Showa Univ J Med Sci **32**(2), 125～133, June 2020

Original

Simple Stratification of Hepatocellular Carcinoma Surveillance after Direct-acting Antiviral Therapy for Chronic Hepatitis C

Tianpeng WANG, Masashi SAKAKI*, Yuki ICHIKAWA,
Yumi OTOYAMA, Yoko NAKAJIMA, Ikuya SUGIURA,
Jun ARAI, Atsushi KAJIWARA, Shojiro UOZUMI,
Yuu SHIMOZUMA, Manabu UCHIKOSHI and Hitoshi YOSHIDA

Abstract: Reports on surveillance systems useful for determining the risk of developing hepatocellular carcinoma (HCC) after direct-acting antiviral (DAA) treatment for hepatitis C have been published. Liver cirrhosis (LC) is a high-risk factor for HCC, but the evaluation frequency necessary for patients with chronic hepatitis (CH) remains unknown. Here, we aimed to identify how frequent CH patients should be evaluated for HCC, with particular emphasis on patients achieving a sustained virological response (SVR) with DAA treatment. Data were collected pre-treatment (Pre) and at the time of SVR for 141 patients with hepatitis C receiving DAA treatment. We defined LC by a platelet (PLT) count $\leq 10\times10^4$/μl, and CH was defined by a PLT count of $> 10\times10^4$/μl. The incidence of HCC in patients with CH after achieving SVR was retrospectively evaluated. 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). Although fibrosis-4 (FIB-4) index, the presence of α-fetoprotein, and prothrombin time were significant risk factors for HCC in patients with CH in the univariate analysis, only the Pre-FIB-4 index was an independent predictive factor for HCC development in the multivariate analysis ($p=0.04$). An 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 ($p=0.004$). Frequent HCC examination is recommended for FIB-4 index ≥3 CH patients who obtain SVR after DAA treatment.

Key words: FIB-4 index, hepatocellular carcinoma, chronic hepatitis C, direct-acting antiviral, platelet count

Introduction

Direct-acting antiviral (DAA) therapy is the first therapeutic choice for eradicating hepatitis C virus (HCV), since it has been shown to enable a sustained virological response (SVR) in almost 100% of the patients receiving it[1–4]. However, key issues remain, including treatment

Department of Medicine, Division of Gastroenterology, Showa University School of Medicine, 1–5–8 Hatanodai, Shinagawa-ku, Tokyo 142–8666, Japan.
* To whom corresponding should be addressed.

failure, acquisition of antiviral resistance[5, 6], and development of HCC. Similar to interferon (IFN) therapy, DAA treatment improves liver status and suppresses HCC development[7–11]. Only a few reports have evaluated the risk of HCC developing after DAA treatment. In order to assess the risk of HCC developing after DAA therapy, α-fetoprotein (AFP), fibrosis-4 (FIB-4) index, and post-treatment levels of Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA^+M2BP) have been considered as useful screening biomarkers[7, 8, 12]. However, the risk stratification still remains unclear because of the differences in inflammatory response in liver diseases. As liver cirrhosis (LC) is a well-known high-risk factor of HCC even in cases where treatment with DAA has been successfully administered[13], estimating the incidence of HCC after SVR is challenging, 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 analysis, we tentatively defined LC and CH in the present study using platelet (PLT) counts. Several studies have shown that a PLT count of less than 10^5/μl indicates advanced liver fibrosis and significant cirrhosis[14–16] and is linked to a high incidence of HCC[17–19]. Kang *et al.* showed that a PLT count less than 10^5/μl was associated with a high incidence of underlying liver cirrhosis[20]. We classified liver status according to PLT count so that if the PLT count was higher than 10^5/μl, we considered the liver status as CH, and if the PLT count was lower than 10^5/μl, we considered it as LC.

An HCC detection surveillance algorithm has been published for determining the frequency image examination for LC and CH patients[21]. However, data on improvement of liver function after DAA treatment was not considered. We therefore performed a study to clarify the appropriate frequency of surveillance for HCC detection, with particular emphasis on patients with CH who successfully achieved SVR.

