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【 ORIGINAL ARTICLE 】

Influence of Interferon-free Direct-acting Antiviral Therapy on Primary Hepatocellular Carcinoma Recurrence: A Landmark Time Analysis and Time-dependent Extended Cox Proportional Hazards Model Analysis

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Abstract:

Objective The influence of interferon (IFN)-free direct-acting antiviral (DAA) on hepatocellular carcinoma (HCC) recurrence remains unclear. Previous retrospective analyses revealed that the time interval between HCC curative treatment and IFN-free DAA induction is the critical factor affecting HCC recurrence. Thus, this study aimed to examine the influence of DAA therapy on HCC recurrence considering this interval.

Methods Factors contributing to HCC recurrence were retrospectively analyzed using a landmark time analysis and time-dependent extended Cox proportional hazards model.

Patients After screening 620 patients who were diagnosed with primary HCC from January 2001 to December 2016, 76 patients with early-stage (primary and solitary) disease who received curative treatment and were positive for serum hepatitis C virus RNA were included.

Results HCC recurrence was observed in 8 of 17 (47.1%) patients who had received IFN-free DAA therapy and 45 of 59 (76.3%) who had not. No significant difference was seen between the IFN-free DAA (-) and IFN-free DAA (+) groups in the landmark time and time-dependent Cox proportional hazards model analyses. However, IFN-free DAA therapy tended to decrease the HCC recurrence rate after curative treatment for primary HCC in patients with chronic hepatitis. In addition, IFN-free DAA therapy tended to decrease the second HCC recurrence rate after treatment for the first HCC recurrence.

Conclusion Our results, with a consideration of the time interval between HCC curative treatment and IFN-free DAA induction, showed that IFN-free DAA therapy was not associated with early-stage HCC recurrence after curative treatment.

Key words: hepatitis C, hepatocellular carcinoma, direct-acting antivirals, recurrence, time interval

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Introduction

Interferon (IFN)-free direct-acting antiviral (DAA) therapy has replaced IFN as the standard treatment for hepatitis C virus (HCV) infection based on its high efficacy and tolerability (1, 2). It has also been actively used after curative treatment in patients with concurrent hepatocellular carcinoma (HCC). It is well-characterized that chronic inflammation due to HCV infection accelerates hepatic fibrosis, which promotes HCC development (3-5). Furthermore, several molecular studies have shown that the presence of HCV infection or HCV proteins themselves are involved in hepatic carcinogenesis (6-8). Therefore, the elimination of

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HCV by IFN-free DAA therapy is believed to suppress hepatic carcinogenesis, and its use in patients with HCC to prevent recurrence after curative treatment is acceptable.

However, a controversial Spanish study published in 2016 raised concerns that IFN-free DAA therapy may promote the progression of HCC (9). Since this report, many studies have investigated the influence of IFN-free DAA therapy on HCC progression. Several systematic reviews and meta-analyses have shown that IFN-free DAA therapy does not affect HCC recurrence (10-13). In contrast, single-center studies have shown that IFN-free DAA therapy may have either promotive or suppressive effects on HCC recurrence (14-18). Thus, the studies reported to date present conflicting data.

When investigating HCC recurrence, it is appropriate to analyze the period from the time of curative treatment to recurrence (19). However, several problems plague retrospective analyses on the effect of IFN-free DAA treatment on HCC recurrence. First, various factors must be considered at the primary HCC stage. Even after curative treatment, the risk of HCC recurrence is thought to differ among patients with single or multiple tumors and those with primary or recurrent tumors. Second, the interval between curative treatment and IFN-free DAA therapy is different. HCC recurrence rates are believed to differ according to this recurrence-free interval. When analyzing the effect of IFN-free DAA treatment on HCC recurrence, it is necessary to consider these factors and to adjust for them accordingly.

With the aforementioned gap in research, this study aimed to delineate the effect of IFN-free DAA therapy on HCC recurrence considering the above-mentioned factors. We limited the study to patients with primary and solitary HCC curatively treated with surgery, radiofrequency ablation (RFA), and stereotactic radiotherapy (SRT). Furthermore, to adjust for differences in the recurrence-free interval, we used a landmark time analysis and the time-dependent extended Cox proportional hazards model to retrospectively analyze the effect of IFN-free DAA therapy on HCC recurrence (20, 21).

