Simple stratification of hepatocellular carcinoma surveillance after direct-acting antiviral therapy for chronic hepatitis C

Authors: Tianpeng Wang[1], Masashi Sakaki[1], Yuki Ichikawa[1], Yumi Otoyama[1], Yoko Nakajima[1], Ikuya Sugiura[1], Jun Arai[1], Atsushi Kajiwara[1], Shojiro Uozumi[1], Yuu Shimozuma[1], Manabu Uchikoshi[1], and Hitoshi Yoshida[1]

Affiliations: [1] Division of Gastroenterology, Department of Medicine, Showa University School of Medicine.

1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan

Correspondence and reprint request: Masashi Sakaki, M.D., Ph.D,

Division of Gastroenterology, Department of Medicine, Showa University School of Medicine,

1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan

Email: msakaki@med.showa-u.ac.jp

Tel: +81-3-3784-8535; Fax: +81-3-3784-7553

Running title: (HCC surveillance after DAA therapy for CH-C)

Abstract

Background: There have been few reports on useful surveillance system for determining the occurrence risk of hepatocellular carcinoma (HCC) after direct-acting antiviral (DAA) treatment for hepatitis C. Liver cirrhosis (LC) is a high-risk factor for HCC, but appropriate frequency of evaluation necessary for patients with chronic hepatitis (CH) remains unknown. In order to clarify this problem, we conducted a study how frequent should a CH patient have surveillance of HCC, especially in patients who achieved a sustained virological response (SVR) with DAA treatment.

Methods: Data were collected from both pretreatment (Pre) and SVR time points for 141 patients with hepatitis C recruited from October 1, 2014 through July 31, 2018 who received DAA treatment. We defined LC as a platelet (PLT) count ≤10×10^4/μL, and CH was defined as PLT count of >10×10^4/μL. The incidence of HCC in patients with CH after achieving SVR was retrospectively evaluated.

Results: In total, 128 patients (CH, n=102; LC, n=26) achieved SVR, and 13 developed HCC after SVR during the follow-up period, mean 748 days. Though fibrosis-4 (FIB-4) index, presence of α-fetoprotein, and prothrombin time (PT) % were significant risk factors for HCC in patients with CH with univariate analysis, only Pre-FIB-4 index was an independent predictive factor for HCC development with multivariate analysis (p=0.04). A FIB-4 index ≥3 was a significant risk factor for HCC (p=0.005). The cumulative risk for HCC at 1000 days was 2.6% and 24.2% in the FIB-4 index <3 and FIB-4 index ≥3 groups, respectively (p=0.004).

Conclusion: Frequent HCC examination is recommended for FIB-4 index ≥3 CH patients who have obtained SVR after DAA treatment.

Key words: FIB-4 index, Hepatocellular carcinoma, Chronic hepatitis C, Direct-acting antiviral, Platelet count

1. Introduction

Direct-acting antiviral (DAA) therapy is the first choice of treatment to eradicate hepatitis C virus (HCV), because it can achieve almost 100% of sustained virologic response (SVR) (1-4). However, key issues remain including treatment failure, acquisition of viral resistance (5, 6), and development of HCC. Similar to interferon (IFN) therapy, DAA treatment induces improvement of liver status and suppression of HCC incidence (7-11). Only a few reports have evaluated the occurrence risk of HCC after DAA treatment. In order to assess the risk of HCC after DAA therapy, α-fetoprotein (AFP), the fibrosis-4 (FIB-4) index, and post-treatment levels of Wisteria floribunda agglutinin-positive Mac-2 binding protein ($WFA^{+}M2BP$) have been considered helpful as screening biomarkers (7, 8, 12). However, the risk stratification still remains unclear because inflammatory responses in liver diseases are diverse. As liver cirrhosis (LC) is an obvious high-risk factor of HCC even if treated with DAA (13), estimating the incidence of HCC after SVR is difficult especially in cases of a chronic liver disease that involves mild to moderate inflammation. Because LC and chronic hepatitis (CH) are difficult to distinguish accurately without liver biopsy, here we tentatively defined LC and CH using platelet (PLT) count. Several studies had shown that PLT count of less than 10^5/μL indicate to have advanced liver fibrosis and significant cirrhosis (14-16) and reflect a high incidence of HCC (17-19). Kang et al. shows PLT count less than 10^5 was a high incidence of underlying liver cirrhosis (20). We divided liver status according to PLT count such as if PLT count is more than 10^5/μL, we considered as CH and if PLT count is less than 10^5/μL, we considered as LC. There is a surveillance algorithm for hepatocellular carcinoma determines imaging examination intervals for LC and CH (21). However, improvement of liver function after DAA treatment is not considered. We conducted a study to clarify the frequency of appropriate surveillance period for HCC, especially in patients with CH who successfully achieved SVR.

