Table 2. Cox proportional hazards analysis for recurrence exceeding the Milan criteria (n = 323).

Factors	Univariate analysis	Multivariate analysis	
	p value	p value	(HR, 95% CI)
Age, >65 years	0.644		
Child–Pugh score (B compared with A)	0.098		
AFP, >100 ng/mL	0.0006	0.0059	(1.59, 1.14–2.23)
PIVKA-II, >100 mAU/mL	0.0004	0.211	(1.26, 0.87–1.84)
Tumor size, > 20mm	0.0033	0.012	(1.54, 1.09–2.16)
Tumor number, >2	0.291		
Early recurrence (within 1 year after RFA)	<0.0001	<0.0001	(2.76, 2.05–3.71)

HR = hazard ratio; CI = confidence interval

Table 3. Cox proportional hazards analysis for overall survival (n = 323).

Factors	Univariate analysis	Multivariate analysis
	p value	p value (HR, 95%CI)
Age, >65 years	0.644	
Child–Pugh score (B compared with A)	<0.0001	<0.0001 (2.42, 1.61–3.64)
AFP, >100 ng/mL	<0.0001	0.0003 (2.03, 1.37–3.00)
PIVKA-II, ≥100 mAU/mL	0.136	
Tumor size, > 20mm	0.943	
Tumor number, >2	0.0037	0.056 (1.45, 0.99–2.13)
Early recurrence (within 1 year after RFA)	<0.0001	0.0001 (2.09, 1.43–3.03)

HR = hazard ratio; CI = confidence interval

Figure 1

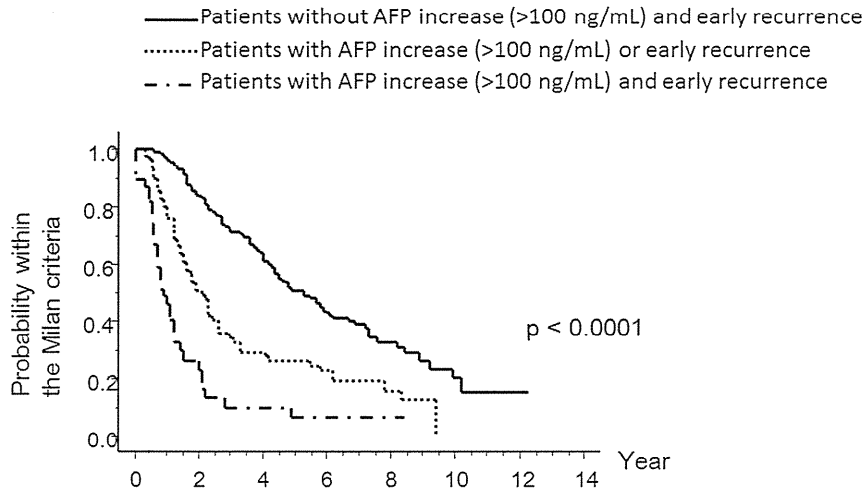

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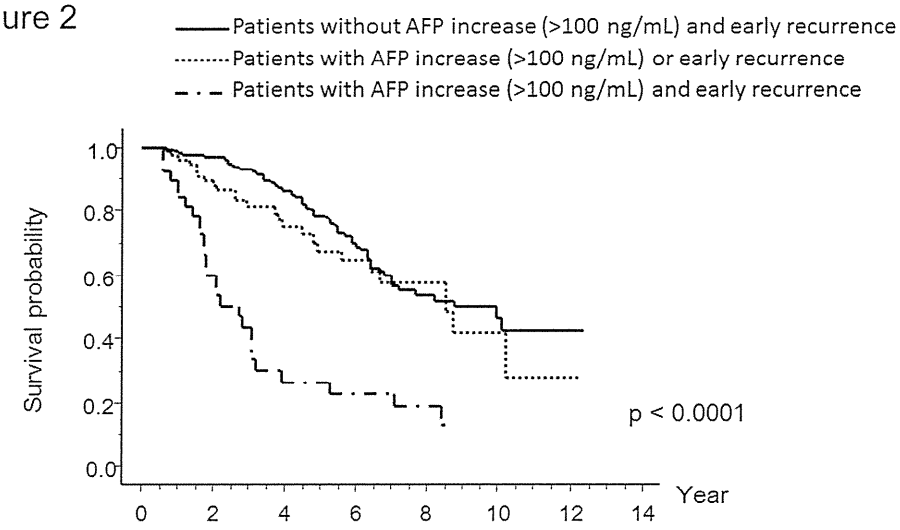
Figure 2



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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Non-invasive prediction of hepatocellular carcinoma development using serum fibrosis marker in chronic hepatitis C patients

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Abstract
Background The FIB-4 index is a simple formula to predict liver fibrosis. This study aimed to evaluate the utility of the FIB-4 index and associated time-course changes as a predictor of hepatocellular carcinoma (HCC) development.
Methods A total of 171 chronic hepatitis C patients who underwent paired liver biopsies and 875 patients who underwent a single liver biopsy (validation group) were investigated during mean follow-up periods of 6.4 and 5.9 years, respectively. All patients had received interferon therapy and had not achieved a sustained virological response. Factors associated with HCC development were analyzed in these patients.
Results HCC developed in 30 patients in the paired biopsy group and 89 patients in the validation group. Univariate analysis demonstrated that the FIB-4 index >3.25 and change in the FIB-4 index per year (ΔFIB-4/year) ≥0.3 were predictive factors for HCC development in both groups. Multivariate analysis in the combined population revealed that these two factors were independent. The hazard ratio (HR) for the FIB-4 index >3.25 was 2.7 ($p < 0.001$) and ΔFIB-4/year ≥0.3 was 1.8 ($p = 0.003$). Patients with a FIB-4 index >3.25 and a ΔFIB-4/year ≥0.3 were defined as high risk, and those with a FIB-4 index ≤3.25 and a ΔFIB-4/year <0.3 were defined as low risk. The HR of HCC development in patients at high risk was 7.3 (95 % confidence interval 4.3–12.5, $p < 0.001$).
Conclusions It was possible to define a group at high risk of developing HCC by intermittently measuring the FIB-4 index and considering time-course changes in this index.

Keywords FIB-4 index · Hepatocellular carcinoma · Chronic hepatitis C · Liver fibrosis · Non-invasive

Introduction

Persistent hepatitis C virus infection induces chronic hepatitis and eventually develops into liver cirrhosis and hepatocellular carcinoma (HCC) [1]. An advanced stage of liver fibrosis in chronic hepatitis C is associated with HCC development and complications such as esophageal variceal bleeding and liver failure [2, 3]. Therefore, accurate evaluation of the stage of liver fibrosis is necessary to predict its progression to liver cirrhosis and HCC development for optimal clinical disease management.

Although the gold standard for evaluating liver fibrosis is liver biopsy [4, 5], it has been reported that this method may be inaccurate because of sampling errors and inter-observer variations [6, 7]. Moreover, because the invasiveness of liver biopsy precludes repeated examinations [8], evaluation of liver fibrosis time-course changes is difficult.

Recently, various non-invasive methods for evaluating liver fibrosis have rapidly improved as alternatives to liver biopsy. Liver fibrosis was reportedly predicted by transient elastography [9, 10], acoustic radiation force impulse imaging [11], and real-time tissue elastography [12]. In

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addition, methods using blood test data, including the aspartate aminotransferase (AST)/alanine aminotransferase (ALT) ratio [13], AST/platelet ratio index [14], the Forns test [15], and the Fibro test [16] have been reported to be useful. These tests have exhibited high accuracy in predicting severe liver fibrosis.