Materials and Methods

Study design and subjects

This study was approved by the Graduate School of Nagasaki University's Research Ethics Committee. Informed consent was obtained from most of the study's participants in accordance with the Declaration of Helsinki, and for those from whom we did not have the opportunity to obtain informed consent, the retrospective nature of the study enabled us to provide information about the research on the hospital's web page and to guarantee these patients the option to refuse participation in the research.

A total of 620 patients who were diagnosed with primary HCC from January 2001 to December 2016 at the Department of Gastroenterology and Hepatology in Nagasaki University Hospital were screened. The diagnosis of HCC was based on the typical findings detected by ultrasonography (US), dynamic computed tomography (CT), magnetic resonance imaging (MRI), abdominal angiography, and/or biopsy findings. The inclusion criteria were as follows: (i) primary and solitary HCC without metastasis and vessel invasion, (ii) positivity for serum HCV RNA at treatment for primary HCC, and (iii) primary HCC treated with surgery, RFA, and SRT curatively. The exclusion criterion was a follow-up period after curative treatment of <6 months.

Curative treatment with surgery was confirmed by findings of resection with a comfortable margin and the absence of HCC recurrence within six months after treatment. Curative treatment with RFA was confirmed by tumor findings contained within the ablation area on either enhanced CT or MRI at one to two weeks after treatment and the absence of HCC recurrence within six months after treatment. Similarly, curative treatment with SRT was confirmed by tumor findings contained in the cautery region on enhanced CT or MRI at three to six weeks after treatment and absence of HCC recurrence within six months after treatment. HCC recurrence was defined as the appearance of enhancement in the arterial phase on either dynamic CT or MRI, and the recurrence-free period was calculated based on the interval between curative treatment and confirmation of recurrence by imaging.

HCC recurrence was investigated by reviewing patients' medical records up to August 2019. Underlying liver diseases, such as chronic hepatitis (CH) and liver cirrhosis (LC), were diagnosed comprehensively based on blood laboratory examinations and US, CT, MRI, and endoscopy findings. The Fib-4 index was calculated based on the blood examination data at the curative treatment for primary HCC (22). The history of antiviral therapy after treatment for primary HCC, including IFN-based and IFN-free DAA therapy, was also extracted from the medical records. Anti-HCV therapy was initiated based on the attending physicians' discretion. Regarding the components of the anti-HCV therapy, IFN-based anti-HCV therapy was given until August 2014, and IFN-free DAA therapy was given after September 2014.

To determine the influence of IFN-free DAA therapy on HCC recurrence, we compared the recurrence rate between patients who received IFN-free DAA therapy and those who did not. The factors contributing to HCC recurrence were also examined.

Statistical analyses

To compare the patients' baseline characteristics, categorical data were summarized by the frequency and rate and analyzed using Fisher's exact test, whereas numerical data were summarized by the median and analyzed using Wilcoxon's rank sum test.

To compare the recurrence rate after curative treatment in the presence or absence of IFN-free DAA therapy, a landmark time analysis was conducted to adjust for the differ-

