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High Incidence of Non-Liver Cancer Following Direct-Acting Antiviral Therapy for Hepatitis C Virus Infection

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Abstract

Objective: To assess the incidence of non-liver cancer following therapy with direct-acting antivirals (DAAs) against hepatitis C in comparison with that following interferon (IFN)-based therapy. The rapid clearance of hepatitis C virus (HCV) by DAAs may induce the normalization of innate immunity and the reduction of cancer immunosurveillance, whereas IFNs have anticarcinogenic effects. It is necessary to note that management for patients can change in response to the shift in treatment from IFNs to DAAs.

Materials & Methods: Patients with chronic HCV infection who had achieved sustained virological response at post-treatment week 12 from February 1992 to June 2017 were recruited. Patient records were examined to identify new cases of cancer, as diagnosed by pathological evaluations, excluding recurrent cancer. The cancer incidence rates were analyzed after adjusting for confounders using a propensity score.

Results: Age and sex significantly differed between patients treated with DAAs (n=371; median age: 70 years, male: 42.0 %) and those treated with IFNs (n=445; median age: 57 years, male: 61.1 %). The median follow-up times for the DAA and IFN groups were 1.8 and 6.2 years, respectively. Nineteen and 25 cases of non-liver cancer were diagnosed in the DAA and IFN groups, respectively. The cumulative incidence rates after one and two years were 2.3 % and 4.2 %, respectively, in the DAA group and 0.2 % and 1.1 %, respectively, in the IFN group. The incidence rates in the DAA group were significantly higher than those in the IFN group (P=0.030).

Conclusions: Our findings suggest that patients on DAA therapy must be more carefully examined for high incidence of non-liver cancer after therapy initiation than those on IFN therapy.

Keywords: Direct-acting antiviral therapy; Hepatitis C; Inverse probability of treatment weighting; Non-liver cancer; Sustained virological response

Abbreviations: AFP: Alpha-Fetoprotein; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; AUC: Area Under the Curve; BMI: Body Mass Index; CI: Confidence Intervals; DAA: Direct-Acting Antiviral; DLBCL: Diffuse Large B Cell Lymphoma; eGFR; Estimated Glomerular Filtration Rate; HCC: Hepatocellular Carcinoma; HCV: Hepatitis C Virus; HR: Hazard Ratios; IFN: Interferon; MPN: Myeloproliferative Neoplasms; NHL: Non-Hodgkin Lymphoma; IPTW: Inverse Probability of Treatment Weighting; Ref.: Reference; SVR: Sustained Virological Response

Introduction

The occurrence and recurrence of hepatocellular carcinoma (HCC) have been reported frequently at an early stage in patients with hepatitis C virus (HCV) who have undergone interferon (IFN)-free DAA therapy [1,2]. The underlying mechanisms may include the rapid clearance of HCV, the normalization of innate immunity, and the reduction of cancer immunosurveillance [3,4]. These changes may in turn affect the occurrence or development of cancer in other organs.

In contrast, IFN-α/β have direct anticarcinogenic effects [5-7]. IFNs were replaced by DAAs in antiviral therapy of HCV and in almost all the patients following DAA therapy the rapid clearance of HCV was achieved. If the incidence rate of non-liver cancer in patients on DAA therapy is higher than in patients on IFN therapy, then it would be necessary to consider changing the management of patients after the initiation of antiviral therapy. Because of the high efficacy and safety profile of DAAs, a prospective study to compare DAA therapy with IFN therapy could not be carried out. We therefore conducted a retrospective, single-center, cohort study to compare cancer incidence rates in organs other than the liver between patients treated with IFN-free DAA therapy and those treated with IFN-based therapy.

Methods

Study design

This was a retrospective, single center, cohort study.

Patients

Patients with chronic HCV infection who received antiviral therapy were identified from our hospital records. The inclusion criteria in this study were as follows: antiviral therapy initiated between February 24, 1992 and June 16, 2017 at our hospital, and achievement of sustained virological response (SVR) at 12 weeks after end of treatment. Patients who had relapse of HCVRNA after SVR12 were excluded.

In patients with history of either HCC or non-liver cancers, curative resection or complete response was confirmed by pathological evaluation or medical imaging before the initiation of anti-viral therapy.

Control variables

Baseline patient demographics and characteristics were determined at the time of treatment initiation which included age, sex, body mass index (BMI), cigarette smoking, habitual alcohol intake, diabetes, history of previous IFN therapy, HCC, and non-liver cancer. Habitual alcohol intake was defined as a daily alcohol consumption > 20 g.

Baseline laboratory values, defined as the value at or closest to the treatment start date, were determined for aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, bilirubin, hemoglobin, platelet count, alpha-fetoprotein (AFP), and estimated glomerular filtration rate (eGFR). A diagnosis of cirrhosis was defined as a FIB-4 index > 3.25. This value had a positive predictive value to confirm the existence of a significant fibrosis of 82.1 % with a specificity of 98.2 % [8].

Patients were considered to have SVR12 if HCV-RNA was undetectable on HCV-RNA tests, including at least one test 12 weeks or more after the end of treatment.

Follow-up

Patients in the cohort were followed weekly, biweekly, or every four to 24 weeks for the first few years and then every six or 12 months from the date of antiviral therapy initiation until January 18, 2018. The follow-up data were obtained during routine clinical care, including medical history, physical examination, laboratory tests, and/or imaging studies such as abdominal ultrasonography, contrast-enhanced computed tomography, and magnetic resonance imaging.

