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ORIGINAL RESEARCH

Repeated Measurement of FIB-4 to Predict Long-Term Risk of HCC Development Up to 10 Years After SVR

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Video Abstract

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Purpose: It is unclear whether and how the long-term risk of hepatocellular carcinoma (HCC) will change in hepatitis C virus (HCV) infected patients who have reached sustained virologic response (SVR) with direct-acting antivirals (DAA). In this study, we assessed the long-term risk of HCC up to 10 years after SVR using fibrosis 4 score (FIB-4) and its dynamic changes.

Patients and Methods: A total of 701 DAA-treated patients who achieved SVR between January 2012 to October 2020 were enrolled in the study. The FIB-4 score of each patient was measured at the date of SVR and each follow-up visit annually. Patients were followed until December 31, 2021, with the longest follow-up time being 9.82 years.

Results: Following SVR, 27 cases of HCC were observed. The annual incidence rate of HCC remained stable with no obvious downward trend. Patients with a FIB-4 >3.25 at baseline or anytime during follow-up were at a higher risk of developing HCC than those whose FIB-4 remained below 3.25. Patients with cirrhosis and patients with no cirrhosis but a FIB-4 >3.25 were at higher risk of developing HCC than patients with no cirrhosis and a FIB-4 ≦3.25.

Conclusion: FIB-4 >3.25 measured at SVR or any time post-SVR was associated with HCC risks. The repeated measurement of FIB-4 revealed a better predictive ability of HCC risks than the simple measurement of FIB-4 at baseline. The additional stratification of patients by combining FIB-4 and cirrhosis leads to more accurately identifying high-risk patients. Surveillance of HCC is recommended for virologically cured patients with a FIB-4 >3.25 at SVR or anytime afterward and patients diagnosed with cirrhosis.

Keywords: chronic hepatitis C, sustained virologic response, direct-acting antivirals, serum biomarker, hepatocellular carcinoma

Plain Language Summary

In this study, we attempted to assess the long-term risk of hepatocellular carcinoma using the fibrosis 4 score (FIB-4) and its dynamic changes after the eradication of the hepatitis C virus. Our findings show that patients with a FIB-4 >3.25 at baseline or anytime during follow-up were at a higher risk of developing HCC. Patients with cirrhosis and patients with no cirrhosis but a FIB-4 >3.25 were at higher risk of developing HCC as well.

Introduction

Hepatitis C virus (HCV) infection has been a severe public health problem for more than 30 years, with an estimated 71 million chronically infected worldwide.[1] Treatments with either interferon (IFN) or with direct-acting antivirals

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(DAA) offer remarkable rates of HCV virus eradication,[2,3] especially with DAA. The new DAA-based regimen has led to a sustained virologic response (SVR) rate of over 90% in patients with HCV infection.[4] With the widespread use of DAA treatment, it is foreseeable that most HCV patients will achieve SVR after their antiviral treatments.

One of the most severe outcomes of HCV infection is the development of hepatocellular carcinoma (HCC). HCC, the most common primary liver malignancy, resulted in approximately 830,000 deaths in 2020 alone.[5] Several novel medical options for HCC treatment have emerged in recent years, including immune checkpoint inhibitors (ICIs),[6,7] regorafenib (REG),[8] and metronomic capecitabine.[9] Despite these new treatment options, HCC continues to be a major threat to the survival of HCV patients.

Multiple studies have shown mitigation of liver fibrosis[10,11] and reduced risk of HCC[12,13] after patients reached SVR with DAA. However, the residual risk of HCC persists years after HCV eradication.[14–16] Therefore, surveillance for HCC is still needed even after patients achieve SVR. Several baseline characteristics, including older age,[15] male gender,[17] presence of cirrhosis,[15,16] excessive alcohol use,[18,19] and diabetes[19] were reported to be related to the risk of HCC development and can be used to narrow down patients in need of surveillance.

However, the majority only take into account the short-term risk of HCC. It is not entirely clear whether the risk of HCC will change over time in virologically cured patients. Fibrosis 4 (FIB-4) score is a non-invasive serum biomarker initially designed for the evaluation of fibrosis.[20,21] Composed of age, platelet counts, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) levels, the FIB-4 score is easy to measure and calculate. In addition, it has been reported to be associated with the degree of liver fibrosis and the risk of HCC.[22–24] With the repeated measurement of FIB-4, it is possible to observe HCC risk changes over time and thus identify patients needing surveillance at any time after SVR.

The current study aimed to examine the association between FIB-4 score and its dynamic changes with the risk of HCC in virologically cured patients with no cirrhosis and compensated cirrhosis. We also determined whether the repeated measurement of FIB-4 could provide a good prediction of HCC and identified high-risk patients who require surveillance post-SVR.

