38/1/96/8/48/4
Most recent DAA therapy n (SVR/non-SVR)	
DCV+ASV	252 (245/7)
SOF/LDV	459 (457/2)
SOF+RBV	331 (327/4)
OBV/PTV/r	75 (75/0)
OBV/PTV/r+RBV	11 (11/0)
EBR+GZR	84 (84/0)
BCV/DCV/ASV	5 (3/2)
GLE/PIB	237 (237/0)
SOF/VEL+RBV	7 (7/0)
Cirrhosis n (%)	395 (27.0)
Fatty liver n (%)	225 (15.4)
Diabetes mellitus n (%)	299 (20.4)
Insulin n (%)	59 (4.0)
Hypertension n (%)	593 (40.5)
Dyslipidemia n (%)	195 (13.3)
Alcohol n (%)	
none	775 (53.0)
< 30 g (Male) or < 20 g (Female)	207 (14.1)
30-59 g (Male) or 20-59 g (Female)	75 (5.1)
> 60 g	44 (3.0)
NA	360
History of smoking	
Yes (current and previous smoking)/ Never/NA	447/566/448
History of IFN-based therapy n (% Naïve)	
Naïve/Experienced/NA	1136/316/9 (77.7)
History of DAA therapy n (% Naïve)	
Naïve/Experienced/NA	1336/101/22 (91.4)
Number with DAA therapy	
1/2/3/4/5/6/NA	1336/85/11/1/1/2/23
Observation period (days) median (IQR)	1167 (644-1673)

ASV: asunaprevir; BCV: beclabuvir; BMI: body mass index; DAA: direct-acting antiviral; DCV: daclatasvir; EBR: elbasvir; GLE: glecaprevir; GZR: grazoprevir; HCV: hepatitis C virus; IFN: interferon; IQR: interquartile range; LDV: ledipasvir; NA: not available; OBV: ombitasvir; PIB: pibrentasvir; PTV: paritaprevir; r: ritonavir; RBV: ribavirin; RFA: radiofrequency ablation; sero: serogroup; SOF: sofosbuvir; SVR: sustained virological response; TACE: transcatheter arterial chemo embolization; VEL: velpatasvir. †Data were not available in 266 patients, ‡Hepatic malignancies comprised hepatocellular carcinoma and intrahepatic cholangiocarcinoma.

Development of extrahepatic malignancies

After the start of DAA therapy, 96 (including 8 instances of recurrence) extrahepatic malignancies were diagnosed (Table 2). The most frequent extrahepatic malignancy was lung cancer followed by gastric and colorectal cancer. Cancer recurrence was also found among several cancer types. The incidence rates of total malignancies in overall and female patients after DAA therapy were significantly greater than expected in the general population (11) based on SIRs (Table 3). Similarly, the incidence rates of overall and female lung cancer after DAA therapy were significantly greater than expected in the general population (11) based on SIRs (Table 3). The rates of the second- and third-most frequent extrahepatic malignancies of gastric and colorectal cancer were not significantly different from those in the general population (11) based on SIRs (data not shown).

Development of hepatic malignancies

After the start of DAA therapy, a total of 189 (with 122 recurrences) and 8 (with 7 recurrences) hepatic malignancies were diagnosed in SVR and non-SVR patients, respectively. These hepatic malignancies comprised 185 HCCs (120 recurrences) and 5 ICCs (2 recurrences) in SVR patients and 7 HCCs (6 recurrences) and 1 ICC (1 recurrence) in non-SVR patients.

Given that the rate of non-SVR was very low (1.1%) and SVR has been established to affect the development of HCC, the predictive risk factors associated with the occurrence and recurrence of hepatic malignancies and their cumulative incidences were evaluated exclusively in SVR patients. The characteristics of SVR patients as well as the occurrence and recurrence of hepatic malignancies in these patients are shown in Supplementary Table 1.