2. Methods

2.1. Study population

In total, 141 patients with hepatitis C virus (HCV) liver disease were treated with IFN-free DAA therapy from October 1, 2014 through July 31, 2018 in a single center hospital. Among them, 131 patients (93%) achieved “SVR-12” were defined as cases with serologically HCV negative for more than 12 weeks after the treatment. Three patients who developed HCC before SVR were excluded because they may have had HCC already before beginning the DAA treatment. Thus, 128 patients who had achieved SVR, including 10 patients who developed HCC after SVR, were included in the analysis.

This study was conducted in compliance with the Declaration of Helsinki and Ethical Guidelines for Medical and Health Research Involving Human Subjects. The Ethics Committee of Showa University Hospital approved the study protocol (Permit Number: 2461).

2.2. Patient characteristics

We collected baseline data, including age, sex, and HCV genotype, and extracted relevant laboratory test results at both pretreatment (Pre) and after the SVR time points. With respect to PLT counts, we defined LC as a PLT count less than 10^5/μL and CH as more than 10^5/μL. The FIB-4 index (22), aspartate aminotransferase (AST) to PLT ratio index (APRI) (23), albumin-bilirubin (ALBI) grade (24), and WFA+M2BP, which is an M2BP glycan isomer (M2BPGi) (25), were used as surrogate markers of liver fibrosis and liver function. The formula is shown in Figure 1.

2.3. Incident HCC

We identified incident cases of HCC after patients had achieved at least SVR-12. All patients who achieved SVR were followed at 3- to 6-month intervals with ultrasonography, helical dynamic computed tomography (CT), or gadoxetate disodium-enhanced magnetic resonance imaging (EOB-MRI). We diagnosed HCC by dynamic CT or EOB-MRI.

2.4. Statistical analysis

The Wilcoxon rank sum test was used to evaluate the background of patients treated with DAA. All P-values<0.05 on two-tailed testing were considered significant. The risk factors of HCC development after DAA treatment were evaluated using Cox proportional hazards model analysis. HCC occurrence rate was evaluated by Kaplan-Meier curves. The cumulative incidence of HCC was evaluated by the log-rank test. Data were analyzed statistically using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Patient characteristics

The characteristics of 128 patients with SVR and SVR data are shown in Table 1. DAA treatment significantly induced improvement of liver inflammation and fibrosis markers during SVR but did not affect PLT counts. This suggests that PLT count can reflect the actual liver status regardless of HCV presence. Furthermore, PLT counts can clearly divide the status of liver problems between patients with CH and those with LC, as shown in Table 2. Seven patients with CH and three patients with LC developed HCC after achieving SVR.

3.2. Risk factors associated with hepatocellular carcinoma

The factors from Pre and post SVR following DAA treatment were evaluated because the Pre data included both an inflammatory response with the presence of HCV and cumulative liver damage. In