The FIB-4 index is a simple formula used for predicting liver fibrosis based on the standard biochemical values (AST, ALT and platelet count) and age, and is reported to be significantly useful for predicting advanced liver fibrosis [17–19]. Because the FIB-4 index can be repeatedly calculated using age and general biochemistry results, it offers the advantage of easy follow-up of time-course changes with repeated measurements. We have reported that time-course changes in the FIB-4 index correlate with changes in liver fibrosis, and advancement of liver fibrosis can be predicted by changes in the FIB-4 index [20].

Progression of liver fibrosis in chronic hepatitis C is closely associated with the high risk of developing HCC [2, 3]. In addition, we have reported that the risk of developing HCC increases with aging [21]. Because the FIB-4 index correlates with liver fibrosis and considers age, it is possible that it can also be used to predict the risk of developing HCC. In this study we investigated the significance of the FIB-4 index and time-course changes in the FIB-4 index as predictors of HCC development.

Methods

Paired biopsy group

Study subjects comprised 314 chronic hepatitis C patients who underwent liver biopsies twice between 1991 and 2010 at Musashino Red Cross Hospital. The average interval between two biopsies was 4.9 ± 2.9 years. The subject characteristics were detailed previously [20]. All patients were treated by interferon after the first liver biopsy and had non-sustained virological response. They underwent the second biopsy and were treated again by interferon. After excluding 110 patients who achieved a sustained virological response with the second interferon therapy, 171 patients were followed-up ≥1 year and were included in this analysis. Exclusion criteria comprised the follows: (1) co-infection with hepatitis B virus or human immunodeficiency virus, (2) alcohol abuse, (3) the presence of nonalcoholic steatohepatitis, (4) the presence of HCC at entry, (5) interval between paired biopsies <1.5 years, and (6) length of biopsy sample <15 mm. The relationship between HCC development and the FIB-4 index at liver biopsy or change in the FIB-4 index between the two liver biopsies was investigated. To determine the optimal cut-off values of change in the FIB-4 index for prediction of HCC development, patients with HCC development within 10 years were considered. Time zero was set at the date of the second biopsy.

Single liver biopsy group (validation group)

A total of 1,377 patients received interferon therapy after liver biopsy at Musashino Red Cross Hospital between 1991 and 2010 and were followed-up for ≥1 year after treatment. Of those in follow-up, 875 patients who exhibited non-sustained virological response were included in the validation group. Exclusion criteria were the same as those for the paired biopsy group. Because these patients did not undergo a second liver biopsy, change in the FIB-4 index was calculated between the liver biopsy and 1, 2 and 3 years after the end of interferon therapy. The relationship between HCC development and the FIB-4 index at liver biopsy or change in the FIB-4 index was investigated. Time zero was set at the date of liver biopsy.

Ethical approval

Written informed consent was obtained from each patient in the paired biopsy group and in the validation group, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki and was approved by the institutional ethics review committees.

Histological evaluation

Liver biopsy specimens were obtained using 13G needles laparoscopically, or by percutaneous ultrasound-guided liver biopsy using 15G needles. Specimens were fixed, paraffin-embedded, and stained with hematoxylin–eosin and Masson's trichrome. A minimum 15 mm biopsy sample was required for diagnosis. All liver biopsy samples were independently evaluated by two senior pathologists who were blinded to the clinical data. Fibrosis staging was categorized according to the METAVIR score: F0, no fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with few septa; F3, numerous septa without cirrhosis; and F4, cirrhosis [22]. When staging was inconsistent between the two pathologists, an appropriate stage was determined by discussion between the two. Fibrosis progression was defined as a 1 point or more increase in the METAVIR score, and fibrosis non-progression was defined as no change or a 1 point or more decrease in the METAVIR score.

HCC surveillance and diagnosis

Ultrasonography and a blood test including tumor markers were performed every 3–6 months for HCC surveillance. When tumor marker levels showed an abnormal rise and/or

abdominal ultrasonography suggesting a lesion suspicious for HCC, contrast-enhanced computed tomography, magnetic resonance imaging or angiography were performed. HCC was diagnosed for tumors showing vascular enhancement at an early phase with washout at a later phase. Tumor biopsy was used to diagnose tumors with non-typical imaging findings.

Clinical and biological data

The age and gender of the patients were recorded. Serum samples were collected within 1 month prior to the liver biopsy. The following variables were obtained by analyzing the serum samples: AST, ALT, and platelet count. The FIB-4 index was calculated according to the following formula: FIB-4 index = age [years] × AST [IU/L]/(platelets [10^9/L] × ALT [IU/L]$^{1/2}$). Cutoff value of the FIB-4 index was set at 3.25 according to the previously established value for the prediction of advanced fibrosis [17]. Change in the FIB-4 index per year (ΔFIB-4 index/year) in the paired biopsy group was calculated by the following formula: ΔFIB-4 index/year = (the FIB-4 index at the second liver biopsy − the FIB-4 index at the first liver biopsy)/the interval between paired biopsies (years). ΔFIB-4 index/year in the validation group was calculated similarly between the liver biopsy and 1, 2, and 3 years after the end of interferon therapy. Change in AST, ALT, platelets per year (ΔAST/year, ΔALT/year, ΔPlatelets/year) were calculated similarly.

Statistical analysis

Categorical data were compared using the Chi-square and Fisher's exact test. Distributions of continuous variables were analyzed using the Student's *t* test or the Mann–Whitney *U* test. A *p* value of <0.05 was considered statistically significant. The cumulative incidence curve was determined by the Kaplan–Meier method and differences among groups were assessed using a log-rank test. Receiver operating characteristic (ROC) curves were constructed, and the area under the ROC curve (AUROC) was calculated. Optimal cut-off values were selected using Youden's index. Factors associated with HCC risk were determined by the Cox proportional hazard model. Statistical analyses were performed using the Statistical Package for the Social Sciences software version 15.0 (SPSS Inc., Chicago, IL, USA)

Results

Patient characteristics

Table 1 shows the characteristics of patients in the paired biopsy group and validation group. There were no significant differences in the FIB-4 index between the two groups. Mean follow-up periods were 6.4 years in the paired biopsy group and 5.9 years in the validation group, respectively. HCC developed in 30 patients (14 %) in the

Table 1 Patient characteristics

	Paired biopsy group		Validation group	*p* value*	*p* value**
	First biopsy	Second biopsy			
Patients (*n*)	171		875		
Age (SD) (years)	56.1 (8.5)	60.8 (8.1)	58.0 (10.3)	0.02	<0.001
Gender [*n* (%)]					
Female	95 (56)		493 (56)		
Male	76 (44)		382 (44)	0.85	
Fibrosis stage [*n* (%)]					
F0–1	67 (39)	57 (33)	388 (44)		
F2	57 (34)	60 (35)	269 (31)		
F3	43 (25)	43 (25)	186 (21)		
F4	4 (2)	11 (7)	32 (4)	0.41	0.04
AST (SD) (IU/L)	68.3 (38.2)	60.3 (38.7)	62.9 (35.4)	0.08	0.51
ALT (SD) (IU/L)	90.8 (63.2)	68.3 (54.0)	77.9 (52.7)	0.008	0.06
Platelets (SD) (10^9/L)	159 (48)	153 (51)	157 (50)	0.71	0.28
FIB-4 index	2.90 (1.6)	3.38 (1.9)	3.20 (2.1)	0.08	0.28
Interferon response (relapse/no response/ND)		71/100	335/366/174		0.15
HCC development [*n* (%)]		30 (14)	89 (10)		0.01
Follow-up period (SD) (years)		6.4 (2.7)	5.9 (2.8)		0.04

AST aspartate aminotransferase, *ALT* alanine aminotransferase, *ND* not determined

* Comparison between paired biopsy group at first biopsy and validation group

** Comparison between paired biopsy group at second biopsy and validation group

paired biopsy group and 89 patients (10 %) in the validation group during the follow-up.