Material and Methods

Patients and Follow-Up

A total of 1042 patients with chronic HCV infection from the Chronic Hepatitis C Research Program of Jiangsu (CHCRPJ) received DAA treatment from January 2012 to October 2020 in Jurong people's hospital, China. SVR was defined as a serum HCV RNA viral load below the lower detection limit at least 12 weeks after treatment. Of these patients, 796 achieved SVR. Patients diagnosed with HCC or hepatic decompensation (defined as the occurrence of ascites, encephalopathy, variceal bleeding, or hepatorenal syndrome) prior to SVR and patients who lack a FIB-4 score at baseline were excluded. Eventually, 701 patients were included in the study. The flowchart of patient selection is shown in Figure 1.

The index date of the study was defined as the date of SVR. Patients were followed until the study outcome, death or 31/12/2021, whichever came first. The study outcome was new cases of HCC after the index date. Information on HCC occurrence before and after treatment was obtained from hospital inpatient and outpatient diagnoses. HCC was diagnosed according to the American Association for the Study of Liver Diseases (AASLD) guidelines.[25] Laboratory tests were carried out at the date of SVR and annually after that when patients returned for their follow-up visit.

Written informed consent was obtained from all patients who participated in the study. The study protocol conformed to the ethical guidelines of the Declaration of Helsinki, and the institutional ethics review committee of Nanjing Medical University approved the study.

Baseline Characteristics

Baseline characteristics at SVR, including age, gender, cirrhosis, diabetes, hepatitis B virus (HBV) co-infection, and alcohol use, were collected. The birthdate and gender of each patient were obtained from their identity profiles. Information about cirrhosis, HBV co-infection, and diabetes was retrieved from the inpatient and outpatient diagnoses before SVR. Cirrhosis was diagnosed based either on a transient elastography score >14 kPa, a liver biopsy showing

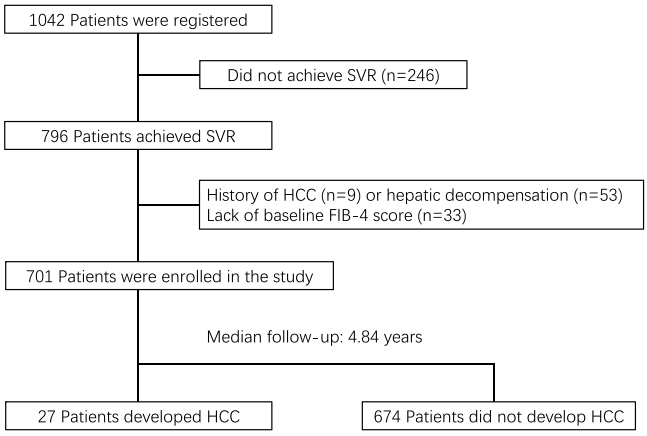

Figure 1 Flow chart of patient selection.
Abbreviations: SVR, sustained virological response; HCC, hepatocellular carcinoma; FIB-4, fibrosis 4 score.

Metavir F4, or clinical evidence. Information about alcohol use was collected through telephone inquiries. Excessive alcohol use was defined as >20 g/day in females and >30 g/day in males.

Fibrosis-4 (FIB-4) Scores at Baseline and During Follow-Up

Baseline FIB-4 score was calculated using the laboratory test results done at the same time as SVR, according to the following formula: (Age × AST)/ (Platelet count×√ALT).[26] FIB-4 score during follow-up was calculated based on the laboratory test results performed at the annual visit of each patient. The cutoff value for FIB-4 level was set at 3.25, which was previously established as the threshold of advanced fibrosis[21] and was shown to be associated with HCC risks.[23,27]

The changes of FIB-4 over time were modeled in two ways. First, we analyzed the FIB-4 score of patients at baseline and the last follow-up, then we categorized patients into four groups. For patients who developed HCC during follow-up, the last follow-up was defined as the time of HCC occurrence.

(a) Patients with a FIB-4 ≦3.25 at baseline and last follow-up were classified in the unchanged low-risk group.
(b) Patients who dropped from FIB-4 >3.25 at baseline to FIB-4 ≦3.25 at the last follow-up were classified in the declined group.
(c) Patients with a FIB-4 ≦3.25 at baseline and a FIB-4 >3.25 at the last follow-up were categorized as the increased group.
(d) Patients with a FIB-4 >3.25 at baseline and last follow-up were categorized as the unchanged high-risk group.

Second, we attempted to examine the impact of changes in FIB-4 score throughout the entire study period. To achieve that, we analyzed FIB-4 level (categorized as >3.25 or ≦3.25) as a time-dependent covariate. Each patient was analyzed under the low FIB-4 category when their FIB-4 score was below 3.25 and under the high FIB-4 category when their FIB-4 score was above 3.25.

Statistical Analyses

Continuous variables were presented as mean (standard deviation), and categorical variables were presented as count (percentage). Continuous variables were compared using the Student's *t*-test, and categorical variables by the Chi-square test or the Fisher exact test when appropriate.