Predictive risk factors associated with the occurrence of hepatic malignancies after DAA therapy in SVR patients

Univariate and multivariate analyses were performed to identify predictive risk factors associated with HCC occurrence after DAA therapy. Only the presence of liver cirrhosis was found to be a predictive factor identified by multivariate analysis (Supplementary Table 2).

Predictive risk factors associated with the recurrence of hepatic malignancies after DAA therapy in SVR patients

Univariate and multivariate analyses were performed to identify predictive risk factors associated with the recurrence of hepatic malignancies after DAA therapy. The insulin use, ALBI score, and PIVKA-II level were found to be predictive risk factors in multivariate analyses (Supplementary Table 2).

Comparison of the cumulative incidence between the occurrence and recurrence of hepatic malignancies in SVR patients

The cumulative incidence of the occurrence of hepatic malignancies was significantly lower than that of the recurrence of hepatic malignancies (5-year incidence:

Table 2. — **Development of extrahepatic malignancies after DAA therapy**

Malignancies	n	SVR/ non-SVR	occurrence/ recurrence	Alcohol: none/light† /moderate‡/heavy§/NA	Diabetes mellitus yes/no	Smoking: never/current/ previous/NA
Total	96	94/2	88/8	56/17/9/0/14	19/77	37/17/23/19
Lung cancer	19	18/1	19/0	12/1/1/0/5	5/14	4/5/6/4
Gastric cancer	17	16/1	16/1	9/3/4/0/1	3/14	8/1/5/3
Colorectal cancer	15	15/0	15/0	8/5/1/0/1	2/13	8/3/3/1
Breast cancer	8	8/0	8/0	5/0/0/0/3	0/8	4/0/0/4
Pancreatic cancer	6	6/0	5/1	4/2/0/0/0	3/3	3/1/1/1
Esophageal cancer	5	5/0	4/1	4/1/0/0/0	3/2	1/2/2/0
Prostatic cancer	5	5/0	5/0	2/2/0/0/1	1/4	1/1/1/2
Biliary tract cancer	4	4/0	4/0	3/0/0/0/1	0/4	1/1/1/1
Bladder cancer	4	4/0	3/1	3/1/0/0/0	0/4	0/2/2/0
Uterine cancer	2	2/0	0/2	2/0/0/0/0	1/1	2/0/0/0
Malignant lymphoma	2	2/0	2/0	0/1/0/0/1	0/2	1/0/1/0
Kidney cancer	2	2/0	1/1	1/1/0/0/0	1/1	1/0/0/1
Leukemia	1	1/0	1/0	0/0/1/0/0	0/1	0/0/1/0
Tongue cancer	1	1/0	0/1	0/0/1/0/0	0/1	1/0/0/0
Laryngeal cancer	1	1/0	1/0	0/0/1/0/0	0/1	0/1/0/0
Ovarian cancer	1	1/0	1/0	1/0/0/0/0	0/1	1/0/0/0
Brain cancer	1	1/0	1/0	1/0/0/0/0	0/1	1/0/0/0
Ethmoid sinus cancer	1	1/0	1/0	0/0/0/0/1	0/1	0/0/0/1
Myerodysplastic syndrome	1	1/0	1/0	1/0/0/0/0	0/1	0/0/0/1

DAA: direct-acting antiviral; NA: not available; SVR: sustained virological response. †< 30 g (Male) or < 20 g (Female), ‡ 30-59 g (Male) or 20-59 g (Female), §> 60 g.

Table 3. — **SIR of total extrahepatic malignancies and lung cancer after DAA therapy**

	Observed	Expected	SIR	95% CI
Total				
Overall	88	70.0	1.3	1.0†-1.5
Male	46	42.3	1.1	0.8-1.5
Female	42	27.1	1.5	1.1-2.1
Lung cancer				
Overall	19	10.4	1.8	1.1-2.8
Male	10	7.0	1.4	0.7-2.6
Female	9	3.4	2.7	1.2-5.1

CI: confidence interval; DAA: direct-acting antiviral; SIR: standarized incidence ratio. †The lower limit value is 1.008.