contrast, DAA treatment can induce an immediate improvement of inflammation at the point of the SVR (26). The SVR point may reflect true accumulated liver damage. Multivariate analyses that took into consideration fibrosis parameters such as FIB-4 index, APRI, and ALBI grade were performed at each pretreatment and post SVR points. At the pretreatment point, prothrombin time (PT) %, AFP, and FIB-4 index were significantly different between the non-HCC and HCC groups in the univariate analysis, and only the FIB-4 index was an independent predictor of HCC in the multivariate analysis (p=0.04) (Table 3). At the SVR point, PT%, AST, AFP, and FIB-4 index were extracted as parameters predictive of HCC incidence by univariate analysis. Although there were no significant differences, the FIB-4 index tended to be an independent predictor of HCC in the multivariate analysis (p=0.05) (Table 4). The receiver operating characteristic (ROC) curve for the FIB-4 index at the pretreatment point is shown in Figure 2. The area under the ROC curve was 0.799. Based on this result, the FIB-4 index of 3 was defined as the optimum cut-off value. Moreover, the FIB-4 index ≥3 group was significantly associated with a high risk of HCC incidence (hazard ratio [HR]=11.7; 95% confidence interval [CI], 2.0-220.2; p=0.0046). In addition, the cumulative risk for HCC at 1000 days was 2.6% in the FIB-4 index <3 group compared with 24.2% for the FIB-4 index ≥3 group, and the cumulative HCC incidence was significantly higher in the FIB-4 index ≥3 group than that in the FIB-4 index <3 group on the log-rank test (p=0.004) (Figure 3).

4. Discussion

IFN-free DAA treatment can induce HCV clearance in almost 100% of cases without severe complications. Although viral clearance in serologically is achieved, we should keep in mind the possible occurrence of HCC even though the SVR reduces risk of HCC (9, 10). In the present study, we evaluated the risk of HCC after IFN-free DAA treatment. Generally, liver fibrosis is a high-risk factor for HCC; hence, intensive follow-up for patients with LC is necessary even if SVR has been achieved. Moreover, not every patient with CH undergoes close imaging examination because the accumulated liver injury and fibrosis are different with each case. There are some reports about the predictors of HCC occurrence such as WFA+M2BP, FIB-4 index, AFP, and ALBI (7, 8) (27). However, inflammatory responses in liver diseases are diverse and risk stratification is still unclear. For this reason, this study evaluated the HCC risk of patients with CH and determined the high-risk group. However, to evaluate the status of liver inflammation and fibrosis in patients with CH and LC without using liver biopsy is difficult. As well-known, liver biopsy is a harmful procedure that can cause complications, such as pain, hemorrhage, and even mortality (28). With those reasons, liver biopsy is not commonly performed for those patients especially with DAA treatment. The patients with DAA have higher SVR rate and lower adverse reaction rates compared to those with IFN therapy. Therefore, we used PLT counts to differentiate CH from LC. Our data support the use of PLT counts to define liver problems, by classifying the values into CH and LC groups; moreover, DAA treatment did not affect the PLT count.

In the CH group, PT%, AFP, and FIB-4 index at both pretreatment and SVR points were risk factors of

HCC development, but only pretreatment FIB-4 index was identified as an independent risk factor of HCC incidence by multivariate analysis. Moreover, a FIB-4 index ≥3 was a predictive factor of HCC incidence and showed significant occurrence risk of HCC in the log-rank test. These results suggest that clarifying the liver status based on PLT count and stratifying the risk of HCC incidence using the FIB-4 index enables convenient CH follow-up based on the risk of HCC development after DAA therapy. The follow-up time interval after DAA therapy depends on physicians' decision based on their experience. Generally, patients with CH do not necessarily undergo every two to three months' surveillance for HCC like LC patients. However, even CH patients, periodical inspections are necessary for HCC. Problem is, how often and which marker is most efficient. We believe our study can suggest appropriate markers and follow-up period considering carcinogenic risk of HCC after DAA therapy. In other words, patients with HCV, a PLT count >10^5/μL before DAA treatment, and with a FIB-4 index ≥3 are at a high risk of HCC incidence and should be monitored frequently.

The present study has some limitations. The sample size analyzed was small, and the observation period was relatively short. Nevertheless, our approach is considered non-invasive, simple, and useful for stratification of CH and LC patients for the risk of HCC onset. More evaluations are required with more cases for longer observation period.

5. Conclusion

FIB-4 index ≥3 CH patients who have obtained SVR after DAA treatment are considered to be a high risk of hepatocellular carcinoma, and frequent surveillance is recommended..

Acknowledgments

None

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

References

1) Lawitz E, Mangia A, Wyles D, et al. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **368**(20): 1878-87.
2) Jacobson IM, Gordon SC, Kowdley KV, et al. Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**(20): 1867-77.
3) Mizokami M, Yokosuka O, Takehara T, et al. Ledipasvir and sofosbuvir fixed-dose combination with and without ribavirin for 12 weeks in treatment-naive and previously treated Japanese patients with genotype 1 hepatitis C: an open-label, randomised, phase 3 trial. *Lancet Infect Dis* 2015; **15**(6): 645-53.