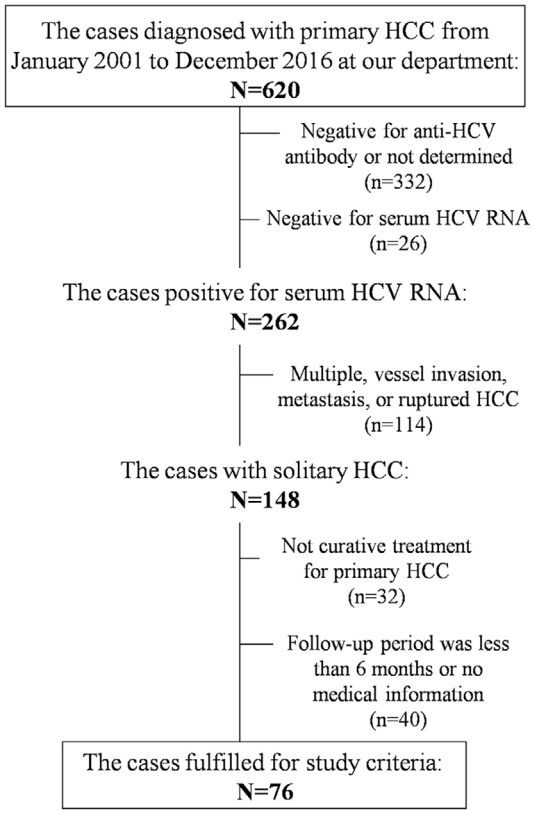

Figure 1. Flowchart illustrating the method of screening patients for the present study.

ence in the recurrence-free interval after curative HCC treatment (23). The analysis was performed by setting the landmark time to 1 year and 2 years. To determine the factors contributing to HCC recurrence, a time-dependent, extended Cox proportional hazards model was developed. The time period between primary HCC treatment and HCC recurrence was set as the objective variable, while the age, sex, primary HCC diameter, underlying liver disease (CH and/or LC), treatment for primary HCC (surgery, RFA, or SRT), administration of IFN-free or IFN-based anti-HCV therapy after primary HCC treatment, and the presence of early enhancement of primary HCC were set as explanatory variables. The administration of IFN-free DAA therapy was set as the time-dependent explanatory variable (20, 21, 24). The time-dependent expanded Cox proportional hazards model was analyzed as follows:

$h(t, X(t))= h0(t)exp [\beta 1*Age\ (years)+\beta 2*Gender\ (Man/Woman)+\beta 3*HCC\ diameter\ (cm)+\beta 4*Underlying\ liver\ disease\ (CH/LC)+\beta 5*therapy\ (Operation/\ RFA/\ SRT)+\beta 6*IFN\text{-}free\ DAA\ therapy\ (t)+\beta 7*IFN\text{-}based\ therapy+\beta 8*early\ phase\ enhancement\ of\ primary\ HCC]$

The software programs SAS for Windows Release ver. 9.4 (SAS Institute, Cary, USA) and R version 3.3.1 (R Foundation for Statistical Computing, Vienna, Austria) were used for the analyses. A two-tailed $p<0.05$ indicated a significant difference.

Results

Characteristics of the study population

A total of 76 patients met the inclusion criteria and were included in the final analysis (Fig. 1). The baseline characteristics of the patients are presented in Table 1. IFN-free DAA therapy was administered to 17 patients (22.4%) during the follow-up period [IFN-free DAA (+) group] but not to the remaining 59 patients (77.6%) [IFN-free DAA (-) group]. Within the IFN-free DAA (+) group, 8 patients received daclatasvir and asunaprevir combination therapy for 24 weeks and 9 received sofosbuvir and ledipasvir combination therapy for 12 weeks. The median duration from curative treatment for primary HCC to induction of IFN-free DAA therapy was 0.72 (0.31-10.4) years. All patients completed their course of treatment and achieved a sustained virologic response (SVR). No significant differences in the sex, age at the diagnosis with primary HCC, underlying liver disease, or Fib4 index were found between the two groups. The diameter of the primary HCC in the IFN-free DAA (+) group was significantly less than that in the IFN-free DAA (-) group. Furthermore, no significant difference in the treatment for primary HCC was found between the groups. IFN therapy was administered to 8 of 59 patients (13.6%) in the IFN-free DAA (-) group and 1 of 17 (5.9%) in the IFN-free DAA (+) group during the follow-up period. Two patients in the IFN-free DAA (-) group achieved SVR by IFN therapy.

Influence of IFN-free DAA therapy on recurrence after curative treatment for primary HCC

HCC recurrence was observed in 45 of 59 (76.3%) patients in the IFN-free DAA (-) group and in 8 of 17 (47.1%) in the IFN-free DAA (+) group. The median periods from curative treatment for primary HCC to recurrence in the IFN-free DAA (-) and IFN-free DAA (+) groups were 1.72 and 3.10 years, respectively.