9.1% with the former patients, 72.2% with the latter patients; p < 0.001, log-rank test) (Fig. 1A).

Differences in the occurrence of hepatic malignancies according to the presence of liver cirrhosis in SVR patients

The cumulative incidence of the occurrence of hepatic malignancies in patients with liver cirrhosis was significantly greater compared with those without liver cirrhosis (5-year incidence: 30.5% of the former patients, 3.4% of the latter patients; p < 0.001, log-rank test) (Fig. 1B).

Differences in the recurrence of hepatic malignancies according to insulin use in SVR patients

The cumulative incidence of the recurrence of hepatic malignancies in patients using insulin was significantly higher than those not using insulin (3-year incidence: 83.3% in the former patients, 59.1% in the latter patients; p = 0.010, log-rank test) (Fig. 1C).

Differences in the recurrence of hepatic malignancies according to the baseline ALBI score in SVR patients

We investigated the cutoff level as the optimal baseline ALBI score in SVR patients to predict the recurrence of hepatic malignancies based on a receiver operating characteristic analysis, as mentioned above. The cutoff level was determined to be -2.37. The cumulative recurrence of hepatic malignancies in patients with a baseline ALBI score ≥ -2.37 was significantly higher than in those with a baseline ALBI score < -2.37 (3-year incidence: 78.7% of the former patients, 51.8% of the latter patients; p = 0.002, log-rank test) (Fig. 1D).

Differences in the recurrence of hepatic malignancies according to the baseline PIVKA-II levels in SVR patients

We investigated the cutoff level as the optimal baseline PIVKA-II level in SVR patients to predict the recurrence of hepatic malignancies based on receiver