The annual incidence rate of HCC (per 1000 person-years [PY]) and the corresponding 95% confidence interval (95% CI) were calculated assuming a Poisson distribution. The annual incidence rate of HCC between subgroups and between years after SVR was compared using two-way ANOVA with Tukey post-hoc analysis. The cumulative incidence of HCC

was evaluated using the Kaplan-Meier method, and the differences between subgroups were compared with the log-rank test.

Univariate and multivariate Cox proportional hazards regression models were used to estimate the effects of potential factors on the risk of HCC occurrence. Variables found to be statistically significant in univariate analysis ($P < 0.05$) were included in the multivariate analysis. HCC risks were also calculated according to the change in FIB-4 score at the last follow-up and based on FIB-4 coded as a time-dependent covariate using Cox regression models. Patients not developing HCC were censored on 31/12/2021 or at the date of death.

Statistical significance was defined as $P < 0.05$. Data analysis was performed using R software, version 4.1.2 (R Foundation for Statistical Computing).[28]

Results

Baseline Characteristics of Patients

The baseline characteristics of the study patients are shown in Table 1. Among the 701 patients, the majority (75.5%) were female. The mean age was 56.7 years old (SD = 8.3 years). 14.4% of the patients had cirrhosis at baseline. 24.5% of the patients had diabetes, 12.0% had excessive alcohol consumption, and 1.4% had HBV co-infection. A total of 273 patients (39.0%) had a FIB-4 score above 3.25 at the initiation of treatment. Aside from cirrhosis status and FIB-4 level, there were no significant differences in baseline factors between patients who developed HCC and patients who did not.

The median follow-up time for the overall study population was 4.84 years, with the 25th and 75th percentiles of the follow-up time being 3.45 years and 6.65 years, respectively. The patient with the longest follow-up period was followed for up to 9.82 years.

Incidence of HCC Following SVR

There were 27 cases of HCC following SVR. HCC incidence rate was 2.86 per 1000 PY during the first year, gradually increasing to 15.59 per 1000 PY in the third year and then decreasing afterward (Figure 2A). The cumulative incidence at 1, 2, 3, 4, and 5 years after SVR were 0.3%, 1.0%, 2.4%, 3.0%, and 3.3%, respectively.

We also plotted the annual incidence of HCC based on the presence or absence of baseline cirrhosis due to the possible difference between these two subgroups. The HCC incidence rate was stable in patients without cirrhosis, ranging from 1.67 per 1000 PY to 12.63 per 1000 PY. Patients with baseline cirrhosis had a noticeably higher annual incidence rate, with the highest incidence rate (33.98 per 1000PY) occurring three years after SVR (Figure 2B). There was also a slight rebound in the incidence rate in the fifth year after SVR (21.09/1000PY). In general, there was no significant downward trend in the annual incidence of HCC following SVR. The two-way ANOVA analysis discovered

Table 1 Baseline Characteristics of Patients

Characteristics	All Patients (N=701)	Patients with HCC (N=27)	Patients Without HCC (N=674)	*P* value
Age, y, mean (SD)	56.7 (8.3)	56.6 (8.1)	56.7 (8.3)	0.931
Gender (%)				
Male	172 (24.5)	6 (22.2)	166 (24.6)	0.955
Female	529 (75.5)	21 (77.8)	508 (75.4)	
Cirrhosis (%)	101 (14.4)	8 (29.6)	93 (13.8)	0.044
HBV co-infection (%)	10 (1.4)	1 (3.7)	9 (1.3)	0.849
Diabetes (%)	172 (24.5)	8 (29.6)	164 (24.3)	0.690
Excessive alcohol use (%)	84 (12.0)	2 (7.4)	82 (12.2)	0.657
FIB-4 (%)				
≦3.25	428 (61.1)	10 (37.0)	418 (62.0)	0.016
>3.25	273 (39.0)	17 (63.0)	256 (38.0)	

Abbreviations: HBV, hepatitis B virus; FIB-4, fibrosis 4 score.

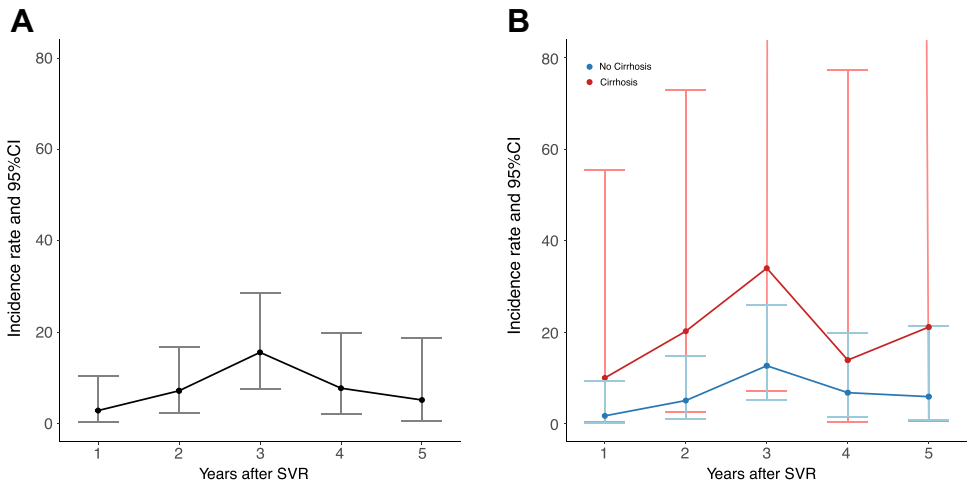

Figure 2 The annual incidence rate of HCC in virologically cured patients after SVR in the overall cohort (**A**) and the subgroups defined according to the presence of cirrhosis (**B**).