Fig. 2 shows the landmark time analysis of the recurrence rates in the two groups. The landmark time of Fig. 2A was set at one year. Sixty-one patients had no HCC relapse for one year after the curative treatment. Among them, eight patients were treated with IFN-free DAA therapy during that time. Similarly, the landmark time of Fig. 2B was set at two years. Thirty-nine patients had no HCC relapse for two years after the curative treatment. Among them, seven patients were treated with IFN-free DAA therapy during that time. Furthermore, no significant difference was found between the IFN-free DAA (-) and IFN-free DAA (+) groups in the landmark time analysis set at 1 year and 2 years.

Influence of IFN-based therapy on recurrence after curative treatment for primary HCC

There have been many reports about the influence of IFN-based therapy on HCC recurrence after curative treatment

Table 1. Characteristics of the Study Population.

Number (%) or Median (range)	IFN-free DAA (-) (n=59)	IFN-free DAA (+) (n=17)	p value
Sex: male/female	33 (55.9)/26 (44.1)	9 (52.4)/8 (47.1)	0.953
Age at diagnosis with primary HCC, years	72.0 (55-86)	74.0 (50-85)	0.557
Underlying liver disease, CH/LC	24 (40.7)/35 (59.3)	5 (29.4)/12 (70.6)	0.576
Fib4 index	4.90 (1.19-17.8)	5.46 (1.45-15.1)	0.837
Diameter of the primary HCC, cm	2.0 (0.7-8.0)	1.4 (0.6-3.4)	0.004
Diameter of the primary HCC, ≤2.0 cm/>2.0 cm	34 (57.6)/25 (42.4)	16 (94.1)/1 (5.9)	0.012
Early phase enhancement of primary HCC, (-)/(+)	6 (10.1)/53 (89.8)	5 (29.4)/12 (70.6)	0.118
Therapy for primary HCC			
Surgery	21 (35.6)	5 (29.4)	0.319
RFA	35 (59.3)	9 (52.9)	
SRT	3 (5.1)	3 (17.6)	
IFN therapy induction during follow up period, (-)/(+)	51 (86.4)/8 (13.6)	16 (94.1)/1 (5.9)	0.662
IFN-free DAA therapy regimen			
DCV/ASV		8 (47.1)	
SOF/LDV		9 (52.9)	
Interval between HCC treatment and IFN-free DAA therapy, years		0.72 (0.31-10.4)	

Categorical data are presented as numbers (percentages) of patients and numerical data as median (range).

CH: chronic hepatitis, DCV/ASV: daclatasvir and asunaprevir combination therapy, HCC: hepatocellular carcinoma, IFN-free DAA: interferon free direct-acting antiviral therapy, LC: liver cirrhosis, RFA: radiofrequency ablation, SOF/LDV: sofosbuvir and ledipasvir combination therapy, SRT: stereotactic radiotherapy

for primary HCC (25-27). In this study, nine patients received IFN-based therapy after curative treatment for primary HCC, and only two patients achieved SVR. Among them, one patient with chronic hepatitis did not develop HCC recurrence after IFN-based therapy. The other patient with liver cirrhosis developed HCC at 6.5 years after curative treatment (4.5 years after the IFN treatment); however, a second instance of recurrence was not observed in the subsequent 2.4 years. In contrast, HCC continued to recur during the follow-up period in patients who did not achieve SVR by IFN-based therapy, except for in one patient who subsequently received IFN-free DAA therapy. No recurrence has been observed in patients who received both IFN-based and IFN-free DAA therapies. On comparing the recurrence rates after curative treatment for primary HCC by the landmark time analysis set at 1 year, no significant difference in the recurrence rate was found between patients treated with IFN-based therapy and those without IFN-based therapy (p=0.319, data not shown).

The analysis of risk factors for HCC recurrence after curative treatment

We analyzed the factors contributing to HCC recurrence using a time-dependent expanded Cox proportional hazards model that considers the time to induction of IFN-free DAA therapy. Multivariate analysis results are shown in Table 2. No significant factors, including a history of IFN-free DAA therapy, were found among the study participants.

In addition, we analyzed the influence of IFN-free DAA therapy on HCC recurrence according to the subgroup analysis using a time-dependent extended Cox proportional hazards model. As shown in Fig. 3, IFN-free DAA therapy was not significantly associated with HCC recurrence after curative treatment in any of the subgroups. However, IFN-free DAA therapy in the presence of CH exhibited a trend towards decreased HCC recurrence rates (hazard ratio=0.142, p=0.372).