4) Chayama K, Suzuki F, Karino Y, et al. Efficacy and safety of glecaprevir/pibrentasvir in Japanese patients with chronic genotype 1 hepatitis C virus infection with and without cirrhosis. *J Gastroenterol* 2018; **53**(4): 557-65.
5) Fridell RA, Wang C, Sun JH, et al. Genotypic and phenotypic analysis of variants resistant to hepatitis C virus nonstructural protein 5A replication complex inhibitor BMS-790052 in humans: in vitro and in vivo correlations. *Hepatology* 2011; **54**(6): 1924-35.
6) Vermehren J, Sarrazin C. The role of resistance in HCV treatment. *Best Pract Res Clin Gastroenterol* 2012; **26**(4): 487-503.
7) Nagata H, Nakagawa M, Asahina Y, et al. Effect of interferon-based and -free therapy on early occurrence and recurrence of hepatocellular carcinoma in chronic hepatitis C. *J Hepatol* 2017; **67**(5): 933-9.
8) Watanabe T, Tokumoto Y, Joko K, et al. Predictors of hepatocellular carcinoma occurrence after direct-acting antiviral therapy in patients with hepatitis C virus infection. *Hepatol Res* 2019; **49**(2): 136-46.
9) Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol* 2017.
10) Calvaruso V, Cabibbo G, Cacciola I, et al. Incidence of Hepatocellular Carcinoma in Patients With HCV-Associated Cirrhosis Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2018; **155**(2): 411-21 e4.
11) Carrat F, Fontaine H, Dorival C, et al. Clinical outcomes in patients with chronic hepatitis C after direct-acting antiviral treatment: a prospective cohort study. *Lancet* 2019.
12) Kobayashi M, Suzuki F, Fujiyama S, et al. Sustained virologic response by direct antiviral agents reduces the incidence of hepatocellular carcinoma in patients with HCV infection. *J Med Virol* 2017; **89**(3): 476-83.
13) Kanwal F, Kramer J, Asch SM, et al. Risk of Hepatocellular Cancer in HCV Patients Treated With Direct-Acting Antiviral Agents. *Gastroenterology* 2017; **153**(4): 996-1005 e1.
14) Giannini E, Borro P, Botta F, et al. Serum thrombopoietin levels are linked to liver function in untreated patients with hepatitis C virus-related chronic hepatitis. *J Hepatol* 2002; **37**(5): 572-7.
15) Pradella P, Bonetto S, Turchetto S, et al. Platelet production and destruction in liver cirrhosis.*J Hepatol* 2011;**54**(5): 894-900.
16) Wang CS, Yao WJ, Wang ST,et al. Strong association of hepatitis C virus (HCV) infection and thrombocytopenia: implications from a survey of a community with hyperendemic HCV infection. *Clin Infect Dis.* 2004; **39**(6): 790-6.
17) Ikeda K, Arase Y, Saitoh S, et al. Prediction model of hepatocarcinogenesis for patients with hepatitis C virus-related cirrhosis. Validation with internal and external cohorts. *J Hepatol.* 2006; **44**(6): 1089-97.