Methods

2.1. Study population

In total, 141 patients with HCV-related liver disease were treated with IFN-free DAA therapy from October 1, 2014 through July 31, 2018 in a single-center hospital. Among these, 131 patients (93%) achieved "SVR-12," which means that these patients were 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 prior to 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 performed in compliance with the Declaration of Helsinki and the 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 collated baseline data, including data on age, sex, and HCV genotype, and extracted relevant laboratory test results at both pre-treatment (Pre) and post-SVR time points. With

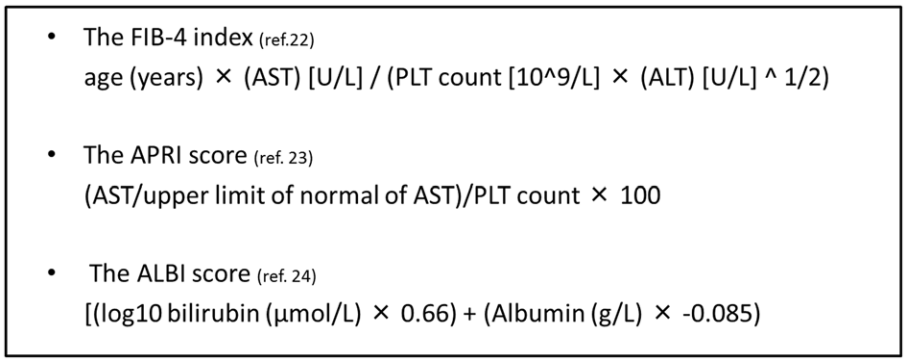

Fig. 1. The formulas for calculation of surrogate markers of liver fibrosis and liver function

respect to PLT counts, we defined LC by a PLT count less than 10^5/μl and CH by 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 formulas for calculating these surrogate markers are shown in Figure 1.

2.3. Incident cases of HCC

Incident cases of HCC were defined as those patients who developed HCC after having 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), and gadoxetate disodium-enhanced magnetic resonance imaging (EOB-MRI). The diagnosis of HCC was established 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 for HCC development after DAA treatment were evaluated using Cox proportional hazards model. The HCC occurrence rate was evaluated by Kaplan-Meier curves. The cumulative incidence of HCC was evaluated by the log-rank test. Statistical analysis was performed using JMP® Pro 14.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

3.1. Patient characteristics

The characteristics of the 128 patients with SVR and SVR data are shown in Table 1. DAA treatment significantly reduced liver inflammation and fibrosis markers during SVR without, however, affecting PLT counts. This suggests that the PLT count reflects the actual liver status regardless of HCV presence. Furthermore, PLT count can be used as a proxy to differentiate between the liver status of patients with CH and those with LC, as shown in Table 2. Seven

Table 1. Clinical characteristics at pre-and post-DAA treatment time points of the 128 HCV patients achieving SVR

Male/Female (*n*)	59/69		
Age (median)	65.6 (68)		
	Pre	SVR	*p* value
Platelet count ($\times 10^4$/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). SVR: sustained virological response

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

	CH ($n = 102$) PLT > 10×10^4/μl	LC ($n = 12$) PLT ≤ 10×10^4/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).

patients with CH and three patients with LC developed HCC after achieving SVR.

3.2. Risk factors associated with hepatocellular carcinoma (HCC)

Data from pre- and post-SVR following DAA treatment were evaluated because the pre-SVR records covered data on both the inflammatory response in the presence of HCV and cumulative liver damage. In contrast, DAA treatment can induce an immediate improvement of

Table 3. Factors affecting HCC incidence in patients with CH at the time before DAA treatment

	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. Factors affecting HCC incidence in DAA-treated patients with CH at the time of SVR

	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).

inflammation at the time of SVR[26]. The SVR point may reflect true accumulated liver damage. Multivariate analyses were performed for data obtained pre-treatment and post-SVR points, taking into consideration fibrosis parameters such as FIB-4 index, APRI, and ALBI grade. At the pre-treatment point, prothrombin time (PT)%, AFP, and FIB-4 index significantly deferred 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 time of SVR, PT%, AST, AFP, and FIB-4 index were identified as parameters predictive of HCC incidence by univariate analysis. Although there were no significant differences, the FIB-