Influence of IFN-free DAA therapy on second HCC recurrence after curative treatment for primary HCC

Some previous studies reported that anti-HCV therapy following HCC treatment prevented a second HCC recurrence but not a first recurrence (25, 27-31). We therefore analyzed the period until the second recurrence after treatment for first HCC recurrence in our study population.

The period until the second recurrence after treatment for first HCC recurrence was analyzed using the Kaplan-Meier method in patients followed for more than six months after the treatment for the first recurrence. As shown in Fig. 4A, no significant difference was found between the IFN-free DAA (-) and (+) groups (p=0.068). Similarly, no significant difference was noted in those who had received curative treatment for the first HCC recurrence (p=0.115) (Fig. 4B). However, IFN-free DAA therapy tended to reduce the frequency of a second HCC recurrence.

Discussion

In the present study, we analyzed the effect of IFN-free DAA therapy on HCC recurrence in patients with primary, solitary tumors that had been treated curatively. Although several studies have investigated this issue, to our knowledge, none have limited their analysis to patients with primary, solitary HCC. Our results showed that IFN-free DAA

therapy did not have a significant effect on early-stage HCC recurrence. However, IFN-free DAA therapy tended to reduce the HCC recurrence rate after curative treatment for primary HCC in patients with CH and a second instance of HCC recurrence after treatment for the first HCC recurrence.

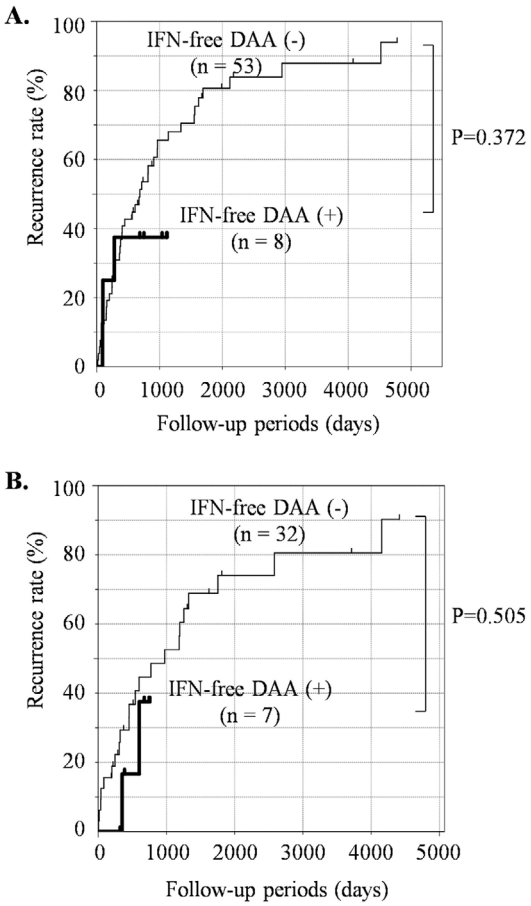

Figure 2. Cumulative HCC recurrence rate after curative treatment for primary and solitary HCC in the presence or absence of IFN-free DAA therapy (landmark time analysis). The HCC recurrence rate in patients who had received IFN-free DAA therapy [IFN-free DAA (+), bold line] or had not [IFN-free DAA (-), thin line] was analyzed by a landmark time analysis set at 1 year (A) and 2 years (B). HCC: hepatocellular carcinoma, IFN-free DAA: interferon-free direct-acting antiviral therapy

The precise effects of IFN-free DAA therapy on HCC recurrence after curative treatment remain unclear. Some retrospective studies have shown that the progression of hepatic fibrosis, tumor-specific factors at the time of curative treatment (such as numbers and size), and number of previous treatments sessions are associated with the HCC recurrence rate after IFN-free DAA therapy (17, 18, 32). In this study, we limited the analysis to patients with primary, solitary HCC cases that had been treated curatively to minimize the influence of tumor-specific factors. Therefore, although the sample size was limited, the analysis focused more on the effect of IFN-free DAA therapy itself on HCC recurrence than other studies.