18) El-Serag HB, Kanwal F, Davila JA, et al. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. *Gastroenterology* 2014; **146**(5): 1249-55 e1.
19) Velazquez RF, Rodriguez M, Navascues CA, et al. Prospective analysis of risk factors for hepatocellular carcinoma in patients with liver cirrhosis. *Hepatology* 2003; **37**(3): 520-7.
20) Kang CM, Choi GH, Kim DH, et al. Revisiting the role of nonanatomic resection of small (< or = 4 cm) and single hepatocellular carcinoma in patients with well-preserved liver function. *J Surg Res.* 2010; **160**(1): 81-9.
21) Kokudo N, Takemura N, Hasegawa K, et al. Clinical practice guidelines for hepatocellular carcinoma: The Japan Society of Hepatology 2017 (4th JSH-HCC guidelines) 2019 update. *Hepatol Res.* 2019; **49**(10): 1109-1113.
22) Vallet-Pichard A, Mallet V, Nalpas B, et al. FIB-4: an inexpensive and accurate marker of fibrosis in HCV infection. comparison with liver biopsy and fibrotest. *Hepatology* 2007; **46**(1): 32-6.
23) Wai CT, Greenson JK, Fontana RJ, et al. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology* 2003; **38**(2): 518-26.
24) Johnson PJ, Berhane S, Kagebayashi C, et al. Assessment of liver function in patients with hepatocellular carcinoma: a new evidence-based approach-the ALBI grade. *J Clin Oncol* 2015; **33**(6): 550-8.
25) Kuno A, Ikehara Y, Tanaka Y, et al. A serum "sweet-doughnut" protein facilitates fibrosis evaluation and therapy assessment in patients with viral hepatitis. *Sci Rep* 2013; **3**: 1065.
26) Singh S, Facciorusso A, Loomba R, Falck-Ytter YT. Magnitude and Kinetics of Decrease in Liver Stiffness After Antiviral Therapy in Patients With Chronic Hepatitis C: A Systematic Review and Meta-analysis. *Clin Gastroenterol Hepatol* 2018; **16**(1): 27-38 e4.
27) Casadei Gardini A, Foschi FG, Conti F, et al. Immune inflammation indicators and ALBI score to predict liver cancer in HCV-patients treated with direct-acting antivirals. *Dig Liver Dis* 2018.
28) Rockey DC, Caldwell SH, Goodman ZD, et al. American Association for the Study of Liver D. Liver biopsy. *Hepatology* 2009; **49**(3): 1017-44.

Legends for Tables and figures

Table 1. Clinical characteristics of 128 patients with HCV at pre-treatment and after DAA therapy with SVR.

Table 2. The characteristics of patients with CH and LC based on baseline PLT count.

Table 3. Factors affecting HCC incidence in patients with CH at pre-treatment.

Table 4. Factors affecting HCC incidence in DAA-treated patients with CH at the SVR.

Figure 1. The formula of liver fibrosis and liver function markers.

Figure 2. The ROC curve for the FIB-4 index at the pre-treatment.

Figure 3. Cumulative incidence (%) of HCC in patients with CH who achieved SVR with DAA treatment compared to the FIB-4 index at pre-treatment. The cumulative incidence increases significantly in patients with CH and a FIB-4 index ≥3 (p=0.004, log-rank test).

Table 1.

Male/Female (n)		59/69	
Age (median)		65.6(68)	
	Pre	SVR	*p*-value
Platelet count (×10⁴/μL)	16.5 (6.6)	17.3 (6.3)	0.29
Prothrombin time (%)	86.5 (10.8)	88.5 (9.7)	0.18
Total bilirubin (mg/dL)	0.8 (0.3)	0.8 (0.3)	0.63
AST (U/L)	50.9 (29.1)	25.2 (8.9)	<0.001
ALT (U/L)	51.7 (41.3)	18.4 (10.2)	<0.001
Albumin (g/dL)	4.1 (0.4)	4.2 (0.3)	<0.002
AFP (ng/mL)	11.3 (29.1)	4.2 (3.2)	<0.001
M2BPGi	3.0 (2.6)	1.4 (1.2)	<0.001
FIB-4 index	3.72 (2.62)	2.84 (1.81)	<0.01
APRI	1.43 (1.35)	0.64 (0.48)	<0.001
ALBI	-2.71 (0.36)	-2.85 (0.29)	<0.002

Data are expressed as the mean (standard deviation).

Table 2.

	CH (n=102) PLT >10×10⁴/μL	LC (n=12) PLT ≤10×10⁴/μL	*p*-value
Male/Female	49/53	10/16	<0.005
Age	64.9 (11.4)	68.4 (10.1)	0.21
Prothrombin time (%)	88.8 (9.9)	77.2 (9.3)	<0.001
Total bilirubin (mg/dL)	0.76 (0.25)	0.99 (0.35)	<0.003
AST (U/L)	48.4 (28.6)	60.4 (29.6)	<0.02
ALT (U/L)	50.3 (43.2)	57.1 (32.9)	0.07
Albumin (g/dL)	4.1 (0.3)	3.8 (0.5)	<0.001
AFP (ng/mL)	7.8 (21.5)	25.0 (46.7)	<0.001
M2BPGi	2.24 (1.63)	5.68 (3.45)	<0.001
FIB-4 index	2.76 (1.50)	7.51 (2.67)	<0.001
APRI	1.02 (0.76)	3.07 (1.83)	<0.001
ALBI	-2.79 (0.29)	-2.39 (0.43)	<0.001

Data are expressed as the mean (standard deviation).