Prediction of HCC development by a single-point assessment in the paired biopsy group

The incidence of HCC development was compared between patients with F0–2 and F3–4 at the second liver biopsy. The 3-year, 5-year, and 7-year cumulative incidence of HCC was 13.3, 26.6, and 39.4 %, respectively, in patients with F3–4, which was significantly higher than those with F0–2 (1.7, 4.9, and 7.3 %, respectively; $p < 0.001$, Fig. 1a). Similarly, using the FIB-4 index at the second biopsy, the 3-year, 5-year, and 7-year cumulative incidence of HCC after interferon therapy was 1.0, 5.5 and 6.9 %, respectively, in patients with a FIB-4 index ≤ 3.25, whereas it was 11.9, 20.9, and 32.0 %, respectively, in those with a FIB-4 index >3.25 ($p < 0.001$, Fig. 1b).

Prediction of HCC development by time-course changes in FIB-4 index in the paired biopsy group

HCC development was compared with time-course changes in the fibrosis stage from repeated liver biopsies. For this analysis, 4 patients who were diagnosed as having cirrhosis at the first liver biopsy were excluded. The cumulative incidence of HCC was not significantly different between patients with fibrosis progression and those without (Fig. 1c). In contrast, when time-course changes in the FIB-4 index (ΔFIB-4/year) were considered, HCC developed more frequently in patients with large time-course changes in the FIB-4 index. Of 30 patients with HCC development, 28 patients developed HCC within 10 years. The AUROC of the ΔFIB-4 index/year for prediction of HCC development within 10 years was 0.61. Using a cut-off value for a ΔFIB-4/year of 0.3, the sensitivity and specificity for the prediction of HCC

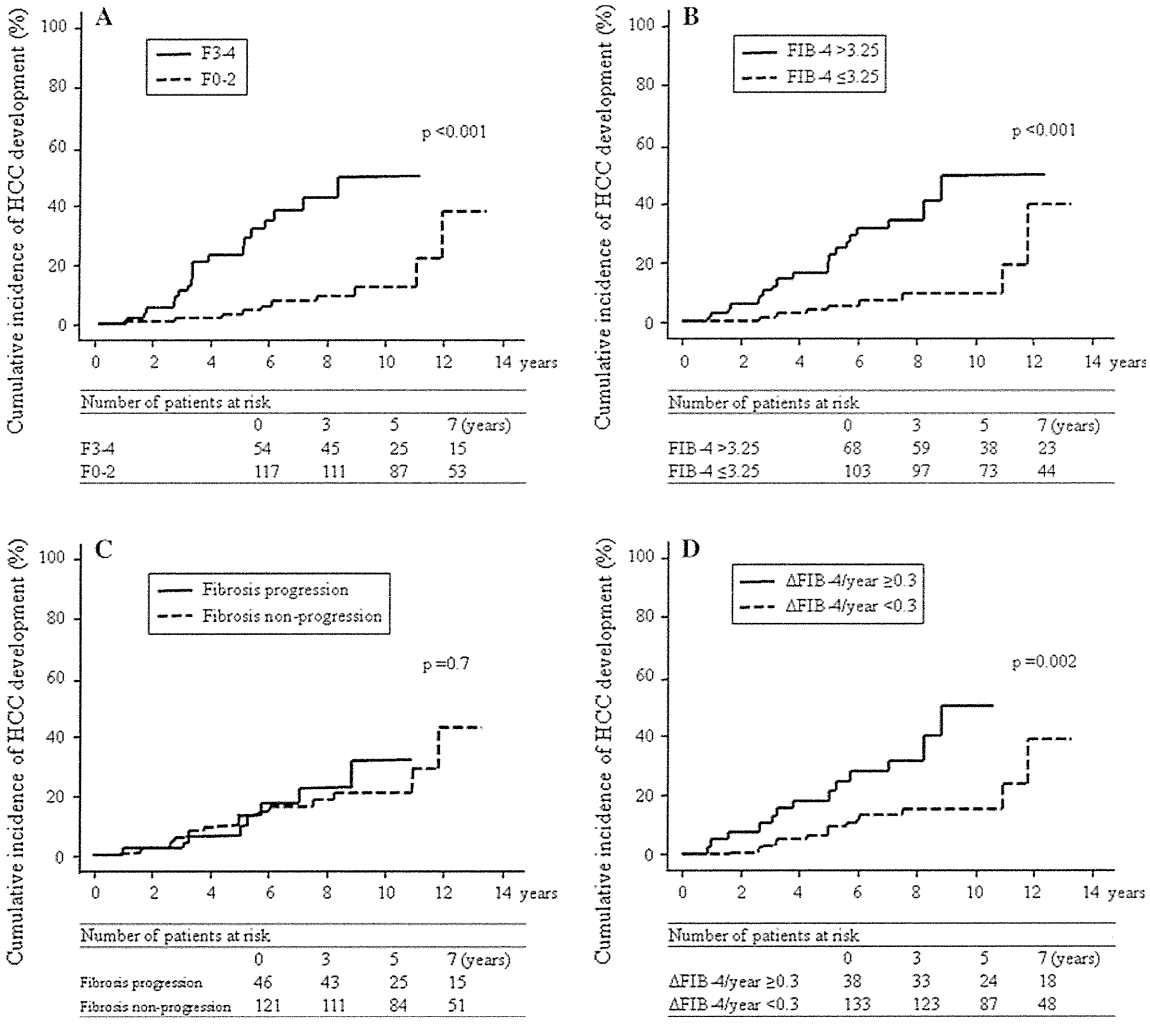


Fig. 1 Cumulative incidence of HCC development in the paired biopsy group. Patients were categorized into two groups according to **a** fibrosis stage, **b** FIB-4 index, **c** time-course change in fibrosis stage, and **d** time-course change in the FIB-4 index (ΔFIB-4/year)

development was 46 and 82 %, and the 3-year, 5-year and 7-year cumulative incidence of HCC was 13.2, 21.9, and 28.4 %, respectively, in patients with a ΔFIB-4/year ≥0.3, whereas it was 3.1, 9.6, and 13.1 % in those with a ΔFIB-4/year <0.3 ($p = 0.002$, Fig. 1d).

Validation by independent patients

The cumulative HCC incidence rate was similarly examined in the validation group using the FIB-4 index at the time of biopsy. In the group with a FIB-4 index >3.25, the 3-year, 5-year, and 7-year cumulative incidences of HCC were 3.9, 11.2, and 22.0 %, respectively, whereas, in the group with a FIB-4 index ≤3.25, the 3-year, 5-year, and 7-year cumulative incidences of HCC were 0.6, 4.0, and 6.4 %, respectively. The rate was significantly higher ($p < 0.001$) in the group with a FIB-4 index >3.25.