Outcome

The study outcome was the incidence of cancer in organs other than

the liver following antiviral therapy. Recurrent cancers were excluded from the outcomes in this study. Patient records were examined to identify new cases of cancer, as diagnosed through pathological evaluation. The dates of pathological diagnosis and last follow-up were determined based on the records.

Hypothesis

Our hypothesis was as follows: Cancer incidence rate, excluding liver cancers, in patients with SVR following IFN-free DAA therapy (DAA group) will be significantly higher than in patients with SVR following IFN-based therapy (IFN group).

Statistical analysis

Quantitative variables were expressed as the median (interquartile range), and qualitative variables were expressed as percentages. The Mann-Whitney test was used for intergroup comparisons of quantitative variables, and the chi-square test was used to compare categorical data. The cumulative cancer incidence rate was calculated using the Kaplan-Meier estimator followed by the log-rank test. Univariate and multivariate Cox regression models were used to assess contributing factors for cancer incidence and to compute hazard ratios (HR) and their 95 % confidence intervals (CI). Variables in the multivariate Cox models were selected using the stepwise method. The proportional hazards assumption was tested using the Schoenfeld residuals test. Goodness of fit for the multivariate Cox models was assessed by plotting the cumulative sums of martingale residuals. All tests were two-sided, and the threshold for statistical significance was set to $P < 0.05$.

Propensity score analysis was used to reduce the effect of potential confounders. If the number of events is low relative to the number of confounders, propensity score analysis is beneficial in regression analyses [9]. The propensity score is defined as the probability that a patient is assigned to a particular treatment condition (DAA versus IFN) based on the observed baseline covariates. The propensity score is calculated based on a multivariate logistic regression model. Adequacy of the model is checked with area under the curve (AUC) and the Hosmer-Lemeshow test. The distribution of the propensity scores was evaluated by kernel density estimation.

Four statistical methods of applying the propensity score have been described, including matching, weighting, covariate, and stratification adjustment [10]. Because all patients in the cohort could not be analyzed using pair-matching methods, we used other methods for analyzing all patients.

Inverse probability of treatment weighting (IPTW) [10,11]

IPTW uses weights based on the propensity score to create a synthetic sample in which the distributions of measured baseline covariates are independent of treatment assignment. Cumulative incidence rates were compared between the groups using the adjusted Kaplan-Meier estimator followed by the log-rank test with an IPTW method, i.e. weighting patients who underwent DAA therapy by 1/ propensity score, whereas patients who underwent IFN-based therapy were weighted by 1/(1 - propensity score). To examine the changes in confounder distribution due to IPTW, we calculated the standardized difference between the two groups for the unweighted sample and for the sample following application of IPTW [12].

Covariate adjustment [10]

In this method, the outcome is set as the dependent variable, and Cox regression analysis is performed using treatment status and estimated propensity score, which integrated the baseline covariates into one variable, as independent variables.

Stratification [10,11]

Patients were divided into five equal-sized groups using the quintiles of the propensity score. Within each stratum, the effect of treatment on outcomes was estimated by comparing outcomes directly between the two groups using Cox regression analysis. The stratum-specific estimates of treatment effect were pooled across strata to estimate an overall treatment effect. Stratification can be conceptualized as a meta-analysis of a set of quasi-randomized control trials.

To compare the incidence of non-liver cancer between the background population and our study patients, estimated annual incidence rate after antiviral therapy initiation was calculated by relating the incidence in the cohort to an age- and sex-matched material from the Japanese general population [13].

Data analysis was performed using IBM SPSS software version 20.0 and 23.0 (IBM Japan, Tokyo, Japan), JMP software version 12.1 (SAS Institute Japan, Tokyo, Japan), and R software version 3.2.5 (R Foundation for Statistical Computing, Vienna, Austria; www.r-project.org).

Ethical considerations

This study was conducted according to the Declaration of Helsinki and the ethical guidelines for epidemiological research designed by the Japanese Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour, and Welfare. The study was also approved by the ethics committee of Kyoto Medical Center (Institutional Review Board approval number: 16-099). We obtained passive consent from patients who were informed by a notice posted in the hospital and on its website and ensured the opportunity to opt out.

Results

Patients

In total, 816 patients who achieved SVR12 were recruited (Figure 1). In the DAA group (371 patients), 82 patients received asunaprevir/daclatasvir therapy, 101 received sofosbuvir/ribavirin (RBV) therapy, 166 received ledipasvir/sofosbuvir therapy, 10 received paritaprevir/ombitasvir/ritonavir therapy, two received paritaprevir/ombitasvir/ritonavir/RBV therapy, and 10 grazoprevir/elbasvir therapy. In the IFN group (445 patients), 57 patients received IFN-α or IFN-β monotherapy, 31 received IFN-α/RBV therapy, 68 received pegylated interferon (pegIFN)-α monotherapy, 212 received pegIFN-α/RBV therapy, and 77 received single DAA/pegIFN-α or pegIFN-λ/RBV therapy.

Three hundred ninety-nine patients underwent 401 courses of interferon (IFN)-free direct-acting antiviral (DAA) therapy. Six hundred eighty-seven patients underwent 725 courses of IFN-based therapy. SVR, sustained virological response.

In the Japanese public health systems, IFN-α and IFN-β monotherapies have been approved by the Ministry of Health and Welfare since January 1992 and March 1992, respectively. Other IFN-based therapies have been approved since December 2001 or later. Asunaprevir/daclatasvir therapy for patients infected with HCV genotype 1b has been approved by the Ministry of Health, Labour, and Welfare since September 2014. Other IFN-free DAA therapies have been approved since May 2015 or later. Therefore, IFN-based therapies were initiated between February 24, 1992 and June 19, 2015, and IFN-free DAA therapies were initiated between September 25, 2014 and June 16, 2017.