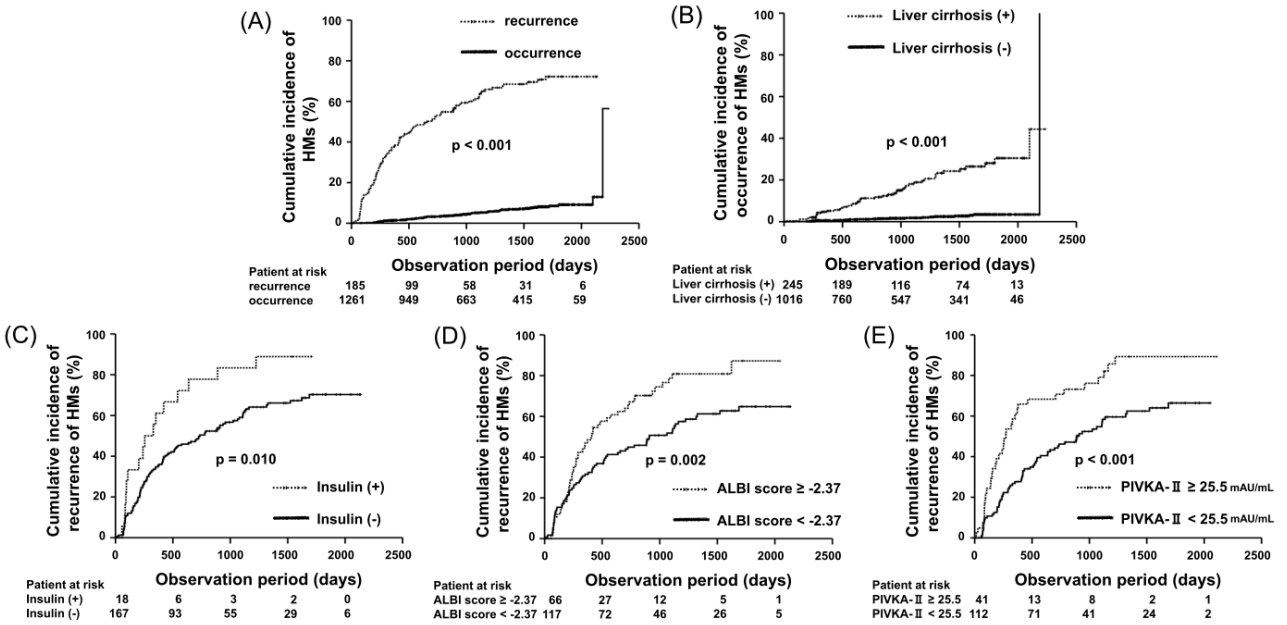

Figure 1. — Cumulative incidence of occurrence and recurrence of hepatic malignancies (HMs) after the most recent direct-acting antiviral therapy in patients with sustained virological response. (A) Cumulative incidence rates of occurrence and recurrence of HMs. (B) Cumulative incidence rates of occurrence of HMs according to the presence of liver cirrhosis. (C) Cumulative incidence rates of recurrence of HMs according to insulin use. (D) Cumulative incidence rates of recurrence of HMs according to the baseline ALBI score. (E) Cumulative incidence rates of recurrence of HMs according to the baseline PIVKA-II level. ALBI score: albumin-bilirubin score; HMs: hepatic malignancies; PIVKA-II: protein induced by vitamin K absence or antagonist-II.

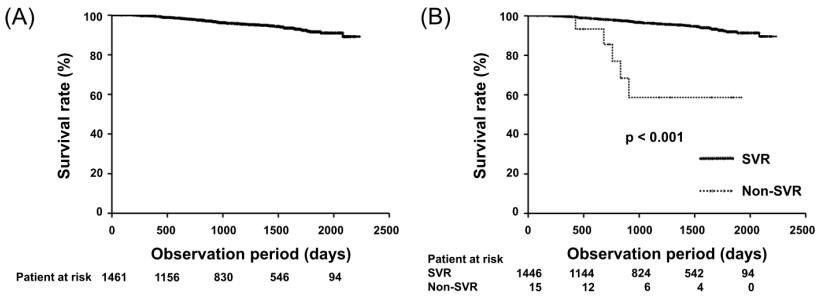

Figure 2. — The overall survival and survival according to the therapeutic efficacy.(A) Overall survival. (B) Survival according to the therapeutic efficacy. SVR: sustained virological response.

operating characteristic analysis, as mentioned above. The cutoff level was determined to be 25.5 mAU/mL. The cumulative recurrence of hepatic malignancies in patients with a baseline PIVKA-II ≥ 25.5 mAU/mL was significantly higher than in those with a baseline PIVKA-II < 25.5 mAU/mL (3-year incidence: 79.1% of the former patients, 54.8% of the latter patients; $p < 0.001$, log-rank test) (Fig. 1E).

Development of autoimmune diseases

A total of 8 (0.5%) cases of autoimmune diseases were diagnosed after the start of DAA therapy. The autoimmune diseases included RA in six patients, euthyroid Graves' disease in one patient, and membranoproliferative glomerulonephritis (MPGN) in one patient (six females and two males in total). Sofosbuvir-based regimens and daclatasvir+asunaprevir combination therapy were administered to five and three cases, respectively. One patient in whom VKH disease deteriorated as an original complication developed new-onset RA, which we previously reported (5). Euthyroid Graves' disease was diagnosed by chance during the detailed examination of hypertension that was discovered while the patient was followed up after DAA therapy. However, the attending physicians deemed there to be a possible causal relationship between DAA therapy and these autoimmune diseases in five cases, including four with RA and one with MPGN. One reason for this judgment was that the onset of autoimmune diseases and the start

Table 4. — **Development of autoimmune diseases that may be associated with DAA therapy**