Time-course changes in the FIB-4 index and HCC incidence were examined with the cut-off value of a ΔFIB-4/year at 0.3. Cumulative incidence of HCC development was examined using the ΔFIB-4/year calculated by using data at 1 year after the interferon therapy. The 3-year, 5-year, and 7-year cumulative incidences of HCC were 2.8, 15.0, and 20.1 %, respectively, in patients with a ΔFIB-4/year ≥0.3, whereas they were 1.4, 4.3, and 9.8 % in patients with a ΔFIB-4/year <0.3. The cumulative incidence rate was significantly higher ($p = 0.008$) in the group with a ΔFIB-4/year ≥0.3. Similarly, using the ΔFIB-4/year calculated by using data at 2 years after the interferon therapy, the 3-year, 5-year, and 7-year cumulative incidences of HCC were 3.3, 11.1, and 16.9 %, respectively, in patients with a ΔFIB-4/year ≥0.3, whereas they were 1.4, 5.3, and 10.7 % in patients with a ΔFIB-4/year <0.3 ($p = 0.04$). Using the ΔFIB-4/year calculated by using data at 3 years after the interferon therapy, the 3-year, 5-year, and 7-year cumulative incidences of HCC were 4.4, 9.7, and 17.1 %, respectively, in patients with a ΔFIB-4/year ≥0.3, whereas they were 1.4, 5.0 and 10.4 % in patients with a ΔFIB-4/year <0.3 ($p = 0.005$). Because similar results were obtained by the ΔFIB-4/year at 1, 2, and 3 years after the interferon therapy, the ΔFIB-4/year calculated by using data at 1 year after the interferon therapy was used for subsequent analysis.

Factors associated with HCC development

Univariate analysis demonstrated factors that increase the hazard ratio (HR) for the development of HCC (Table 2). In the paired biopsy group for advanced liver fibrosis detected by liver biopsy, a high FIB-4 index level, and a ΔFIB-4/year ≥0.3 were risk factors for HCC development. Compared with patients with a FIB-4 index ≤3.25, the HR of those with a FIB-4 index >3.25 was 4.8 [95 % confidence interval (CI) 2.0–10.7, $p < 0.001$]. In terms of change in fibrosis stage, there was no significant difference between the progression and non-progression groups. In contrast, in terms of change in the FIB-4 index, compared with patients with a ΔFIB-4/year <0.3, the HR of those with a ΔFIB-4/year ≥0.3 was 3.1 (95 % CI 1.3–5.7, $p = 0.002$). Similar results were obtained in the validation group; a FIB-4 index >3.25 and a ΔFIB-4/year ≥0.3 were risk factors for HCC development (Table 2). These two groups of patients were combined and univariate and multivariate analysis were performed (Table 3). Because AST, ALT, platelets and age are contained in the FIB-4 index, these factors were excluded in the multivariate analysis. Multivariate analysis revealed that gender, fibrosis stage, the FIB-4 index and ΔFIB-4/year were independent factors associated with HCC development. The HR of HCC development with a FIB-4 index >3.25 and a ΔFIB-4/year ≥0.3 was 2.7 (95 % CI 1.7–4.2, $p < 0.001$) and 1.8 (95 % CI 1.2–2.6, $p = 0.003$), respectively. ΔAST/year, ΔALT/year, and ΔPlatelets/year were not associated with HCC development.

Evaluation of HCC risk by a combining the FIB-4 index and ΔFIB-4/year in the whole group

Multivariate analysis demonstrated that a high FIB-4 index level by single-point assessment and a time-course increase in the FIB-4 index were independent risk factors for HCC development. Their combined risk was examined in four groups, with the cut-off values of a FIB-4 index at 3.25 and a ΔFIB-4/year at 0.3. Patients with a FIB-4 index ≤3.25 and a ΔFIB-4/year <0.3 were defined as the low risk group. Patients with a FIB-4 index ≤3.25 and a ΔFIB-4/year ≥0.3 were defined as the intermediate risk-1 group. Similarly, patients with a FIB-4 index >3.25 and a ΔFIB-4/year ≥0.3 were defined as the high risk group. Patients with a FIB-4 index >3.25 and a ΔFIB-4/year <0.3 were defined as the intermediate risk-2 group. The 3-year, 5-year, and 7-year cumulative incidence of HCC in patients within the high risk group was 7.6, 21.0, and 30.0 %. Similarly, the 3-year, 5-year, and 7-year cumulative incidence of HCC was 4.1, 9.6, and 21.1 % in patients within the intermediate risk-2 group. It was 0.8, 10.1, and 13.8 % in the patients within the intermediate risk-1 group and 0.6, 2.9, and 4.8 % in patients within the low risk group ($p < 0.001$, Fig. 2). The HR of HCC development in patients at high risk was 7.3 (95 % CI 4.3–12.5, $p < 0.001$, Table 4). Sensitivity of prediction for HCC development by the liver biopsy and the FIB-4 index was 58 and 61 %, respectively. The combined risk classification by the FIB-4 index and the ΔFIB-4/year had higher sensitivity (72 %, Table 5).

Table 2 Factors associated with HCC development in the paired biopsy group and the validation group

Risk factor value	Paired biopsy group		Validation group	
	Hazard ratio (95 % CI)	*p* value	Hazard ratio (95 % CI)	*p* value
Risk factor at baseline[a]				
Age (by every 10 years)	1.3 (0.8–2.1)	0.3	1.9 (1.5–2.5)	<0.001
Gender				
Female	1		1	
Male	1.5 (0.7–3.2)	0.2	1.5 (0.9–2.2)	0.06
Fibrosis stage				
F0/F1/F2	1		1	
F3/F4	5.4 (2.5–11.3)	<0.001	4.7 (3.1–7.1)	<0.001
AST (by every 1× ULN)	1.3 (1.1–1.5)	0.01	1.4 (1.1–1.7)	<0.001
ALT (by every 1× ULN)	1.1 (1.0–1.3)	0.04	1.1 (0.9–1.2)	0.1
Platelets (10^9/L)				
≥150	1		1	
<150	3.0 (1.3–6.5)	0.006	2.6 (1.6–4.5)	<0.001
FIB-4 index				
≤3.25	1		1	
>3.25	4.8 (2.2–10.7)	<0.001	3.8 (2.4–5.8)	<0.001
Change of risk factor				
ΔAST/year (IU/L)				
<0	1		1	
≥0	0.9 (0.4–1.9)	0.8	1.4 (0.8–2.4)	0.2
ΔALT/year (IU/L)				
<0	1		1	
≥0	0.8 (0.4–1.8)	0.6	0.8 (0.4–1.6)	0.6
ΔPlatelets/year (10^9/L)				
>−0.5	1		1	
≤−0.5	2.4 (1.1–5.0)	0.01	0.7 (0.4–1.3)	0.3
ΔFIB-4/year				
<0.3	1		1	
≥0.3	3.1 (1.5–6.6)	0.002	1.8 (1.2–2.9)	0.008
Fibrosis stage change				
Non-progression	1			
Progression	1.2 (0.5–2.7)	0.7		

AST aspartate aminotransferase, *ALT* alanine aminotransferase

[a] Data at the second biopsy was used for the paired biopsy group

Discussion

Recently, non-invasive methods substituting liver biopsy for the diagnoses of liver fibrosis have been developed. It has been elucidated that non-invasive liver fibrosis markers are related to HCC development and mortality [23–25]. In addition, it was reported that after interferon therapy for chronic hepatitis C, some non-invasive liver fibrosis markers correlated with HCC development [26]. However, it remains unclear whether time-course changes in these markers correlate with HCC development and mortality.