Abbreviations: SVR, sustained virological response; CI, confidence interval; FIB-4, fibrosis 4 score; HCC, hepatocellular carcinoma.

a substantial difference in the HCC incidence rate between the two subgroups ($P = 0.006$). No difference was found between years after SVR (all $P > 0.05$) in the Tukey post-hoc test.

Baseline Factors Associated with the Risk of HCC

Univariate and multivariate Cox regression models were used to evaluate baseline factors that might contribute to the development of HCC. Gender, excessive alcohol use, diabetes, cirrhosis, HBV co-infection, and FIB-4 score were included in the analysis. In the univariate analysis, cirrhosis (hazard ratio [HR] = 2.98; 95% CI = 1.29–6.86) and FIB-4 >3.25 (HR = 3.00; 95% CI = 1.37–6.56) were associated with a higher risk of HCC in patients achieving SVR. Significant variables ($P < 0.05$) in the univariate analysis were included in the multivariate analysis, where FIB-4 >3.25 (HR = 2.51; 95% CI = 1.10–5.72) was identified as an independent factor that contributed to the risk of HCC (Table 2) whereas cirrhosis was not.

Figure 3 depicts the cumulative incidence of HCC in patients who achieved SVR based on risk factors identified in the Cox regression analysis. The incidence of HCC was significantly higher in patients with a FIB-4 >3.25 at baseline than in those with a FIB-4 ≦3.25 (Figure 3A, $P = 0.0044$). Similarly, the incidence of HCC after SVR was higher in patients with cirrhosis (Figure 3B, $P = 0.0085$).

Table 2 Baseline Factors Associated with HCC Risk in Patients Achieving SVR

Characteristics	Univariate		Multivariate	
	HR (95% CI)	*P* value	HR (95% CI)	*P* value
Gender, male vs female	1.13 (0.46,2.80)	0.791	–	–
Excessive alcohol use, no vs yes	0.64 (0.15,2.69)	0.538	–	–
Diabetes, no vs yes	1.45 (0.63,3.32)	0.377	–	–
Cirrhosis, no vs yes	2.98 (1.29,6,86)	0.010	2.11 (0.88,5.08)	0.095
HBV co-infection, no vs yes	3.04 (0.41,22.46)	0.275	–	–
FIB-4, ≦3.25 vs >3.25	3.00 (1.37,6.56)	0.006	2.51 (1.10,5.72)	0.029

Abbreviations: HCC, hepatocellular carcinoma; CI, confidence interval; HR, hazard ratio; SVR, sustained virologic response; HBV, hepatitis B virus; FIB-4, fibrosis 4 score.

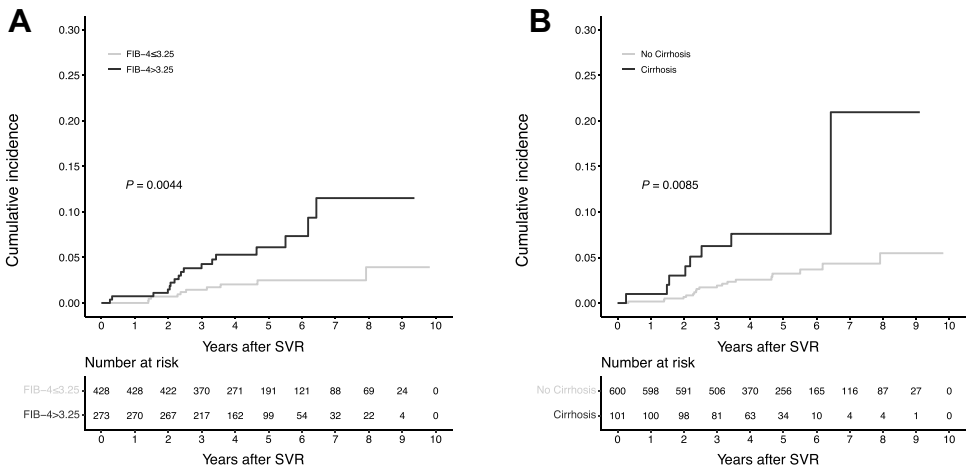

Figure 3 Cumulative incidence curves of HCC in virologically cured patients after SVR according to different subgroups: (**A**) FIB-4 level; (**B**) cirrhosis.
Abbreviations: SVR, sustained virological response; FIB-4, fibrosis 4 score; HCC, hepatocellular carcinoma.