Table 3.

	non-HCC (n=95)	HCC (n=7)	*p*-value	
			Univariate analysis	Multivariate analysis
Male/Female	44/51	5/2		
Age	64.4 (11.4)	72.1 (8.4)		
Prothrombin time (%)	89.6 (9.6)	80 (8.8)	0.01	0.05
Total bilirubin (mg/dL)	0.75 (0.25)	0.86 (0.31)		
AST (U/L)	48.4 (29.4)	48.4 (13.8)		
ALT (U/L)	50.9 (44.4)	42.1 (20.6)		
Albumin (g/dL)	4.1 (0.3)	3.9 (0.3)		
AFP (ng/mL)	7.6 (22.2)	11 (5.9)	0.004	0.89
M2BPGi	2.21 (1.59)	2.71 (2.45)		
FIB-4 index	2.68 (1.51)	3.77 (0.80)	0.009	0.04
APRI	1.01 (0.78)	1.10 (0.38)		
ALBI	-2.81 (0.29)	-2.60 (0.29)		

Data are expressed as the mean (standard deviation).

Table 4.

	non-HCC (n=95)	HCC (n=7)	*p*-value	
			Univariate analysis	Multivariate analysis
Prothrombin time (%)	90.5 (11.2)	83.3 (11.4)	0.04	0.45
Total bilirubin (mg/dL)	0.74 (0.28)	0.89 (0.25)		
AST (U/L)	24.6 (9.3)	27 (3.7)	0.04	0.64
ALT (U/L)	18.0 (11.0)	16.4 (6.3)		
Albumin (g/dL)	4.3 (0.3)	4.1 (0.4)		
AFP (ng/mL)	3.9 (3.6)	5.7 (2.2)	0.009	0.35
M2BPGi	0.95 (0.44)	2.26 (1.42)		
FIB-4 index	2.23 (1.27)	3.12 (0.66)	0.006	0.05
APRI	0.49 (0.26)	0.57 (0.20)		
ALBI	-2.91 (0.26)	-2.71 (0.28)		

Data are expressed as the mean (standard deviation).

Figure 1.

- The FIB-4 index (ref.22)
  age (years) × (AST) [U/L] / (PLT count [10^9/L] × (ALT) [U/L] ^ 1/2)

- The APRI score (ref. 23)
  (AST/upper limit of normal of AST)/PLT count × 100

- The ALBI score (ref. 24)
  [(log10 bilirubin (μmol/L) × 0.66) + (Albumin (g/L) × -0.085)

Figure 2.