Table 1 shows patients' baseline characteristics in both the DAA and IFN groups. There were significant differences between DAA- and IFN-treated patients in all their characteristics, except BMI and diabetes. Of note, DAA-treated patients were older, while IFN-treated patients had a higher prevalence of males, former or current cigarette smoking, and habitual drinking. Figure 2 shows the age and sex distributions in the study cohort. In addition, DAA-treated patients had the higher prevalence of cirrhosis. Some data were missing for smoking and

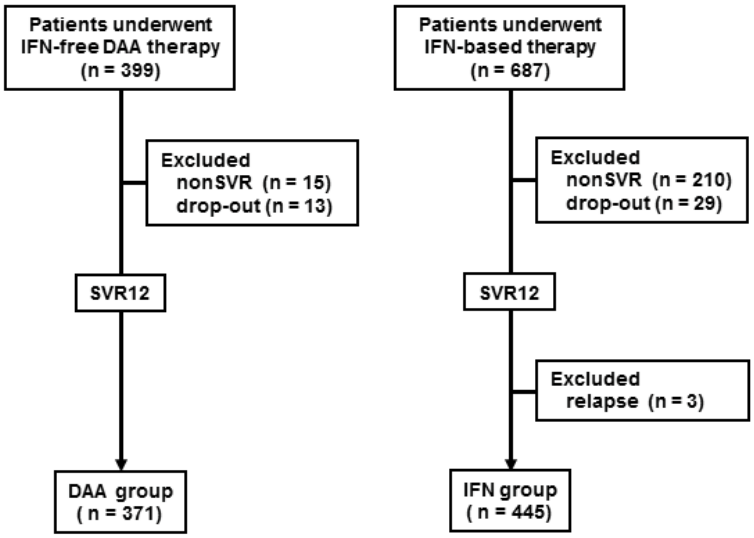


Figure 1: Study cohort

Table 1: Baseline characteristics of patients who achieved SVR12.

Variable	DAA group (n=371)		IFN group (n=445)		*P*-value
Age (years)	70	(60-76)	57	(46-65)	< 0.001
Male	156	(42.0)	272	(61.1)	< 0.001
Body mass index (kg/m^2)	22.8	(20.2-25.3)	23.1	(20.9-24.7)	0.360
Former or current smoking	155	(41.8)	262	(58.9)	< 0.001
Habitual alcohol intake	77	(20.8)	121	(27.2)	0.033
Diabetes Mellitus	80	(21.6)	76	(17.1)	0.108
History of IFN therapy	96	(25.9)	76	(17.1)	0.003
History of HCC	59	(15.9)	49	(11.0)	0.048
History of non-liver cancer	41	(11.1)	27	(6.1)	0.011
AST (IU/L)	44	(29-63)	51	(35-80)	< 0.001
ALT (IU/L)	38	(23-62)	63	(40-106)	< 0.001
Albumin (g/dL)	4.0	(3.6-4.2)	4.2	(3.9-4.4)	< 0.001
Bilirubin (mg/dL)	0.8	(0.6-1.1)	0.8	(0.6-1.0)	0.035
Hemoglobin (g/dL)	13.1	(12.1-14.1)	13.9	(13.0-15.0)	< 0.001
Platelet count (x10^4/μL)	14.9	(11.0-19.5)	16.2	(12.0-20.5)	0.008
AFP (ng/mL)	5	(3-9)	5	(3-10)	0.007
eGFR (mL/min./1.73 m^2)	67.9	(58.1-78.7)	82.2	(71.9-95.8)	< 0.001
FIB-4 index > 3.25	184	(49.6)	150	(33.7)	< 0.001
Child-Pugh score > 5	69	(18.6)	28	(6.3)	< 0.001

Values are presented as number (percentage) or as medians (interquartile range). SVR12, sustained virological response at post-treatment week 12; DAA, direct acting antiviral; IFN, interferon; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate.

alcohol use in the records – of the total 816 patients, cigarette smoking was unknown in five patients and alcohol consumption was unknown in two patients. Among other critical covariates, prothrombin time was not tested in six patients before treatment and was not available in eight patients who underwent anticoagulant therapy. In 16 patients, neither HCV serotype nor HCV genotype was tested.