HCC Risks Stratified by Baseline FIB-4 and Cirrhosis

In addition, we examined the association between baseline FIB-4 level together with cirrhosis and HCC risks. The incidence rate of HCC was the highest in patients with baseline cirrhosis and a FIB-4 >3.25 (18.63/1000PY). After adjustment for other baseline characteristics by the Cox regression model, patients with cirrhosis and a FIB-4 >3.25 had a 6.56-fold higher risk of developing HCC than patients with no cirrhosis and a FIB-4 ≦3.25 (adjusted HR = 6.56; 95% CI = 2.13–20.19). Patients with baseline cirrhosis and a FIB-4 ≦3.25, as well as patients with no cirrhosis and a FIB-4 >3.25, were also at higher risk of HCC compared with patients with no cirrhosis and a FIB-4 ≦3.25 (adjusted HR = 5.50 and 3.19, respectively) (Table 3).

Figure 4 depicts the impact of FIB-4 level on the HCC-free survival of patients stratified by baseline cirrhosis. Among patients with no cirrhosis at baseline, the incidence of HCC was significantly higher in patients with a FIB-4 >3.25 than in patients with a FIB-4 ≦3.25 (Figure 4A, $P = 0.012$). No difference was observed in HCC incidence between patients with different FIB-4 levels among patients with cirrhosis (Figure 4B, $P = 0.96$).

Changes in FIB-4 Over Time and Its Effect on HCC Risks

We compared the FIB-4 scores of patients at baseline and the last follow-up visit. Of the 701 patients included in this study, the FIB-4 score at the last follow-up was available in 514 patients. In total, 56.4% of patients remained at low risk, and 18.9% remained at high risk at the last follow-up. The FIB-4 score decreased in 20.0% of patients and increased in only 4.7%. The incidence rate was highest in the increased group (29.60/1000PY), followed by the unchanged high-risk group (15.16/1000PY) and the declined group (12.52/1000PY). Patients who experienced an elevation in FIB-4 score had the highest risk of developing HCC (adjusted HR = 9.88; 95% CI = 2.56–38.14). Also, HCC risks remained high in patients with a baseline FIB-4 >3.25 regardless of whether the FIB-4 score had dropped at the last follow-up (adjusted HR = 3.97; 95% CI = 1.24–12.74) or not (adjusted HR = 4.99; 95% CI = 1.44–17.26) (Table 4).

Table 3 Association Between Baseline FIB-4 Level and HCC Risk Stratified by the Presence of Cirrhosis in Patients Achieving SVR

Characteristics	Number of Patients (%)	Number Who Developed HCC (%)	Patient-Years	HCC Per 1000 Patient-Years	Crude HR (95% CI)	Adjusted HR (95% CI)†
No cirrhosis with FIB-4≦3.25	402 (67.0)	8 (2.0)	2274	3.52	1	1
No cirrhosis with FIB-4>3.25	198 (33.0)	11 (5.6)	1009	10.90	3.06 (1.23,7.61)	3.19 (1.27,7.99)
Cirrhosis with FIB-4≦3.25	26 (25.7)	2 (7.7)	128	15.63	4.40 (0.93,20.80)	5.50 (1.13,26.81)
Cirrhosis with FIB-4>3.25	75 (74.3)	6 (8.0)	322	18.63	5.12 (1.75,14.92)	6.56 (2.13,20.19)

Note: †Adjusted for gender, excessive alcohol use, diabetes, and HBV co-infection.
Abbreviations: HCC, hepatocellular carcinoma; CI, confidence interval; HR, hazard ratio; SVR, sustained virologic response; FIB-4, fibrosis 4 score.

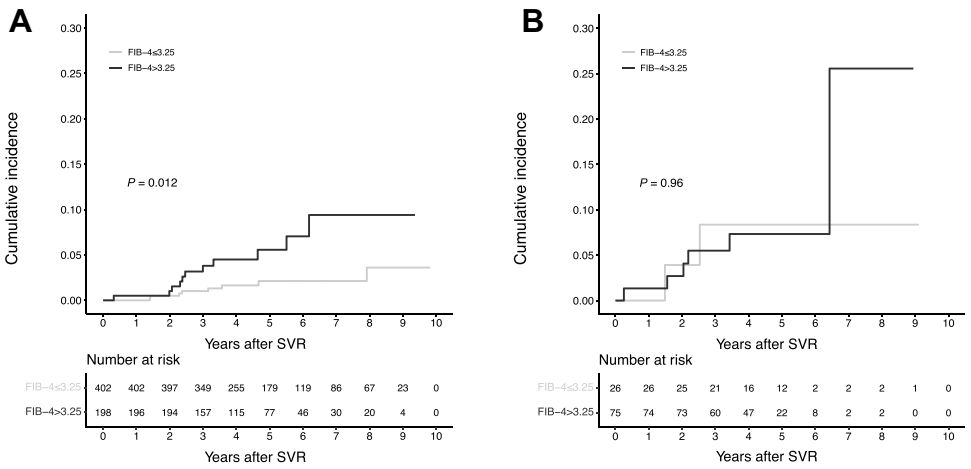

Figure 4 Cumulative incidence curves of HCC among virologically cured patients with different FIB-4 levels, stratified by cirrhosis: (**A**) cirrhosis; (**B**) without cirrhosis.
Abbreviations: SVR, sustained virological response; FIB-4, fibrosis 4 score; HCC, hepatocellular carcinoma.