Number	1	2	3	4	5
Diagnosis	RA	RA	RA	RA	MPGN
Age	73	50	79	66	72
Gender	Female	Male	Female	Male	Female
History of IFN-based therapy	Peg-IFN	none	none	none	none
Personal history of autoimmune disease	none	VKH disease	none	none	none
Family history of autoimmune disease	RA: mother	none	none	none	none
DAA	SOF+RBV	SOF+RBV	SOF+RBV	SOF+RBV	DCV+ASV
DAA treatment period (weeks)	12	12	12	12	24
Efficacy of DAA	SVR	SVR	SVR	SVR	SVR
Time to onset (months)	6	5	10	5	3
Time to the diagnosis (months)	9	11	22	19	13

ASV: asunaprevir; DAA: direct-acting antiviral; DCV: daclatasvir; IFN: interferon; MPGN: membranoproliferative glomerulonephritis; Peg-IFN: pegylated interferon; RA: rheumatoid arthritis; RBV: ribavirin; SOF: sofosbuvir; SVR: sustained virological response; VKH disease: Vogt-Koyanagi-Harada disease.

of DAA therapy occurred within one year in all cases and within six months in four cases.

A summary of the development of autoimmune diseases that may be associated with DAA therapy is shown in Table 4. However, the incidence rate of RA as the most frequent immune-related complication could not be compared with those rates in previous studies or in the general population for the reasons described below.

Overall survival in all patients

The 1-, 3-, and 5-year overall survival rates were 99.7%, 95.9%, and 91.6%, respectively (Fig. 2A).

Survival according to therapeutic efficacy in overall patients

The survival rate in SVR patients was significantly higher than that in non-SVR patients (5-year survival: 91.9% of the former patients, 58.7% of the latter patients; $p < 0.001$, log-rank test) (Fig. 2B).

Primary cause of death

The primary cause of death is shown in Table 5. The number of deaths was 68 (4.6%), and SVR patients accounted for 64 of these deaths (4.4%). The primary cause of death was malignancy in 41 (60.2%), including 25 hepatic malignancies, 3 biliary tract cancers, 3 liver failures, and 24 others. After excluding patients with a history of hepatic malignancies, 21 of the 48 total deaths (43.8%) were due to malignancies. Lung and colorectal cancers were the most common causes of cancer death aside from hepatic malignancies. Regarding extrahepatic malignancies, the mortality rates of total, male, and female malignancies after DAA therapy were not significantly different from those in the general population (12) based on SMRs (data not shown).

Table 5. — **Primary cause of death**

Causes	All	SVR/non-SVR	Occurrence/Recurrence
Total	68	64/4	NA
Malignancies	41	39/2	18/23
Hepatocellular carcinoma	22	21/1	4/18
Intrahepatic cholangiocarcinoma	3	3/0	1/2
Colorectal cancer	4	4/0	4/0
Lung cancer	4	3/1	4/0
Biliary tract cancer	3	3/0	3/0
Malignant lymphoma	1	1/0	1/0
Ovarian cancer	1	1/0	0/1
Kidney cancer	1	1/0	0/1
Pancreatic cancer	1	1/0	1/0
Bladder cancer	1	1/0	0/1
Liver failure	3	2/1	NA
Others	24	23/1	NA

NA: not applicable; SVR: sustained virological response.

Discussion

To date, few reports have been published outside Japan regarding the development of extrahepatic malignancies after DAA therapy (2-4). A previous study based on precedent case reports (4) noted that the incidence rates of several cancers, including lymphoma, after DAA therapy were reportedly higher than expected in the general population (2). However, methodological problems were likely present in that study, so the results should be carefully interpreted. For example, regarding lymphoma, the incidence rate (i.e., the annual incident number per 100,000 people in a single facility) in Israel was simply compared to the European age-standardized rate (13), so the age structure in the study population was not considered. In our study, malignant lymphoma

developed after DAA therapy in two cases, which was consistent with previous studies (2,3).