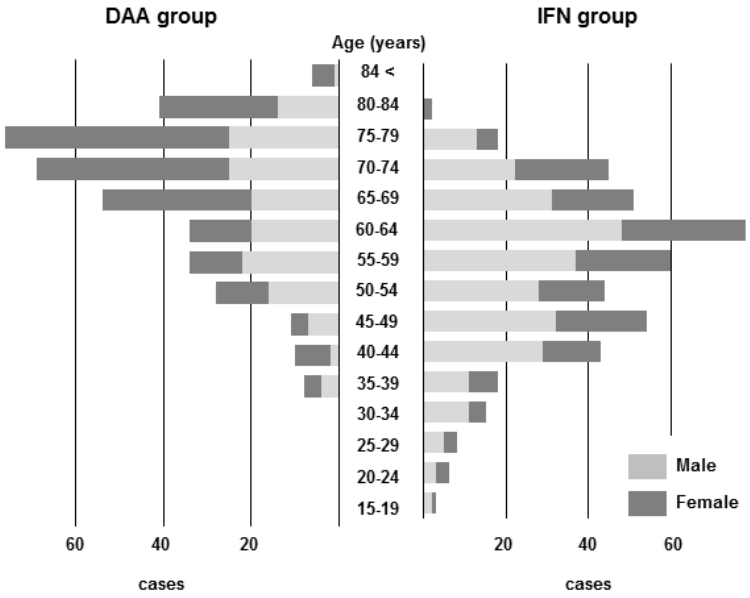
Outcome

The median follow-up times of the DAA and IFN groups were 1.8 (interquartile range; 1.3 -2.2) and 6.2 (3.7-9.5) years, respectively. In total, 44 patients received a cancer diagnosis. In the DAA group, there were 19 cases of cancer incidence in organs other than the liver; cancer occurred most frequently in the gastrointestinal tract, followed by the urinary organs, and the hematopoietic organs (Table 2). The median period between the initiation of DAA therapy and the time of diagnosis was 1.0 years. In the IFN group, there were 25 cases of cancer incidence in organs other than the liver; cancer occurred most frequently in the gastrointestinal tract, followed by the respiratory organs, hematopoietic organs, and urinary organs. The median period between the initiation of IFN therapy and the time of diagnosis was 5.6 years. In all cases, pathological diagnoses were obtained. Forty out of 44 patients underwent pathological examinations at our hospital. The remaining four patients consisted of one with pancreatic cancer, one with lung cancer, one with non-Hodgkin lymphoma, and one with eyelid cancer, all of whom had pathological diagnoses from other institutions.

We indicated baseline characteristics of patients who received non-liver cancer diagnosis after the initiation of anti-viral therapy in the two groups (Table 3).

Conventional statistical analyses

The non-liver cancer incidence in the DAA group was 0.0280 per person-year, and that in the IFN group was 0.0075 per person-year. Figure 3 shows the cumulative cancer incidence rate for the DAA and IFN groups. The cumulative incidence rates after one and two years were 2.3 % (95 % CI 1.1-4.5 %) and 4.2 % (95 % CI 2.4-7.4 %),



DAA: Direct acting antiviral; IFN: Interferon.

Figure 2: Age and sex distributions in the study cohort.

Table 2: Primary organs in cancer incidence after the antiviral therapy initiation.

Primary Organs		DAA group	IFN group
Gastrointestinal tract			
	Esophagus		1
	Stomach	3	3
	Colorectum	4	4
	Extrahepatic bile tract	2	
	Pancreas	1	2
Urinary organs			
	Kidney	2	
	Urinary bladder	3	2
	Prostate		1
Respiratory organs			
	Trachea		1
	Lung	1	4
Hematopoietic organs			
	MPN, unclassifiable	1	
	DLBCL	1	1
	non-DLBCL NHL		3
Head and Neck			
	Eyelid		1
	Tongue	1	
Skin			1
Unknown			1

DAA, direct acting antiviral; IFN, interferon; MPN, myeloproliferative neoplasms; DLBCL diffuse large B cell lymphoma; NHL, non-Hodgkin lymphoma.

respectively, in the DAA group, and 0.2 % (95 % CI 0.0-1.6 %) and 1.1 % (95 % CI 0.5-2.7 %), respectively, in the IFN group. The differences in the incidence between the two groups were significant ($P < 0.001$) using the log-rank test.

Cumulative cancer incidence rates were calculated via the Kaplan-Meier estimator. A significant difference between the DAA and IFN groups was detected by the log-rank test ($P < 0.001$). DAA, direct acting antiviral; IFN, interferon.

Table 3: Baseline characteristics of patients with non-liver cancer incidence.

Variable	DAA group (n=19)		IFN group (n=25)	
Age (years)	72	(70-78)	62	(51-70)
Male	9	(47.4)	19	(76.0)
Body mass index (kg/m^2)	22.7	(21.1-25.1)	23.2	(21.7-24.0)
Former or current smoking	8	(42.1)	20	(80.0)
Habitual alcohol intake	2	(10.5)	5	(20.0)
Diabetes Mellitus	3	(15.8)	4	(16.0)
History of IFN therapy	5	(26.3)	2	(8.0)
History of HCC	4	(21.1)	6	(24.0)
History of non-liver cancer	1	(5.3)	1	(4.0)
AST (IU/L)	57	(31-84)	53	(41-71)
ALT (IU/L)	44	(28-78)	81	(45-116)
Albumin (g/dL)	3.8	(3.4-4.1)	4.2	(3.9-4.4)
Bilirubin (mg/dL)	1.1	(0.8-1.1)	0.8	(0.5-1.0)
Hemoglobin (g/dL)	12.9	(11.9-13.5)	13.8	(12.9-14.7)
Platelet count (x10^4/μL)	12.8	(9.3-15.8)	13.7	(10.5-21.6)
AFP (ng/mL)	5	(3-14)	5	(4-12)
eGFR (mL/min./1.73 m^2)	62.4	(58.3-69.3)	80.8	(75.4-91.2)
FIB-4 index > 3.25	15	(78.9)	12	(48.0)
Child-Pugh score > 5	5	(26.3)	0	(0.0)

Values are presented as number (percentage) or as medians (interquartile range). SVR12, sustained virological response at post-treatment week 12; DAA, direct acting antiviral; IFN, interferon; HCC, hepatocellular carcinoma; AST, aspartate aminotransferase; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate.