Previously, we reported that time-course changes in the FIB-4 index correlated with liver fibrosis progression [20]. Because the FIB-4 index correlates with liver fibrosis, a risk factor for HCC development, and it considers age, another risk factor, it was presumed that the index could be closely correlated with HCC development. In this study, the significance of the FIB-4 index and time-course changes in the FIB-4 index were investigated in relation to HCC development.

The most important finding in this study was that it was possible to predict HCC development by time-course changes in the FIB-4 index. The cumulative HCC incidence rate was lower in patients with a ΔFIB-4/year <0.3 compared with those with a ΔFIB-4/year ≥0.3. It has been reported that a high level of AST and ALT levels correlate with progression of liver fibrosis, and improved levels prevent HCC development [27–29]. It is also known that liver fibrosis progression and the risk of HCC development is increased with a decrease in platelet count [30].

Table 3 Factors associated with HCC development in the combined population

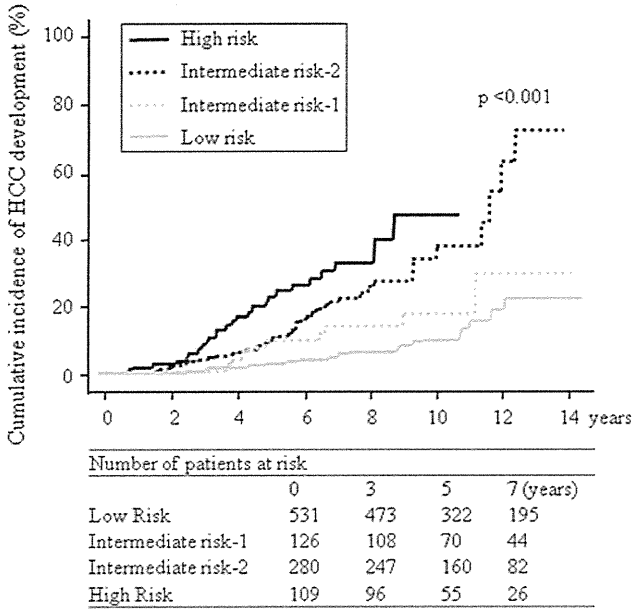
Risk factor value	Univariate		Multivariate	
	Hazard ratio (95 % CI)	*p* value	Hazard ratio (95 % CI)	*p* value
Risk factor at baseline				
Age (by every 10 years)	1.8 (1.4–2.3)	<0.001		
Gender				
Female	1		1	
Male	1.5 (1.0–2.1)	0.03	2.0 (1.4–2.9)	<0.001
Fibrosis stage				
F0/F1/F2	1		1	
F3/F4	4.9 (3.5–7.2)	<0.001	3.0 (2.0–4.6)	<0.001
AST (by every 1× ULN)	1.4 (1.2–1.6)	<0.001		
ALT (by every 1× ULN)	1.1 (1.0–1.2)	0.04		
Platelets (10^9/L)				
≥150	1			
<150	2.7 (1.7–4.1)	<0.001		
FIB-4 index				
≤3.25	1		1	
>3.25	4.0 (2.8–5.9)	<0.001	2.7 (1.7–4.2)	<0.001
Change of risk factor				
ΔAST/year (IU/L)				
<0	1			
≥0	1.3 (0.8–2.1)	0.2		
ΔALT/year (IU/L)				
<0	1			
≥0	0.9 (0.6–1.6)	0.8		
ΔPlatelets/year (10^9/L)				
>−0.5	1			
≤−0.5	1.0 (0.6–1.6)	0.8		
ΔFIB-4/year				
<0.3	1		1	
≥0.3	2.1 (1.4–3.1)	<0.001	1.8 (1.2–2.6)	0.003

AST aspartate aminotransferase, *ALT* alanine aminotransferase

However, time-course changes of AST, ALT, and platelet count were not significantly associated with HCC development in this study. On the other hand, the FIB-4 index, which considers these factors together; had its time-course changes useful for real-time monitoring of disease progression. As the disease advances, the FIB-4 index deteriorates and the risk of HCC development increases.

One advantage of the FIB-4 index is the feasibility of repeated measurements for evaluating disease status. Needless to say, liver biopsy is the gold standard for diagnosis of liver fibrosis and is still important to predict the progression of liver disease. However, there are problems associated with liver biopsies including sampling errors and inter-observer variations [6, 7]. In addition, it is difficult to repeat biopsies, making it challenging to evaluate time-course changes because of the invasiveness of the procedure. In contrast, the FIB-4 index can be calculated using age and general biochemistry results and making it markedly easy to follow up time-course changes. In this study, changes in fibrosis stage between two liver biopsies failed to stratify HCC development. These results suggest that the FIB-4 index, rather than the liver biopsy, was more useful for real-time monitoring of disease advancement.

Correlations of non-invasive liver fibrosis markers including the FIB-4 index with HCC incidence risk have been reported previously [23–26, 31]. A similar result was shown in this study using a single-point assessment of the FIB-4 index. Since the FIB-4 index correlates with liver fibrosis, a high FIB-4 index indicates a high risk for HCC development similar to other liver fibrosis markers. Furthermore, an important fact in this study was that combining the FIB-4 index and time-course changes in the FIB-4 index could stratify patients with high risk of HCC development. A high FIB-4 index level by single-point assessment and a time-course increase in the FIB-4 index were independent risk factors for HCC development. Patients with a low baseline FIB-4 index and a time-course



Number of patients at risk	0	3	5	7 (years)
Low Risk	531	473	322	195
Intermediate risk-1	126	108	70	44
Intermediate risk-2	280	247	160	82
High Risk	109	96	55	26

Fig. 2 Cumulative incidence of HCC development in the combined population. Patients were categorized into four groups using the FIB-4 index and time-course change in the FIB-4 index (ΔFIB-4/year). Low risk: FIB-4 index ≤3.25 and ΔFIB-4/year <0.3, intermediate risk-1: FIB-4 index ≤3.25 and ΔFIB-4/year ≥0.3, intermediate risk-2: FIB-4 index >3.25 and ΔFIB-4/year <0.3, high risk: FIB-4 index >3.25 and ΔFIB-4/year ≥0.3

Table 4 Evaluation of HCC risk by combining the FIB-4 and the ΔFIB-4/year

	Number of patients	Hazard ratio (95 % CI)	*p* value
Low risk	531	1	
Intermediate risk-1	126	2.1 (1.1–4.0)	0.03
Intermediate risk-2	280	4.1 (2.6–6.5)	<0.001
High risk	109	7.3 (4.3–12.5)	<0.001

Low risk: patients with a FIB-4 index ≤3.25 and a ΔFIB-4/year <0.3

Intermediate risk-1: patients with a FIB-4 index ≤3.25 and a ΔFIB-4/year ≥0.3

Intermediate risk-2: patients with a FIB-4 index >3.25 and a ΔFIB-4/year <0.3

High risk: patients with a FIB-4 index >3.25 and a ΔFIB-4/year ≥0.3

improvement in the FIB-4 index had a low risk of HCC development, whereas those with a high baseline FIB-4 index and worsening of the FIB-4 index had a markedly high risk for HCC development. In addition to the utility of predicting liver fibrosis and HCC development by single-point assessment, the combination with real-time monitoring enables stratification of a group with a high risk of HCC development, which is a great advantage of the FIB-4 index over a liver biopsy.