The mechanism underlying the increased incidence rates of extrahepatic malignancies is unclear. Regarding lymphoma, DAA therapy causes a significant reduction in the B-cell component in chronic hepatitis C patients; however, pathological monoclonal B-cell populations can survive in patients with lymphoproliferative disorders (3). Thus, the potential risk might persist even after DAA therapy (3), as noted with HCC. Regarding cancer in general, serum vascular endothelial growth factor (VEGF) levels significantly increase and remain high during DAA therapy, and the addition of patient-derived serum during DAA therapy causes the proliferation of human umbilical vein endothelial cells (14). However, these changes revert to the basal level after DAA therapy has concluded (14). VEGF plays an important role in regulating vasculogenesis and angiogenesis but can be pathogenic under cancerous conditions (15). VEGF mRNA is overexpressed in most human tumors, and its correlation with invasiveness, vascular density, metastasis, recurrence, and prognosis has been established (15). Thus, VEGF overexpression might cause the occurrence and recurrence of malignancies after DAA therapy. However, as overexpression is temporary, the oncogenic action might be limited. Innate immune reconstitution after DAA therapy (6-8) might affect tumor recurrence, but the role might also be limited to *de novo* carcinogenesis given that reconstitution is completed within several months.

However, the increased incidence rates of extrahepatic malignancies after DAA therapy in our study might not reflect true data. For example, according to the Cancer Statistics in Japan by the National Cancer Center Japan, lung cancer morbidity ranked fourth, third, and third among cancers in male, female, and total patients in 2018, respectively (11). Thus, lung cancer in female and total patients occupies a relatively high ranking compared to that in male patients; this imbalance may be reflected in our data. Alternatively, the rate of smoking in the general Japanese population, which is a representative risk factor for lung cancer as well as cancer in general, was 17.8%, 29%, and 8.1% in overall, male, and female patients in 2018, respectively, according to the National Health and Nutrition Survey Japan (16). In our study, the rates of current and previous smoking were at least 30.6% (Table 1), 41.6%, and 57.8% in overall patients, patients with any cancer and those with lung cancer, respectively. This numerically higher smoking rate potentially affected the incidence of lung cancer and total cancer. Another possibility is that the incidence of extrahepatic malignancies might have been overestimated due to an insufficient sample size in our study. Based on our results, we propose one possible strategy in which patients who receive DAA therapy, especially those with a history of smoking, undergo regular medical checkup, such as annual comprehensive medical examination. The strategy includes chest X-ray in most cases, and chest computed tomography may be offered as an optional examination in some institutes. Thus, it would be helpful to screen malignancies, including lung malignancies.

The most important risk factor for HCC is liver cirrhosis, particularly advanced cirrhosis (Child Class B), and a high baseline fibrosis 4 index in patients without cirrhosis (17). The HCC risk also depends on the patient demographics and clinical characteristics (17). The findings of an expert review (17) support our own, with liver cirrhosis and the ALBI score, which is an indicator of advanced liver disease, reported as predictive risk factors for the occurrence and recurrence of hepatic malignancies, respectively. The incidence of HCC was increased by insulin use in patients with diabetes mellitus (18), which is consistent with our results. However, conflicting findings were reported (19). Hyperinsulinemia and excessive serum insulin-like growth factor are associated with the progression of cholangiocarcinoma (20), which supports our data. The finding that baseline PIVKA-II level is a predictive factor associated with the recurrence of hepatic malignancies makes sense, as it is a serum tumor marker of HCC. The effect of first treatments for HCC, including transcatheter arterial chemoembolization, which do not always cure HCC completely, was not a significant risk factor for recurrence of HCC based on multivariate analysis (Supplementary Table 2).