In addition, in a systematic review and meta-analysis of 24 studies (1,820 subjects) conducted by Saraiya (33), the risk of HCC recurrence after IFN-free DAA therapy was higher in patients with a short interval between curative treatment for HCC and IFN-free DAA therapy initiation than those with a long interval. Therefore, assessing the timing of IFN-free DAA therapy after HCC curative treatment is extremely important when retrospectively analyzing the effect of IFN-free DAA therapy on HCC recurrence (19). To take this factor into account, we used a landmark time analysis and time-dependent extended Cox proportional hazards model with this between-treatment interval as the time-dependent variable and showed that IFN-free DAA therapy did not have a significant influence on early-stage HCC recurrence. If conventional Kaplan-Meier and Cox proportional hazards model analyses had been used, we would have concluded that IFN-free DAA decreased the HCC recurrence (data not shown). This discrepancy may be explained by bias during selection for the induction of IFN-free DAA therapy. In other words, IFN-free DAA therapy is more easily given to patients who have survived and remained recurrence-free for a long time and are at a low risk of recurrence. Attention must be given to conventional Kaplan-Meier and Cox proportional hazards model analyses when retrospectively analyzing the effect of IFN-free DAA therapy on HCC recurrence.

In the stratified analysis, the presence of CH exhibited a

Table 2. Multivariate Analysis of Risk Factors Associated with HCC Recurrence.

Variable		Hazard ratio (95% CI)	p value
Sex	female	0.856 (0.445-1.648)	0.642
Age	year	1.007 (0.962-1.053)	0.774
Background	LC	1.383 (0.660-2.895)	0.390
Diameter of primary HCC	cm	1.126 (0.487-2.605)	0.782
Early phase enhancement of primary HCC	(+)	1.372 (0.456-4.128)	0.574
Therapy for primary HCC	RFA	0.937 (0.394-2.229)	0.884
	SRT	1.379 (0.349-5.594)	0.637
IFN-based therapy	(+)	0.921 (0.332-2.550)	0.874
IFN-free DAA therapy	(+)	0.917 (0.358-2.344)	0.856

CI: confidence interval, HCC: hepatocellular carcinoma, IFN-free DAA: interferon-free direct-acting antiviral therapy, LC: liver cirrhosis, RFA: radiofrequency ablation, SRT: stereotactic radiotherapy

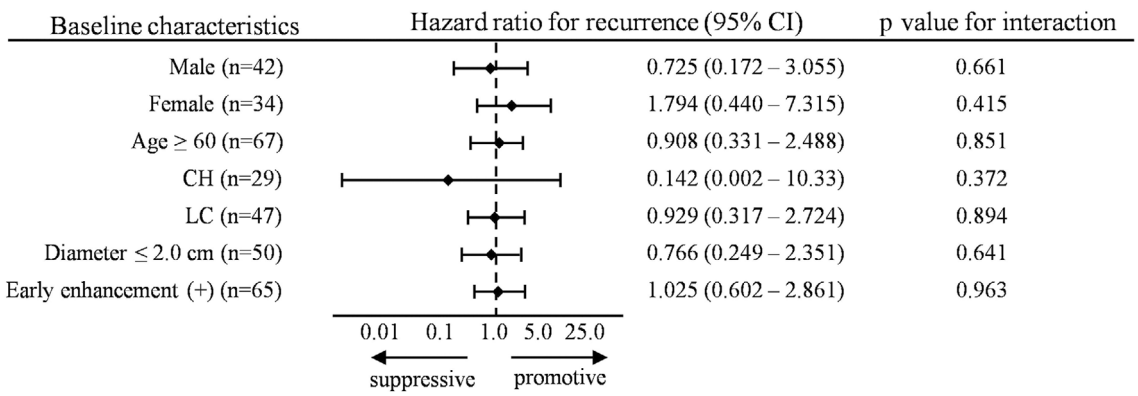

Figure 3. **Subgroup analyses of the influence of IFN-free DAA therapy on recurrence after curative treatment. Subgroup analyses of the hazard ratio of the influence of IFN-free DAA therapy on HCC recurrence by a time-dependent extended Cox proportional hazards model. HCC: hepatocellular carcinoma, IFN-free DAA: interferon-free direct-acting antiviral therapy**