Association Between FIB-4 as a Time-Dependent Covariate and HCC Risks

We analyzed FIB-4 score (classified as >3.25 or ≦3.25) as a time-dependent covariate. The HCC incidence rate was 4.37 per 1000PY when the FIB-4 level of patients was low and 15.18 per 1000PY when the FIB-4 level of patients was high. During the entire follow-up period, FIB-4 >3.25 was associated with a 3.14-fold higher risk of patients developing HCC (adjusted HR = 3.14; 95% CI = 1.40–7.05).

When taking into account the presence of baseline cirrhosis, we discovered a surprisingly high incidence rate of HCC in patients with cirrhosis when their FIB-4 level was high (23.44/1000PY). The incidence rate was slightly higher in patients with cirrhosis and a FIB-4 score below 3.25 (10.26/1000PY) than in patients with no cirrhosis but a FIB-4 above 3.25 (12.55/1000PY). Among patients with cirrhosis, FIB-4 >3.25 during the follow-up period was associated with a 7.49-fold higher risk

Table 4 Association Between Change in FIB-4 Level at Baseline and Last Follow-Up and HCC Risk in Patients Achieving SVR

Characteristics	Number of Patients (%)	Number Who Developed HCC (%)	Patient-Years	HCC Per 1000 Patient-Years	Crude HR (95% CI)	Adjusted HR (95% CI)[†]
Unchanged, low risk	290 (56.4)	5 (1.7)	1781	2.80	1	1
Declined	103 (20.0)	7 (6.80)	559	12.52	4.31 (1.37,13.60)	3.97 (1.24,12.74)
Increased	24 (4.7)	4 (16.7)	135	29.60	10.59 (2.84,39.46)	9.88 (2.56,38.14)
Unchanged, high risk	97 (18.9)	7 (7.2)	462	15.16	5.08 (1.60,16.10)	4.99 (1.44,17.26)

Note: [†]Adjusted for gender, cirrhosis, excessive alcohol use, diabetes, and HBV co-infection.
Abbreviations: HCC, hepatocellular carcinoma; CI, confidence interval; HR, hazard ratio; SVR, sustained virologic response; FIB-4, fibrosis 4 score.

Table 5 Association Between FIB-4 Level During Follow-Up and HCC Risk Stratified by the Presence of Cirrhosis in Patients Achieving SVR

Characteristics	Patient-Years	HCC Per 1000 Patient-Years	Crude HR (95% CI)	Adjusted HR (95% CI)[†]
No cirrhosis with FIB-4≦3.25	2566	3.90	1	1
No cirrhosis with FIB-4>3.25	717	12.55	3.37 (1.37,8.31)	3.29 (1.33,8.16)
Cirrhosis with FIB-4≦3.25	195	10.26	2.56 (0.56,11.72)	2.83 (0.58,13.72)
Cirrhosis with FIB-4>3.25	256	23.44	5.94 (2.14,16.53)	7.49 (2.56,21.95)

Note: [†]Adjusted for gender, excessive alcohol use, diabetes, and HBV co-infection.
Abbreviations: HCC, hepatocellular carcinoma; CI, confidence interval; HR, hazard ratio; SVR, sustained virological response; FIB-4, fibrosis 4 score.

of HCC (adjusted HR = 7.49; 95% CI = 2.56–21.95). In contrast, no increased risk of HCC development was found in cirrhosis patients when their FIB-4 scores were below 3.25 during follow-up (adjusted HR = 2.83; 95% CI = 0.58–13.72) (Table 5).

Discussion

As a result of the wide use of DAA, almost all patients with HCV infection will experience SVR after antiviral treatment. Multiple studies have shown that SVR is associated with improved clinical outcomes, including the mitigation of fibrosis[10,11] and the reduced risk of HCC.[12,29,30] However, there is still a residual risk of HCC after SVR, so surveillance of patients is required. As described by Wang S et al[31] surveillance and early diagnosis are critical in improving the cure rate of cancer as they facilitate a timely and sufficient subsequent treatment. However, surveillance on all virologically cured patients is impractical. As a result, it is necessary to determine which of the patients are at high risk of developing HCC. In addition, the HCC risks may change over time in the long term as patients age and their fibrosis status changes, further complicating the situation. Several studies have evaluated pre-SVR factors contributing to the short-term risk of HCC in virologically cured patients, but few have addressed the long-term risk of HCC. Therefore, in the current study, we seek to use FIB-4, a simple serum fibrosis biomarker that can be repeatedly measured during follow-up, to evaluate the long-term risk of HCC and ultimately identify high-risk patients who require strict surveillance.