With regard to diagnosis capabilities for liver fibrosis, it has been reported that other non-invasive liver fibrosis markers have higher diagnostic capabilities than the FIB-4 index [32, 33]. However, the FIB-4 index has several advantages. Although, it has been reported that transient elastography has high diagnostic capabilities when it comes to liver fibrosis, measurements are sometimes impossible in patients with severe obesity [34]. Reproducibility of transient elastography was reportedly reduced in patients with steatosis, increased body mass index, and lower degrees of liver fibrosis [35]. Moreover, these modalities for measurement of elasticity of the liver using ultrasonography are not widely available, especially in countries where resources are limited. In contrast, the FIB-4 index can be determined by a general blood test, and it can be measured in almost all patients. The parameters required for calculation are only age, AST, ALT, and platelet count, which are measured during the routine examination of patients with liver disease. Therefore, additional blood collection is unnecessary, and the index can be calculated at no extra cost.

Table 5 Sensitivity of prediction for HCC development

	Patients with HCC development	Patients without HCC development
F0–2	50	724
F3–4	69	203
	Sensitivity: 58 %	Specificity: 78 %
FIB-4 index ≤3.25	42	615
FIB-4 index >3.25	77	312
	Sensitivity: 61 %	Specificity: 66 %
Low risk	29	502
Other risk	90	425
	Sensitivity: 72 %	Specificity: 54 %

Other risk: high risk, intermediate risk-2, and intermediate risk-1

In conclusion, it was possible to define a group with a high risk of HCC development by calculating the FIB-4 index and considering time-course changes in the FIB-4 index. Because measurement of the FIB-4 index is simple and easy to repeat, it is useful for non-invasive, real-time monitoring of HCC development.

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Conflict of interest The authors declare that they have no conflict of interest.

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INTERNAL MEDICINE

□ ORIGINAL ARTICLE □

Clinical Characteristics of Patients who Developed Hepatocellular Carcinoma after Hepatitis C Virus Eradication with Interferon Therapy: Current Status in Japan

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Abstract

Objective We attempted to elucidate the clinical features of chronic hepatitis C patients who develop hepatocellular carcinoma (HCC) after achieving a sustained viral response (SVR) to interferon (IFN) therapy.

Methods The clinical features of 130 patients at 19 hospitals who developed HCC after obtaining an SVR were retrospectively reviewed.

Results Overall, 107 (82%) of the 130 patients were men, with 92 (71%) being ≥60 years of age and 76, 38 and 16 developing HCC within 5, 5-10 and 10-16.9 years after IFN therapy, respectively. Before receiving IFN therapy, 92 (71%) patients had cirrhosis and/or a low platelet count ($<15\times10^4$ cells/μL). Lower albumin (<3.9 g/dL) and higher alpha fetoprotein (AFP) (≥10 ng/mL) levels were identified in a multivariate analysis to be independent variables of the development of HCC within five years after IFN therapy. Among 4,542 SVR patients, HCC occurred in 109 (2.4%) during a 5.5-year follow-up period, thus resulting in an occurrence rate of 4.6% for men and 0.6% for women.

Conclusion SVR patients with lower albumin or higher AFP levels require careful assessments to prevent early HCC development after IFN therapy. HCC occurrence within >10 years of IFN therapy is not uncommon, and the risk factors remain uncertain, thus suggesting that all SVR patients should undergo long-term follow-up examinations for HCC development.

Key words: hepatocellular carcinoma, hepatitis C virus, interferon, sustained viral response

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Introduction

Chronic hepatitis C virus (HCV) infection is a common cause of chronic liver disease and hepatocellular carcinoma (HCC) worldwide (1). In Japan, HCC is the third leading cause of cancer-related death, with more than 30,000 deaths occurring in 2010 (2), approximately 70% of which were associated with HCV infection (3). In chronic HCV patients, the risk of HCC has been shown to increase in association with the degree of liver fibrosis, with an annualized rate of HCC developing within a 2-8% cirrhotic background (4-7). It has been reported that interferon (IFN) therapy not only improves hepatic inflammation and fibrosis, but also reduces the incidence of HCC, particularly in patients who achieve a sustained viral response (SVR) (4, 8-10). However, HCC sometimes develops in patients with an SVR, and cases of patients who have developed HCC more than 10 years after completing IFN therapy have been reported (11, 12). The risk factors for HCC in chronic HCV patients are a male sex, older age (13, 14) and the presence of advanced fibrosis (4, 5). Several cohort studies have indicated that these risk factors are also associated with the development of HCC among SVR patients (15-21). However, the number of patients who developed HCC after achieving an SVR in each of these previous studies was limited, and the clinical features of such patients have not been fully clarified. We investigated the clinical features of a large number of patients who developed HCC after undergoing HCV eradication using IFN therapy at a workshop held during the 47th annual meeting of the Liver Cancer Study Group of Japan in July 2011 (President: F. Ichida) on the clinical features of patients who develop HCC after obtaining an SVR in order to compile a more detailed background of such patients.

Materials and Methods

Patients

This study was conducted from February to June 2011 at institutes with which the internal medicine secretaries of the Liver Cancer Study Group of Japan are affiliated. We first investigated the number of SVR patients treated at each institute and, of these, investigated the number of patients who developed HCC after obtaining an SVR. Responses to the initial inquiry were obtained from 25 of 46 hospitals, 22 of which provided the numbers of both SVR and HCC patients. Second, we assessed the characteristics of the patients who developed HCC, and the maximum, minimum and mean follow-up periods of all SVR patients with or without HCC were investigated. Responses were obtained from all 22 hospitals. The characteristics of 144 patients at 22 hospitals were initially reviewed retrospectively; however, three patients who developed HCC before the completion of IFN therapy and 11 patients whose clinical data prior to IFN therapy were unavailable were excluded. Patients positive for the hepatitis B surface antigen were ineligible for this study. Ultimately, 130 patients treated at 19 hospitals were enrolled. Among these patients, 109 were treated at 13 hospitals that also provided follow-up data of SVR patients with or without HCC. Liver cirrhosis and diabetes mellitus were diagnosed based on the clinical data that we had collected. Obesity was defined as a body mass index of ≥25.0 kg/m^2 (22). The SVR patients (4,542) with or without HCC followed up at 13 hospitals at 0.5-21.9 years were analyzed to determine the disease incidence rates.

Statistical analyses

All statistical analyses were performed using the SPSS software package (ver17.0 for Windows; SPSS, Chicago, IL, USA). Clinical differences were evaluated using the chi-square test, and a multivariate analysis was conducted with an ordinal logistic regression analysis using the forced entry method. A p value of <0.05 was considered to be statistically significant.

Results

Patient characteristics at the time of hepatocellular carcinoma occurrence

A total of 107 (82%) of the 130 patients were men; 72 (55%) patients developed HCC in their sixties and 34 (26%) developed HCC in their fifties. Ninety-seven patients (75%) had a solitary tumor at the time of HCC occurrence, and 117 (90%) had one or two tumors. The maximum tumor size was within 3 cm in 102 (78%) patients, and 110 (85%) patients underwent radical treatment.

Period between the completion of IFN therapy and the occurrence of hepatocellular carcinoma

Seventy-six (58%) patients developed HCC within five years of completing IFN therapy and 26 patients developed HCC within one year of completing IFN therapy (Figure). Although the number of patients who developed HCC after >5 years of IFN therapy completion gradually decreased over time (38 patients: >5 and ≤10 years, 16 patients: >10 years), four patients developed HCC 15 years after completing IFN therapy.