To identify the factors associated with cancer incidence outside of the liver after the initiation of antiviral therapy, univariate and multivariate Cox regression analyses were carried out using the data of patients who achieved SVR12 (Table 4). The univariate analysis indicated that treatment condition (DAA versus IFN), age, history of HCC, platelet count, and FIB-4 index were significantly related to non-liver cancer incidence. Multivariate analysis revealed that compared to IFN therapy, DAA therapy (IFN-free) was a significant and independent

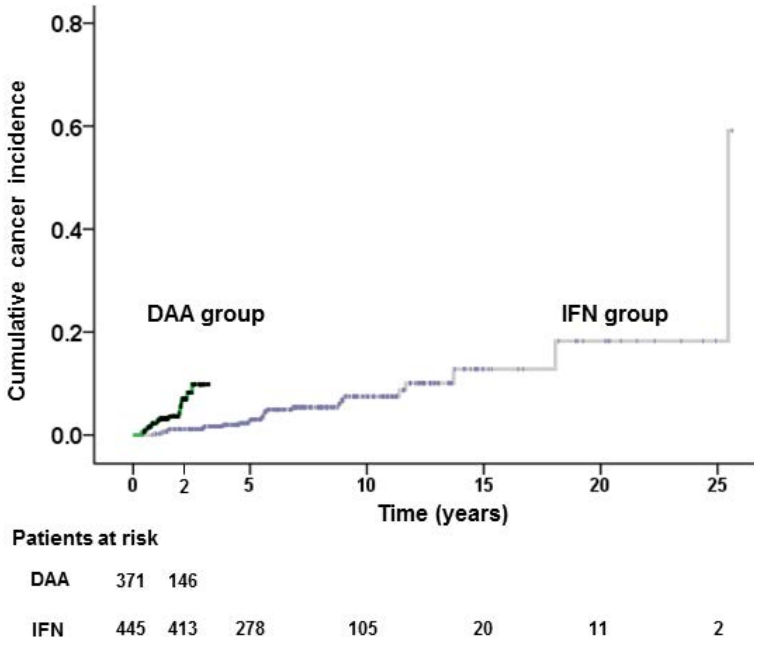


Figure 3: Cumulative non-liver cancer incidence in patients after antiviral therapy initiation.

Table 4: Factors associated with the incidence of non-liver cancer in Cox regression analyses

	Univariate Analysis		Multivariate Analysis	
Factor	**HR (95% CI)**	***P*-value**	**HR (95% CI)**	***P*-value**
DAA (ref. IFN-based)	6.94 (2.67-18.01)	< 0.001	5.54 (2.08-14.75)	0.001
Age > 63 (years)	3.11 (1.64-5.93)	0.001	2.27 (1.12-4.58)	0.023
Male	1.30 (0.70-2.42)	0.405		
BMI > 22.9 (kg/m^2)	1.02 (0.56-1.84)	0.953		
Former or current smoking	1.41 (0.76-2.62)	0.280	2.04 (1.72-3.89)	0.030
Habitual alcohol intake	0.59 (0.26-1.34)	0.208		
Diabetes Mellitus	0.88 (0.39-1.97)	0.752		
History of IFN therapy	1.09 (0.48-2.50)	0.836		
History of HCC	2.43 (1.18-4.99)	0.016		
History of non-liver cancer	0.65 (0.16-2.70)	0.556		
AST > 1.5 x ULN	1.18 (0.64-2.17)	0.596		
ALT > 1.5 x ULN	0.97 (0.53-1.78)	0.921		
Albumin > 4.1 (g/dL)	0.55 (0.29-1.02)	0.059		
Bilirubin > 0.8 (mg/dL)	1.46 (0.81-2.63)	0.214		
Hemoglobin > 13.5 (g/dL)	0.61 (0.33-1.12)	0.108		
Platelet count > 15.7 (x10^4/μL)	0.45 (0.24-0.87)	0.017	0.56 (0.29-1.09)	0.088
AFP > 5 (ng/mL)	0.91 (0.50-1.66)	0.759		
eGFR > 60 (mL/min./1.73 m^2)	0.47 (0.22-1.00)	0.051		
FIB-4 index > 3.25	2.75 (1.48-5.10)	0.001		
Child-Pugh score > 5	1.41 (0.55-3.64)	0.469		

The cut-off values for age, body mass index, albumin, bilirubin, hemoglobin, platelet count, and AFP represented the median values of the entire group of patients. HR, hazard ratio; CI, confidence interval; DAA, direct acting antiviral; ref., reference; IFN, interferon; BMI, body mass index; AST, aspartate aminotransferase; ULN, upper limit of normal; ALT, alanine aminotransferase; AFP, alpha-fetoprotein; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma.

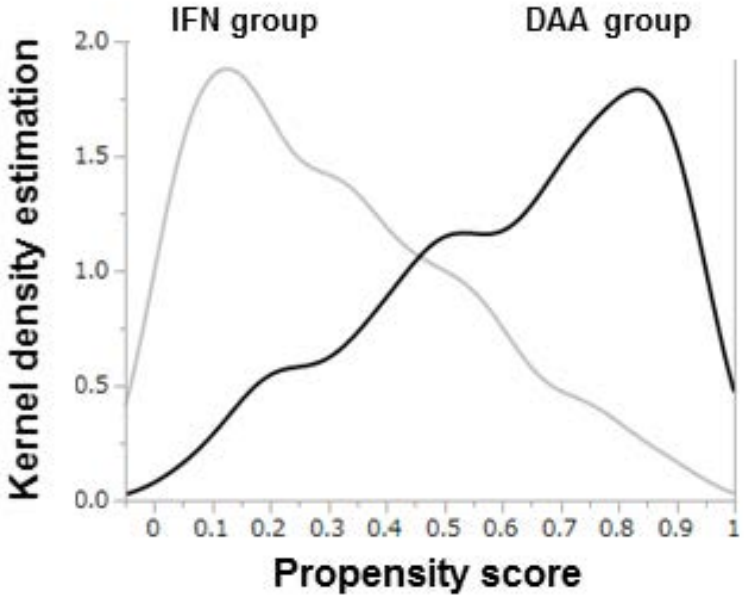
determinant of cancer incidence after adjustments for age, former or current smoking, and platelet count.