In this study, 701 HCV-infected patients treated with DAA were followed for a median of 4.84 years after SVR. The patient with the longest follow-up time was followed for up to 9.82 years. The study's first finding was that the risk of HCC persisted for years after SVR. After inspecting the annual incidence rate of HCC, we found no apparent decline in the residual HCC risks following SVR. The annual incidence rate in the overall cohort was below the cutoff value of 15 per 1000PY, as recommended by the AASLD.[25] Among patients with cirrhosis, however, the annual incidence rate of HCC was higher than the surveillance threshold most of the time, with a slight rebound in the fifth year after SVR. The two-way ANOVA test results revealed a significant difference in HCC incidence rate between patients with or without cirrhosis but no difference between years after SVR. The results above suggest that the risk of HCC after SVR did not reduce over time and that there was a significant difference in HCC risk between different subgroups of patients. The result is consistent with the study investigating HCC risk after HCV eradication in Veterans Health Administration (VHA) patients. The risk of HCC persisted 10 years after SVR and showed no indication of a downward trend in VHA patients.[23]

Secondly, the study found that FIB-4 >3.25 measured at baseline or any time after SVR was associated with HCC risks and that the repeated measurements of FIB-4 presented a better predictive ability of HCC risks than the simple measurement of FIB-4 at baseline. Univariate and multivariate Cox regression analysis showed that FIB-4 >3.25 at baseline was significantly associated with HCC development after HCV eradication. When analyzed as a time-dependent covariant, FIB-4 >3.25 showed a good predicting value of HCC as well. Patients faced a significantly higher risk of HCC when their FIB-4 was above 3.25 at any time during follow-up. The results suggest that FIB-4 measured at any time after treatment can be used to examine HCC risks in patients achieving SVR. Similar results were reported in previous studies as well. In a study evaluating the ability of blood fibrosis tests to predict liver-related events and death, FIB-4 showed good discriminative ability for predicting HCC (with a C-index of 0.884) and was an independent predictor of HCC in the adjusted Cox model.[32] Another study examining the long-time risk of HCC after HCV eradication found that the incidence of HCC was significantly higher in patients with a FIB-4 ≧3.25.[33]

As expected, the FIB-4 level of most patients decreased or remained the same post-SVR. Only 4.7% of the patients experienced an increase in FIB-4 level from a value ≦3.25 to a value >3.25. The increase in FIB-4 level was associated with a 9.88-fold higher risk of HCC. A study focusing on HCC risk after HCV eradication found that an increase in FIB-4 was associated with HCC risk.[23] Though a rise in FIB-4 after SVR is relatively uncommon, the high incidence rate of HCC (29.6/1000PY) in these patients still requires attention. Repeated measurement of FIB-4 after treatment made it possible to identify this small group of patients at risk. In addition, the coefficient of FIB-4 level was larger in the time-dependent analysis than in the model composed of only baseline values, indicating that repeated measurement of FIB-4 was able to capture the effect of FIB-4 as it changes over time. Therefore, the repeated measurement of FIB-4 can better predict HCC risks in SVR patients compared with measuring FIB-4 only at baseline.

Another finding of the current study was that compared to stratifying patients by cirrhosis status at baseline alone, the inclusion of FIB-4 considerably improved the ability to predict HCC risks in patients reaching SVR. When investigating

the risk of HCC occurrence, a number of studies simply stratified patients according to the presence of cirrhosis.[14,34] However, in the current study, baseline cirrhosis was only significantly associated with HCC risks in the univariate model, and HCC still occurred in patients without cirrhosis. Also, multiple studies have shown the changes in liver fibrosis status after patients achieved SVR.[12,22] It appears that the simple stratification based on cirrhosis is not sufficient for predicting HCC. Therefore, the current study combined baseline cirrhosis with FIB-4 and its dynamic changes to stratify virologically cured patients into distinct risk groups. In the baseline analysis, the incidence rate of HCC was the highest in patients with both cirrhosis and a FIB-4 >3.25 (18.63/1000PY). Cirrhotic patients and non-cirrhotic patients with a baseline FIB-4 above 3.25 were at a higher risk of developing HCC than non-cirrhotic patients with a baseline FIB-4 below 3.25. A large cohort study examining HCC risk 10 years after SVR also found that patients with cirrhosis or FIB-4 >3.25 at baseline presented a higher risk of developing HCC.[23] The time-dependent analysis delivered similar results. The incidence rate of HCC was very high when cirrhotic patients had a FIB-4 above 3.25 (23.44/1000PY). Also, when non-cirrhotic patients had a FIB-4 score higher than 3.25, their incidence rate of HCC (12.55/1000PY) was slightly higher than that of cirrhotic patients (10.26/1000PY) when the FIB-4 was below 3.25. These results all suggest that patients faced a significantly higher risk of HCC when their FIB-4 was above 3.25 at any time after HCV eradication, regardless of the presence of baseline cirrhosis. The additional segmentation of patients by combining FIB-4 and cirrhosis leads to more accurate identification of high-risk patients after the eradication of HCV.