Patient characteristics at the start of IFN therapy

Sixteen patients had undergone IFN therapy before 50 years of age and 114 (88%) had undergone IFN therapy after 50 years of age (Table 1). Obesity, alcohol intake (more than 80 g/day for five years), diabetes mellitus and cirrhosis were observed in 34 (26%), 38 (29%), 26 (20%) and 46 (35%) patients, respectively. The platelet count was $<15\times10^4$ cells/μL in 88 (68%) patients, the serum albumin level was <3.9 g/dL in 53 (41%) patients and the alpha fetoprotein (AFP) level was ≥10 ng/mL in 60 (46%) patients. Ninety-two patients (71%) had either cirrhosis or a low platelet count ($<15\times10^4$ cells/μL), and 38 had neither of these fac-

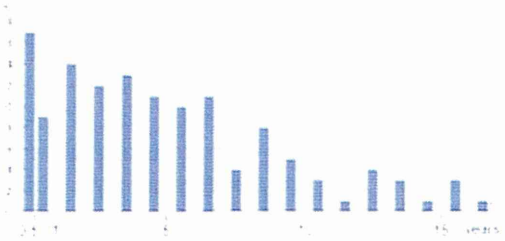

Figure. Patient distribution according to the period between the completion of interferon therapy and the occurrence of hepatocellular carcinoma.

Table 1. Patient Characteristics at the Start of IFN Therapy

	No. of patients
Age (yr)	
38-49	16
50-59	50
60-69	55
≥70	9
BMI (kg/m^2,) ≥25/<25	34/86
Alcohol intake (g/day), ≥80/<80	38/90
Diabetes mellitus, present/absent	26/104
Cirrhosis, present/absent	46/83
Platelet count (× 10^4 per μL), <10 /10- <15 /15- <20 /≥20	26/62/30/9
AST (IU/L), ≥100/<100	43/86
ALT (IU/L), ≥100/<100	54/75
AST/ALT, >1/≤1*	37/72
GGT (IU/L), ≥100/51-99/≤50	41/41/45
Albumin (g/dL), ≤3.5/3.6-3.8/≥3.9	26/27/74
AFP (ng/mL), ≥20/19-10/<10	37/23/49

* Cases with AST>39 IU/L

BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, AFP: alpha fetoprotein

Table 2. Univariate Analysis of Risk Factors Associated with the Development of HCC within Three Periods after Eradication of HCV with IFN

Period since completion of IFN therapy	≤5 years	6-10 years	>10 years	p value
Sex, male/female	58/18	34/4	15/1	0.098
Age at IFN treatment (years), ≥60/<60	45/31	13/25	6/10	0.025
BMI (kg/m^2), ≥25/<25	18/53	11/27	4/12	0.830
Alcohol intake (g/day), ≥80/<80	21/54	13/25	4/12	0.725
Diabetes mellitus, present/absent	14 /62	11/27	1/15	0.142
Cirrhosis, present/absent	29/47	11/26	6/10	0.671
Platelet count (× 10^4 per μL), <15 /≥15	53/21	24/13	11/5	0.767
AST (IU/L), ≥100/<100	23/52	15/23	5/11	0.633
ALT(IU/L), ≥100/<100	28/47	18/20	8/8	0.463
GGT(IU/L), ≥100/<100	24/49	14/24	3/13	0.425
Albumin (g/dL), <3.9/ ≥3.9	38/35	12/26	3/13	0.016
AFP (ng/mL), ≥10/<10	43/20	10/21	7/8	0.003

BMI: body mass index, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, AFP: alpha fetoprotein

tors. No significant differences in obesity, diabetes mellitus or alcohol intake were observed between the patients with and those without either of these background factors.

Risk factors for the development of HCC within five years after IFN therapy

Because 58% of the patients developed HCC within five years of IFN therapy completion and the median period from IFN therapy completion to HCC occurrence was 4.2 years, we compared the background characteristics among the patients who developed HCC within 5, 5-10 and >10 years of IFN therapy completion.

A univariate analysis identified an advanced age (≥60 years) at IFN treatment and lower albumin (<3.9 g/dL) and higher AFP (≥10 ng/mL) levels as significant risk factors associated with HCC development within five years of IFN therapy completion (Table 2). A multivariate analysis of the predictive value of each variable for HCC development within five years identified lower albumin [<3.9 g/dL; odds ratio (OR), 2.604] and higher AFP (≥10 ng/mL; OR, 2.809) levels as being significant (Table 3). No significant factors for HCC development after 10 years of IFN therapy completion were identified.

Patients who started IFN therapy before 50 years of age

Of the 16 patients who received IFN therapy before 50 years of age, all were men (Table 4) and 12 had cirrhosis and/or a low platelet count, while three of the remaining four patients had a background of alcohol intake. Patients with obesity and/or diabetes mellitus were included in these 15 cases, and only one patient had no background of cirrhosis, a low platelet count, alcohol intake, obesity or diabetes mellitus.

Table 3. Multivariate Analysis of Risk Factors Associated with the Development of HCC within 5 Years after HCV Eradication

variable	Odds ratio (95% confidence interval)	p value
Age ≥60 years at IFN treatment	2.147 (0.957-4.816)	0.064
Male sex	0.663 (0.189-2.323)	0.521
Diabetes mellitus	1.303 (0.471-3.604)	0.610
Albumin <3.9 g/dL	2.604 (1.108-6.117)	0.028
AFP ≥10ng/mL	2.809 (1.221-6.456)	0.015

AFP: alpha fetoprotein

Table 4. Patient Characteristics at the Start of IFN Therapy among Patients who Started IFN Treatment before 50 Years of Age

	No.
Male/female	16/0
Age at start of IFN therapy, in years (median)	38–47 (46)
Interval between end of IFN therapy and HCC occurrence, in years (median)	0.1–13 (6.5)
Platelet count, $<15 \times 10^4$ per μL	11
Cirrhosis, present	6
Alcohol intake, ≥80 g/day	9
Diabetes mellitus, present	4
BMI, ≥25.0 kg/m^2	5
None of the above risk factors	1

BMI: body mass index

Table 5. Occurrence of HCC in SVR Patients

	SVR patients			Age in years at last IFN treatment		Follow-up period after IFN treatment (years)		HCC development		
Hospital	Total	Male	Female		Mean		Mean	Total	Male	Female
A	1,193	429	764	15-78	51.0	0.5-18.1	4.5	23	21	2
B	681	392	289	16-77	51.8	0.2-16.9	7.5	17	15	2
C	525	224	301	18-76	50.4	2.0-21.6	9.5	17	14	3
D	487	290	197	15-76	48.5	0.2-21.9	6.0	3	3	0
E	404	227	177	9-85	53.0	0.5-18.0	3.3	9	7	2
F	250	149	101	17-76	50.4	0.5-19.1	5.7	2	2	0
G	193	121	72	17-80	52.7	0.5-15.3	3.7	11	11	0
H	188	97	91	20-75	53.4	0.1-6.9	2.7	4	3	1
I	163	109	54	20-78	54.3	0.2-18.0	3.0	4	3	1
J	155	111	44	18-72	48.0	0.5-14.2	5.5	7	6	1
K	155	85	70	19-79	55.1	0.7-5.8	2.8	2	2	0
L	118	77	41	26-75	49.9	1.0-20.0	5.7	8	6	2
M	30	14	16	37-72	57.5	0.5-6.9	3.3	2	2	0
Total	4,542	2,325	2,217				5.5	109	95	14

Incidence of hepatocellular carcinoma among patients with a sustained response

Between 30 and 1,193 SVR patients were followed up at each of the 13 hospitals. The mean follow-up period was 2.7-9.5 years (Table 5). In 10 of the 13 hospitals, the mean patient age at the time of the last IFN treatment was in the 50s. Among the 4,542 SVR patients, 109 (2.4%) developed HCC within 5.5 years of the last IFN treatment. The HCC occurrence rates for the male and female SVR patients were 4.6% and 0.6%, respectively.