Propensity score analyses

The following variables were forced into the logistic model for estimation of the propensity score: age, sex, BMI, cigarette smoking, habitual alcohol intake, diabetes, history of IFN therapy, HCC and non-liver cancer, ALT, albumin, bilirubin, hemoglobin, platelet count, AFP, and eGFR. The other baseline characteristics (AST, FIB-4 index, and Child-Pugh score) were excluded – AST: its multicollinearity with ALT; FIB-4 index: the formula used to calculate the score included age, ALT, and platelet count; and Child-Pugh score: depended on the level of albumin and bilirubin. The patients with missing data regarding cigarette smoking (n=5) and alcohol consumption (n=2) were excluded from propensity score analysis.

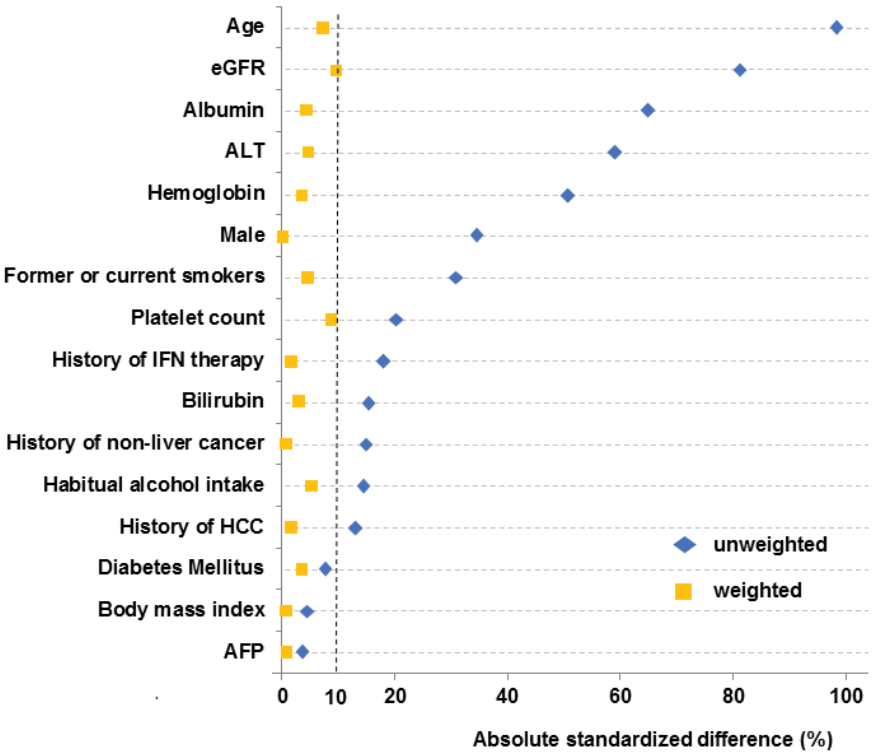
The adequacy of the logistic model was checked with AUC and the Hosmer-Lemeshow test. The AUC was equal to 0.823 (95 % CI, 0.795-0.852), and the *P*-value of the Hosmer-Lemeshow test was 0.202, showing goodness of fit for the model. Figure 4 shows the overlap of the propensity score distributions between the two groups.

To clarify the effects of treatment with DAA and IFN-based therapy on cancer incidence, we applied the adjusted Kaplan-Meier estimator followed by the log-rank test using IPTW [11]. Cumulative cancer incidence rate, excluding liver cancers, in patients with SVR following DAA therapy was significantly higher than in patients with SVR following IFN-based therapy ($P = 0.030$). We calculated the absolute standardized differences for each of the baseline covariates to assess whether covariates were balanced between the DAA and IFN groups in the weighted sample [12]. When using the IPTW model, the largest absolute standardized difference in the weighted sample was 9.9 % (eGFR); all other absolute standardized differences were below 10 % (Figure 5).



DAA: Direct acting antiviral; IFN: Interferon.

Figure 4: Distributions of the propensity score determined by kernel density estimation.



The absolute standardized differences between the DAA and IFN groups for each of the baseline covariates were calculated in the unweighted sample and in the weighted sample. After weighting, there was good balance across all covariates. A vertical line was superimposed denoting a standardized difference of 10 %. eGFR, estimated glomerular filtration rate; ALT, alanine aminotransferase; IFN, interferon; HCC, hepatocellular carcinoma; AFP, alpha-fetoprotein.

Figure 5: Absolute standardized differences in the unweighted and weighted sample.

Further, the treatment and propensity score were added to the multivariate Cox regression analysis to adjust for the measured confounders presented in Table 1, excluding AST, FIB-4 index, and Child-Pugh score. Compared to IFN-based therapy, DAA therapy was significantly related to cancer incidence in organs other than the liver (Table 5).

In the stratified analysis using the Cox regression analysis, the treatment was also significantly related with cancer incidence in organs other than the liver (HR, 4.86; 95 % CI, 1.52-15.49; $P = 0.008$).