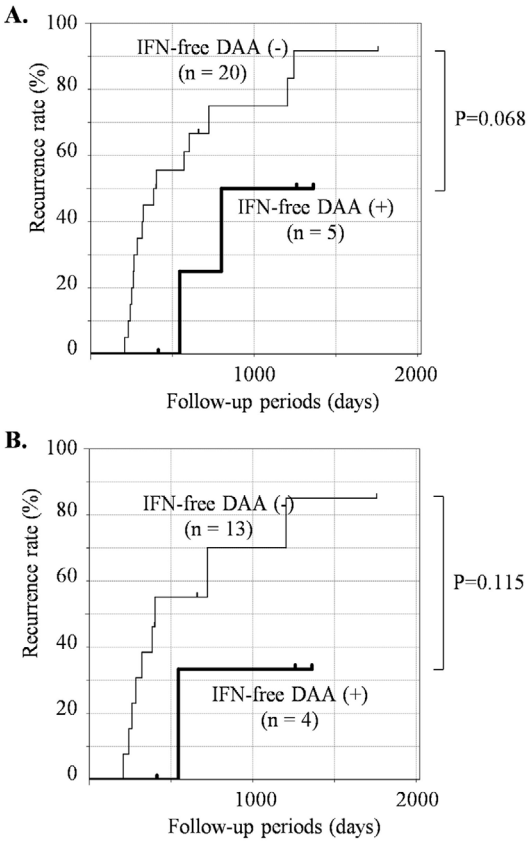

Figure 4. **Cumulative second HCC recurrence rate after treatment for the first HCC recurrence in the presence or absence of IFN-free DAA therapy. The cumulative second HCC recurrence rate after treatment for the first HCC recurrence was analyzed by a Kaplan-Meier analysis (A) in all patients with a first HCC recurrence (n=25) and (B) in the patients who had received curative treatment for the first HCC recurrence (n=17). The patients who received IFN-free DAA therapy [IFN-free DAA (+) group] are represented as a bold line, and those who had not received [IFN-free DAA (-) group] are presented as a thin line. HCC: hepatocellular carcinoma, IFN-free DAA: interferon-free direct-acting antiviral therapy**

trend toward decreased HCC recurrence rates. This may be due to a halt in genetic mutations after IFN-free DAA therapy in the context of CH, in contrast to LC, where such mutations would have already accumulated. In addition, despite the small sample size, the second recurrence rate after treatment for the first HCC recurrence tended to be lower in patients who had been treated with IFN-free DAA therapy after curative treatment for primary HCC than those without IFN-free DAA therapy (Fig. 4). This is similar to the reports that IFN treatment after curative HCC treatment did not affect the first HCC recurrence rate but did have a suppressive effect on the second HCC recurrence rate (25, 27, 28, 30).

However, some limitations associated with this study should be noted. First, IFN-free DAA therapy was initiated based on the attending physicians' discretion. In addition, selection bias cannot be denied for cases receiving IFN-free DAA therapy considered to have a reduced risk of HCC recurrence. Second, as we limited the analysis to patients with primary, solitary tumors, the sample size was relatively small. A prospective study based on large, evenly matched patient groups (in terms of tumor size) is required to definitively determine the effect of IFN-free DAA therapy on HCC recurrence. However, conducting such a study prospectively may face ethical issues due to the efficacy of IFN-free DAA therapy in inhibiting liver function deterioration, leading to an improved HCC prognosis.

Conclusion

IFN-free DAA therapy did not contribute to early-stage HCC recurrence after curative treatment according to a time-dependent extended Cox proportional hazards model analysis. However, a potential promotive effect cannot be definitively excluded, and future studies with larger numbers of patients are required. The time interval between curative treatment for HCC and IFN-free DAA therapy must be taken into account in retrospective analyses, which can be achieved using a landmark time analysis and time-dependent

extended Cox proportional hazards models.

The authors state that they have no Conflict of Interest (COI).

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