Discussion

Several studies have discussed the risk factors for hepatocarcinogenesis following HCV eradication using IFN therapy (19-25). In these studies, the risk factors for HCC development were investigated in SVR patients. In the present

study, we reviewed the clinical features of patients who developed HCC after undergoing HCV eradication. Although the risk factors for hepatocarcinogenesis were not statistically analyzed due to the lack of data for non-HCC patients with an SVR, the characteristics of a relatively large number of patients may provide general information regarding the clinical features and present state of patients who develop HCC after obtaining an SVR in Japan.

At the time of HCC diagnosis, 117 patients had one or two tumors, and the tumor size was ≤3 cm in 102 patients. A total of 110 patients received curative therapy, suggesting that SVR patients are receiving appropriate follow-up care (23) in most of the hospitals that participated in this study.

A majority of the patients were men, received IFN treatment at an older age and had an advanced stage of liver fibrosis, as previously reported (15-21). A considerable number of patients had a background of alcohol intake, obesity and diabetes mellitus prior to IFN treatment, irrespective of the stage of liver fibrosis. These background characteristics have synergistic effects on hepatocarcinogenesis in chronic HCV patients (24-27). Some studies have suggested that alcohol intake is also a risk factor for HCC development among SVR patients (16, 17). Arase et al. (28) recently reported that both alcohol intake and diabetes increase the risk of HCC development in SVR patients without cirrhosis and non-SVR patients with cirrhosis, consistent with the findings of this study. To clarify the associations between these factors, including obesity, prospective cohort studies involving a large number of patients and data on the clinical features of patients who have undergone HCV eradication and individuals with a relatively low volume of alcohol intake, especially female patients, are needed.

An advanced age at HCV eradication is considered to be a risk factor for HCC development in SVR patients (15-21, 29), and most patients in this study were >50 years when they received IFN therapy. Although 16 patients received IFN therapy before 50 years of age, 12 had advanced fibrosis with cirrhosis or a lower platelet count, and three patients were heavy alcohol drinkers before IFN treatment. The number of heavy alcohol drinkers in this group was higher than that of male patients >50 years old (56.3% versus 29.7%, p=0.038), which suggests that alcohol intake may influence hepatocarcinogenesis and the progression of liver fibrosis in this group.

Nagaoki et al. (30) reported an older age at HCV eradication and heavy alcohol intake as risk factors for HCC development within five years of HCV eradication. In this study, an older age at IFN treatment initiation tended to be associated with an increased risk of HCC, and the development of HCC within five years was significantly associated with lower albumin and higher AFP levels at IFN treatment initiation. Kurosaki et al. (31) reported lower albumin levels as one risk factor for HCC development within five years among non-SVR patients without cirrhosis. However, it is uncertain as to why lower albumin levels are correlated with the occurrence of HCC. Regarding the AFP level, Asahina et al. (32) recently reported that the posttreatment AFP level (≥6 ng/mL) is correlated with the development of HCC among IFN-treated patients; however, this is not the case with the pretreatment AFP level. This suggests that patients with a posttreatment AFP level of ≥6 ng/mL have a substantial risk of developing HCC and that, of these patients, those with a pretreatment AFP level of >10 ng/mL have a risk for early HCC development. Therefore, patients with these characteristics should be closely followed up during and after IFN therapy.

In conclusion, our investigation revealed that most patients who develop HCC after obtaining an SVR are men with advanced fibrosis and that lower albumin and higher AFP levels before IFN treatment may increase the risk for HCC development within five years of HCV eradication. In contrast, the risk factors for HCC development after 10 years of IFN treatment are uncertain, and such patients are not rare; therefore, all patients with chronic HCV should receive long-term follow-up care to monitor for the possible development of HCC following HCV eradication.

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

Ethical Considerations

This study was approved by the Medical Ethics Committee of the St. Marianna University School of Medicine. The investigation conformed to the principles outlined in the Declaration of Helsinki.

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α-Fetoprotein Levels After Interferon Therapy and Risk of Hepatocarcinogenesis in Chronic Hepatitis C

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The effects of interferon (IFN) treatment and the post-IFN treatment α-fetoprotein (AFP) levels on risk of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C (CHC) are unknown. To determine the relationship between AFP and alanine transaminase (ALT) levels and HCC risk, a cohort consisting of 1,818 patients histologically proven to have CHC treated with IFN were studied. Cumulative incidence and HCC risk were analyzed over a mean follow-up period of 6.1 years using the Kaplan-Meier method and Cox proportional hazard analysis. HCC developed in 179 study subjects. According to multivariate analysis, older age, male gender, advanced fibrosis, severe steatosis, lower serum albumin levels, nonsustained virological response (non-SVR), and higher post-IFN treatment ALT or AFP levels were identified as independent factors significantly associated with HCC development. Cutoff values for ALT and AFP for prediction of future HCC were determined as 40 IU/L and 6.0 ng/mL, respectively, and negative predictive values of these cutoffs were high at 0.960 in each value. The cumulative incidence of HCC was significantly lower in patients whose post-IFN treatment ALT and AFP levels were suppressed to less than the cutoff values even in non-SVR patients. This suppressive effect was also found in patients whose post-IFN treatment ALT and AFP levels were reduced to less than the cutoff values despite abnormal pretreatment levels. *Conclusion*: Post-IFN treatment ALT and AFP levels are significantly associated with hepatocarcinogenesis. Measurement of these values is useful for predicting future HCC risk after IFN treatment. Suppression of these values after IFN therapy reduces HCC risk even in patients without HCV eradication. (HEPATOLOGY 2013;58:1253-1262)

Hepatocellular carcinoma (HCC), one of the most frequent primary liver cancers,[1,2] is the third most common cause of cancer mortality worldwide.[3] Hepatitis C virus (HCV) infection is a common cause of chronic hepatitis, which progresses to HCC in many patients.[4] In the last two decades, interferon (IFN) therapy has been used to treat chronic hepatitis C (CHC) with the goal of altering the natural history of this disease. Although HCV eradication with IFN therapy for CHC has been shown to prevent HCC,[5-9] HCC sometimes develops even after achieving viral eradication.[5] Because the number of sustained virological responders (SVRs) is increasing along with recent advances in the development of effective anti-HCV therapy, it is very important to determine factors responsible for HCC

Abbreviations: AFP, α-fetoprotein; ALT, alanine aminotransferase; APRI, aspartate aminotransferase to platelet ratio index; CHC, chronic hepatitis C; CT computed tomography; γ-GTP, gamma-glutamyl transpeptidase; HCC, hepatocellular carcinoma; HCV, Hepatitis C virus; IFN, interferon; MRI, magnetic resonance imaging; PEG-, pegylated; RBV, ribavirin; ROC, receiver operator characteristic; SVR, sustained virological response.

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