Estimated annual incidence of non-liver cancer

The left column in Table 6 indicates the reported annual incidence rate of non-liver cancer in males and females of each age group (in five-year increments) in the Japanese general population in 2013 [13]. Using the reported incidences, we calculated the annual incidence rate of non-liver cancer expected for the population with the same age and sex distribution as each of the IFN and DAA groups. The estimated annual incidence rates for the DAA and IFN groups were 1.3 %and 0.8 %, respectively.

Discussion

Recent studies reported the early occurrence and recurrence of HCC in patients with HCV who undergo IFN-free DAA therapy [1,2]. By contrast, a French group reported no increase of HCC recurrence after DAA therapy in prospective cohorts [14]. Other investigators compared the occurrence or recurrence of HCC between patients treated with DAA therapy and those treated with IFN-based therapy and reported that the occurrence and recurrence rates of HCC were similar between the groups [15,16]. However, because the sample size and number of events were small in those studies, it is difficult to conclude that DAA therapy does not induce early occurrence and/or recurrence of HCC.

Chronic HCV infection activates an intrahepatic immune response, leading to increased expression of interferon-stimulated genes and activation of natural killer (NK) cells [3,17,18]. Serti et al. demonstrated that the products of some interferon-stimulated genes in peripheral blood rapidly decreased after the start of DAA therapy, and the elevated NK cell function and change of NK cell phenotype in peripheral blood normalized up to 24 weeks after the end of treatment [3]. Villani et al. demonstrated that DAA therapy induces an early increase in serum vascular endothelial growth factor (VEGF) [4]. VEGF is considered a critical player in tumor angiogenesis and may induce the development of cancer. New blood vessels eventually infiltrate the microscopic tumor mass, thus setting in motion a switch to gradual macroscopic tumor growth [19]; moreover, several antiangiogenic drugs have been approved for the systemic treatment of a variety of advanced and/or metastatic malignancies. Villani et al. did not address the possible origin of the VEGF increase after DAA therapy initiation [4]. However, these studies suggest that the rapid clearance of HCV following DAA therapy may induce the normalization of innate immunity and reduction of cancer immunosurveillance, and stimulation of tumor angiogenesis and its growth. These changes may affect the occurrence or development of non-liver cancer.

The patients in both the DAA and IFN groups underwent rigorous and frequent examinations for several years after the treatment initiation, and the cumulative cancer incidence rate in organs other than the liver was significantly higher in patients treated with DAA therapy than in those treated with IFN-based therapy using the log-rank test for unweighted sample (Figure 3). In this study, however, there were significant differences between patients treated with DAA and those treated with IFN for all characteristics, except BMI and diabetes (Table 1).

Cancer incidence rate has been reported to depend on age and sex in Japan [13]. In particular, cancer incidence is higher in the elderly than

	Multivariate Analysis	
Factor	**HR (95% CI)**	***P*-value**
DAA (ref. IFN-based)	4.95 (1.71-14.33)	0.003
Propensity score	2.61 (0.64-10.58)	0.180

P-value of the treatment condition was based on the Cox regression analysis followed by covariate adjustment using propensity score. DAA, direct acting antiviral; HR, hazard ratio; CI, confidence interval; ref., reference; IFN, interferon.

Table 5: Effect of DAA therapy on non-liver cancer incidence.

	Reported Annual Incidence (%)†		Number of patients DAA group (N = 371)		Number of patients IFN group (N = 445)		Estimated Annual Cancer Cases DAA group (N = 371)	Estimated Annual Cancer Cases FN group (N = 445)	Estimated Annual Incidence (%) DAA group (N = 371)	Estimated Annual Incidence (%) IFN group (N = 445)
	M	**F**	**M**	**F**	**M**	**F**				
Age									**1.309**	**0.814**
> 84	**3.532**	**1.681**	**1**	**5**			**0.119**		**(4.9/371)**	**(3.6/445)**
80-84	**3.413**	**1.422**	**14**	**27**		**2**	**0.862**	**0.028**		
75-79	**2.984**	**1.257**	**25**	**51**	**13**	**5**	**1.388**	**0.451**		
70-74	**2.433**	**1.073**	**25**	**44**	**22**	**23**	**1.081**	**0.782**		
65-69	**1.773**	**0.888**	**20**	**34**	**31**	**20**	**0.657**	**0.727**		
60-64	**1.178**	**0.762**	**20**	**14**	**48**	**30**	**0.342**	**0.794**		
55-59	**0.683**	**0.614**	**22**	**12**	**37**	**23**	**0.224**	**0.394**		
50-54	**0.369**	**0.513**	**16**	**12**	**28**	**16**	**0.121**	**0.186**		
45-49	**0.187**	**0.426**	**7**	**4**	**32**	**22**	**0.03**	**0.153**		
40-44	**0.114**	**0.287**	**2**	**8**	**29**	**14**	**0.025**	**0.073**		
35-39	**0.062**	**0.164**	**4**	**4**	**11**	**7**	**0.009**	**0.018**		
30-34	**0.042**	**0.096**			**11**	**4**		**0.008**		
25-29	**0.029**	**0.048**			**5**	**3**		**0.003**		
20-24	**0.02**	**0.02**			**3**	**3**		**0.001**		
15-19	**0.015**	**0.012**			**2**	**1**		**0**		

Estimated annual incidences of non-liver cancer were calculated based on the same age and sex distribution as each of the DAA and IFN groups using the incidences in the Japanese general population. **†:** According to the report by Hori et al [13]. DAA, direct acting antiviral; IFN, interferon; M, male; F, female.