Finally, the current study identified some patients at high risk of HCC development. Virologically cured patients with a FIB-4 level above 3.25 at SVR or any time afterwards are at a higher risk of developing HCC and should therefore be offered surveillance. Further study will be needed to examine whether the HCC risks will decrease in virologically cured patients experiencing a drop in FIB-4 level. In addition, patients with cirrhosis should continue to be surveilled after SVR, along with patients with no cirrhosis but a FIB-4 >3.25. Surprisingly, no increased risk was found in patients with baseline cirrhosis and a FIB-4 ≦3.25 in the time-dependent analysis. This may be partly explained by the small number of patients with both cirrhosis and a FIB-4 ≦3.25.

Compared with previous studies, the current study holds the following strengths. All patients included in the study were treated with DAA and achieved SVR. The follow-up period was also relatively long, with a median follow-up time of approximately 5 years and a maximum follow-up time of up to 10 years. Previous studies investigating the predictive value of FIB-4 were either done on IFN-treated patients[33] or did not have a sufficiently long follow-up duration of DAA-treated patients.[23,27] Our study demonstrated that the risk of HCC did not decline for a considerable time after DAA treatment and that the dynamic changes in FIB-4 levels were directly associated with HCC risk throughout the follow-up period. Furthermore, the current study combined the dynamic change of FIB-4 and cirrhosis status to divide patients into subgroups further, bringing attention to two particular groups of patients. (1) Patients whose FIB-4 was below 3.25 at the time of SVR but later increased to above 3.25 require strict surveillance as the HCC incidence rate in these patients was extremely high. This specific group of patients was often overlooked since some previous studies only evaluated FIB-4 measured at baseline.[14,35] (2) Patients with baseline cirrhosis but a FIB-4 ≦3.25 at or after SVR still need continuous surveillance despite their relatively low FIB-4 scores. Unlike some studies proposing that patients with a FIB-4 ≦3.25 may be excluded from surveillance,[27,33] our results showed that there was still insufficient evidence to terminate surveillance for patients with low FIB-4 scores but other high-risk factors such as cirrhosis. The incidence rate of HCC in these patients remained relatively high, despite the time-dependent analysis not identifying an increased risk. Further study and a larger population will be needed to investigate the long-term risk of HCC in this distinct group of patients.

The study has some limitations. First, some patients did not return for laboratory tests after SVR as time went by, and the association between FIB-4 change and HCC risks might be underrated or overrated. Second, information about alcohol use was collected through telephone inquiries rather than face-to-face questionnaires, which may have yielded information bias. Third, the current study was a single-center study, and the generalizability of our results to a larger HCV population may be limited. Finally, the study cohort is all Asian and consists mainly of females. It is necessary to conduct validation studies in other cohorts.

The role of FIB-4 in HCC prediction has been thoroughly described in the current study and previous literature. Future studies could put more emphasis on the following aspects. A larger population and a more extended follow-up period will be needed to determine the utility of FIB-4 or other non-invasive biomarkers in identifying cirrhotic patients at low enough risk to

reduce surveillance after SVR. In addition to single non-invasive biomarkers, new risk prediction models constructed based on longitudinal data collected during follow-up, capable of making a more personalized and dynamic prediction of HCC occurrence, will be instrumental in guiding the clinical decisions for HCV patients achieving SVR. Lastly, it is crucial that we concentrate on the urgent need to develop novel methods for HCC treatment. New techniques that tackle cancer in a different approach, such as the OncoCiDia strategy,[31] may hold a curative potential for HCC.

Conclusion

In conclusion, the risk of HCC persists years after SVR. The repeated measurement of FIB-4 presents a better predictive ability of HCC risks than the simple measurement of baseline FIB-4. Patients with a FIB-4 level above 3.25 at SVR or any time after SVR are at a higher risk of developing HCC and should therefore be offered surveillance. By stratifying patients reaching SVR into different risk groups using a combination of FIB-4 and cirrhosis, high-risk patients can be identified more accurately. Patients with cirrhosis should continue to be surveilled after SVR, along with patients with no cirrhosis but a FIB-4 >3.25.

Abbreviations

FIB-4, fibrosis 4 score; HCV, hepatitis C virus; SVR, sustained virologic response; IFN, interferon; DAA, direct-acting antivirals; HCC, hepatocellular carcinoma; ICIs, immune checkpoint inhibitors; REG, regorafenib; AST, aspartate aminotransferase; ALT, alanine aminotransferase; HBV, hepatitis B virus; PY, person-years; CI, confidence interval; HR, hazard ratio; AASLD, American Association for the Study of Liver Diseases; VHA, Veterans Health Administration.

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Disclosure

The authors report no conflicts of interest in this work.

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