RA, as a systematic autoimmune disease (21), and MPGN, as an organ-specific autoimmune disease (22), are extrahepatic manifestations associated with HCV infection. The simplified disease activity index decreased significantly from baseline to 24 weeks after the end of DAA therapy in 11 RA patients (23). MPGN was ameliorated following DAA therapy in some but not all cases (24-26). Our results showed several cases of new-onset RA, one of which we reported previously (5), and new-onset MPGN observed after DAA therapy. To our knowledge, there have been no reports of new-onset RA after DAA therapy except for our previously reported case (5). However, cases of new-onset or worsened MPGN have been reported after achieving SVR following DAA therapy (27-29), which supports our data. The incidence of RA varies among studies, and some incidences change with time (30). As detailed age- and sex-adjusted incidence rates of RA have not yet been reported in Japan, it is difficult to compare the SIRs between our study and previous studies or the general population in Japan. Notably, the possible relationship between the onset of RA/MPGN and DAA therapy was recognized based on temporal context in five cases. A lag occurred between the start of DAA therapy and their diagnosis of autoimmune diseases. Actually, the time lag was within 12 months in only two out of 5 cases. However, a definite diagnosis is likely to take time because autoimmune diseases often develop gradually, and the symptoms or laboratory data at onset are not always sufficient for a definite diagnosis. Thus, we believe that the onset of autoimmune diseases is more important to consider the association between

autoimmune diseases and DAA therapy. However, the incidence of RA after DAA therapy in our study might have been coincidental and not reflective of the real incidence of RA after DAA therapy.

There have been no reports of the development of euthyroid Graves' disease after DAA therapy. However, HCV is lymphocytotropic and can affect many endocrine organs, including the thyroid (31). The titers of thyrotropin-blocking and thyrotropin-stimulating autoantibodies are reportedly affected by HCV and interferon (IFN) therapy (31). Thus, euthyroid Graves' disease might develop, be ameliorated, or be worsened by DAA therapy. However, as the onset of euthyroid Graves' disease occurred 31 months after the start of DAA therapy in the present study, it might have developed by chance.

The mechanism underlying the development of autoimmune diseases after DAA therapy remains unknown. We previously suggested that one of the reasons was that immune reconstitution/restoration due to DAA therapy might be involved in the mechanism (5). The transcription levels of interleukin (IL)1β and chemokine (C-X-C motif) ligand 10/IFN gamma-induced protein 10 (IP-10) and IFN-induced protein with tetratricopeptide repeats 1 are temporally and numerically increased after two and/or four weeks of DAA therapy compared to the baseline (8). However, these levels reverted to the baseline level or lower at the end of therapy and posttreatment week 12, except for IL1β (8), although contradictory findings (7) exist. Type I interferons and IL1β play established and emerging roles in early RA pathogenesis (32), and IP-10 is also associated with RA pathogenesis and disease activity (33,34). Furthermore, heterogeneity in the innate gene expression signature in peripheral blood mononuclear cells exists within RA patients (35). Thus, in some patients, RA may be triggered by temporal changes in the expression of genes associated with RA pathogenesis, whereas RA might be ameliorated by DAA therapy because of heterogeneity in the innate immune system in other patients. The difference in the outcome of MPGN after DAA therapy might be due to individual differences in similar mechanisms.

Malignancy was the primary cause of death in 27.3%, 31.1%, and 23.2% of cases in the overall, male, and female populations, respectively, in 2019 in Japan according to Vital Statistics (36). In our study, even after excluding patients with a history of hepatic malignancies, 43.8% of patients still died of malignancies among the overall patients. This discrepancy may be because our subjects were relatively old compared to the general population. For example, the median age of the subjects in our study was 68 years old, which was much older than the median age of 46 years old in the Japanese population according to the 2015 Population Census (37).

This study was limited by the relatively short follow-up period in some cases, missing data due to the retrospective nature of the study, the lack of an adequate control group that comprised patients with chronic hepatitis C without a past history of DAA therapy, and the lack of regular comprehensive screening of extrahepatic malignancies. Thus, our data should be cautiously interpreted.

In summary, after starting DAA therapy, the incidence rate of extrahepatic malignancies, especially lung cancer, might increase in overall and female patients, and autoimmune diseases, especially RA, might develop. We should thus be alert for the development of these diseases as well as hepatic malignancies after DAA therapy. A nationwide survey or global research effort concerning these complications is needed to confirm our data, and the mechanism and racial differences should be clarified in the near future.

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Conflicts of interest

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