Table 6: Estimated annual incidence of non-liver cancer.

in younger individuals and in men than in women (Table 6). Smoking has also been reported to increase the risk of lung cancer, transitional cell carcinoma of the urinary tract, head and neck cancer, esophageal cancer, gastric cancer, and pancreatic cancer [20]. Consumption of alcohol increases the risk of cancers, including those of the liver, head and neck, esophagus, breast, and colorectum [21]. In the present study, there were more elders and fewer males, smokers, and drinkers in the DAA group than in the IFN group (Table 1 and Figure 2). Age and smoking were adjusted for when using the conventional unweighted multivariate Cox regression analysis, but sex and habitual drinking were not (Table 4). Because the number of events was low relative to the number of confounders, it was not recommended that another variable be forced into the multivariate models. Therefore, we adjusted for the effects of potential confounders, which included age, sex, smoking, and habitual drinking, using propensity score analyses with covariate and stratification adjustment; similar results were obtained from these analyses (Table 5 and Results). In addition, the difference in cancer incidence between the DAA and IFN groups was significant by the adjusted Kaplan-Meier estimator using IPTW method. Austin et al. reported in a systematic literature review that the use of IPTW had increased rapidly in recent years, but that a majority of studies did not formally examine whether weighting balanced the measured covariates between treatment groups [12]. They described a suite of quantitative and qualitative methods that allow one to assess whether measured baseline covariates are balanced between treatment groups in the weighted sample, and suggested that a standardized difference in excess of 10 % may be indicative of meaningful imbalance in covariates between the two groups. In this study, after weighting the absolute standardized differences for age, sex, smoking, and habitual drinking were 8.6 %, 0.0 %, 4.7 %, and 5.9 %, respectively (Figure 5). Our results may indicate that the IPTW method using propensity score created a sample in which the distributions of age, sex, smoking, and habitual drinking were almost similar between the two groups. To support our hypothesis, it is important that all of the results from the conventional multivariate regression adjustment (Table 4) and those from the analyses using the different propensity score methods from the same data set (Table 5 and Results) are the same [10].

IFN-α/β have been reported to have some direct anticarcinogenic effects and to regulate NK cell activity [5-7,22,23]. Ahlenstiel et al. revealed that pegIFN-α/RBV therapy activates NK cells in peripheral blood early after treatment is initiated and up to week 12 of therapy in patients with HCV infection [23]. Most patients who achieved SVR by IFN-based therapy had undergone long-term IFN administration. It is possible that in the IFN group, the exogenous IFN itself inhibited cancer occurrence or development. We calculated the annual incidence rates of non-liver cancer expected for the population with the same age and sex distribution as each of the two groups using the incidences in the Japanese general population. The estimated annual incidence rates for the DAA and IFN groups were 1.3 % and 0.8 %, respectively (Table 6). However, in our study cohort, the cumulative incidence rates of non-liver cancer after one year, using the Kaplan-Meier estimator, were 2.3 % in the DAA group and 0.2 % in the IFN group. It is possible that DAA therapy (IFN-free) may induce the occurrence or development of non-liver cancer and treatment with IFN might inhibit the occurrence or development of non-liver cancer. Our results suggest that the management for patients after the antiviral therapy initiation should be changed in response to the shift in treatment from IFNs to DAAs.

In a large US cohort, HCV infection was positively associated with cancers of the liver, intrahepatic bile duct, extrahepatic bile duct, pancreas, anus, myelodysplastic syndrome, and diffuse large B cell lymphoma, and inverse associations were observed with uterine cancer and prostate cancer [24]. Our study demonstrates that the risk of cancer in patients who undergo DAA therapy may not be restricted to the organs previously known to be related to HCV infection (Table 2).

However, this study also has limitations. Particularly, our study was retrospective and used hospital records for patient data. Therefore, recent cancer incidence in patients with lost follow-up was unknown. Furthermore, it is difficult to accurately estimate cancer incidence in all organs in patients with SVR after the initiation of antiviral therapy. Since cancer detection in organs, other than the liver, can be a challenge in the management of hepatitis, some cases of cancer found after the start of the antiviral therapy might have been diagnosable before the treatment, possibly leading to an overestimation of the incidence in the first few years after the treatment is initiated. For similar reasons, however, some cases of undetected cancer after the start and end of treatment might have led to an underestimation of the incidence. In addition, it has been reported that cancer incidence was correlated with family history of cancers, other viral infections, such as human papillomavirus, and toxins exposure [25-27]. Because the records for these factors were incomplete in this study, the cancer incidence between the two groups could not be adjusted for by these factors. The age-standardized cancer incidence rate has been reported as gradually increasing year by year in Japan [13]. This trend may have affected the difference in cancer incidence between the two groups. However, since the incidence have been rising slowly, the high incidence of non-liver cancer in the DAA group may not only be explained by the changes in cancer incidence over time. Finally, cirrhosis-associated innate and adaptive immune dysfunction has been reported [28]. The prevalence of cirrhosis may affect cancer incidence in the two groups.

In conclusion, our findings suggest that patients on DAA therapy must be more carefully examined for high incidence of non-liver cancer after therapy initiation than those on IFN therapy.

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Author Contributions

All authors collected data. Shinji Katsushima, Naoki Esaka, Bunji Endoh, and Toshiki Komeda contributed to the study conception and design. Shinji Katsushima and Shigeharu Nakano analyzed the data. Shinji Katsushima wrote the original draft. Naoki Esaka edited the manuscript. All authors contributed to review of the manuscript.

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