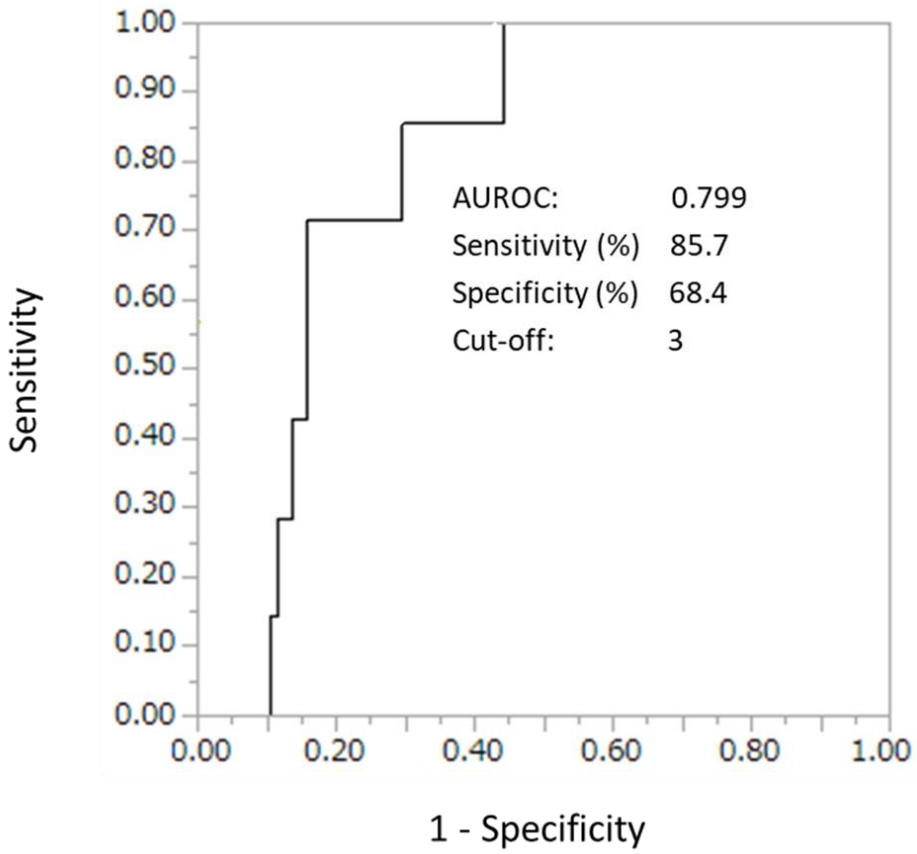
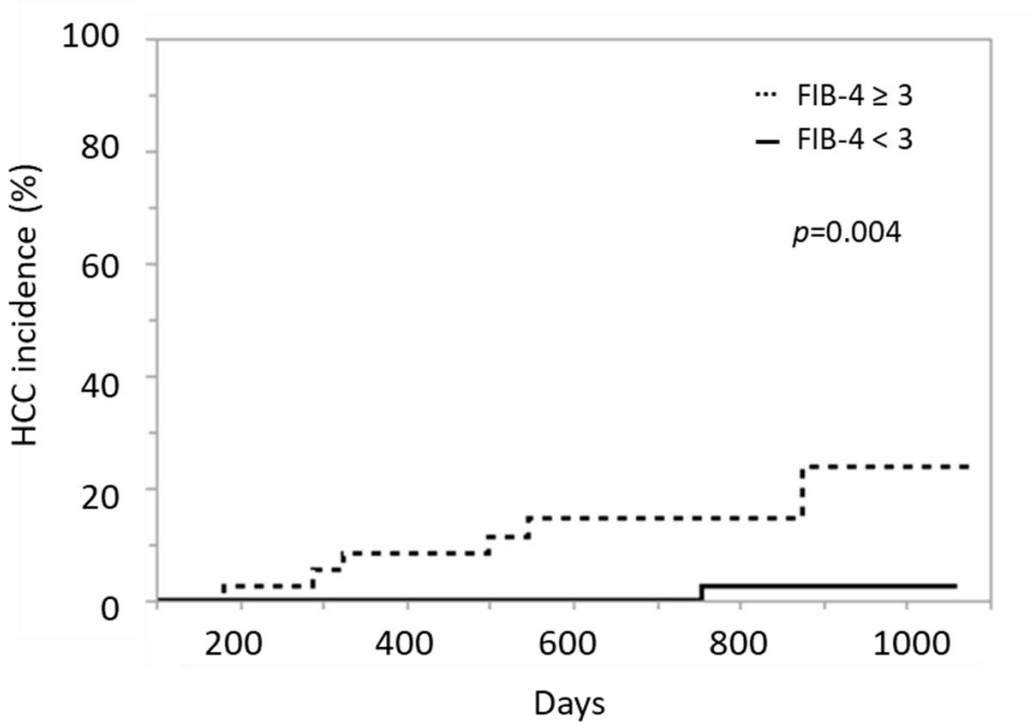

1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Sensitivity
AUROC: 0.799
Sensitivity (%) 85.7
Specificity (%) 68.4
Cut-off: 3
0.00
0.20
0.40
0.60
0.80
1.00
1 - Specificity

Figure 3.


100
80
60
40
20
0
HCC incidence (%)
200
400
600
800
1000
Days
FIB-4 ≥ 3
FIB-4 < 3
p=0.004