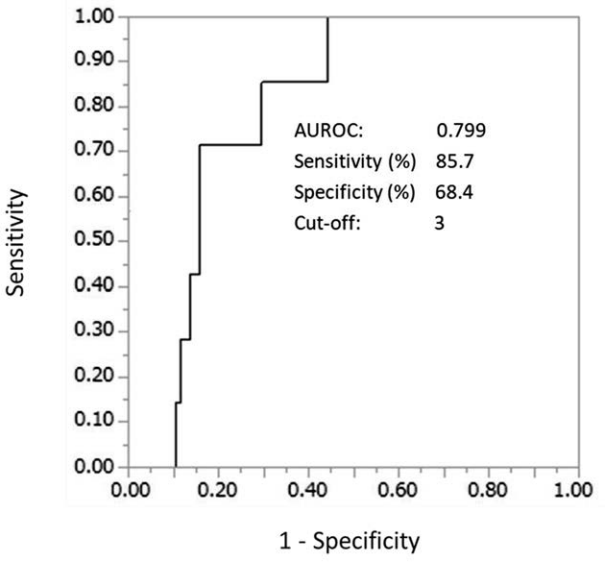

Fig. 2. The ROC curve for the pre-treatment fibrosis-4 (FIB-4) index

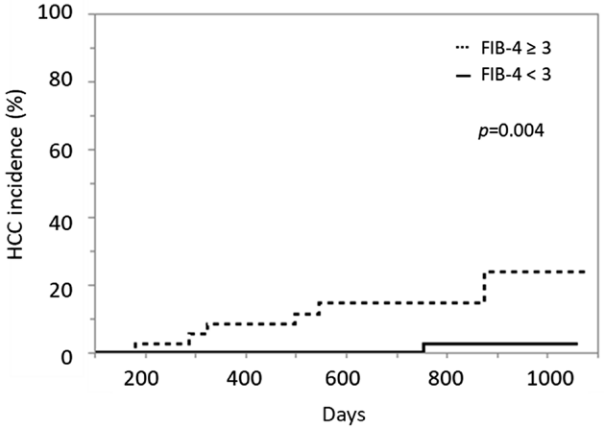

Fig. 3. Cumulative incidence (%) of HCC in patients with CH who achieved a sustained virological response after DAA treatment compared with the pre-treatment fibrosis-4 (FIB-4) index. The cumulative incidence increased significantly in patients with CH and a FIB-4 index ≥ 3 ($p = 0.004$, log-rank test).

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 pre-treatment point is shown in Figure 2. The area under the ROC curve was 0.799. Based on this result, a FIB-4 index of 3 was defined as the optimum cut-off value. Moreover, a FIB-4 index ≥ 3 was significantly associated with a high risk of developing HCC (hazard ratio [HR] = 11.7; 95% confidence interval [CI], 2.0–220.2; $p = 0.0046$). In addition, the cumulative risk for HCC at 1,000 days was 2.6% in the FIB-4 index < 3 group compared with 24.2% in 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).

Discussion

IFN-free DAA treatment can lead to HCV clearance in almost 100% of treated cases without severe complications. Although viral clearance is serologically achieved, it should be kept in mind that HCC could subsequently develop, despite the fact that patients with SVR have a reduced risk of HCC[9, 10]. In the present study, we evaluated the risk of developing 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 tend to differ from case to case. According to scientific literature, there are factors that can be used to predict HCC development, 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 risk of HCC development in patients with CH, and we identified the risk factors for HCC. However, it is challenging to evaluate the extent of liver inflammation and fibrosis in patients with CH and LC in the absence of data from liver biopsy analysis. Liver biopsy is an invasive procedure, which may result in complications, such as pain, hemorrhage, and even mortality[28]. For these reasons, liver biopsy is not commonly performed in this group of patients, particularly not in those who have received DAA treatment. Patients receiving DAA exhibit higher SVR rates and lower adverse reaction rates compared with those receiving IFN therapy.

Here, we used PLT counts to differentiate between CH and LC. Our data support the use of PLT counts to delimit the extent of liver pathology, by linking the values with CH and LC groups; moreover, DAA treatment did not affect the PLT count.

In the CH group, PT%, AFP, and FIB-4 index as measured pre-treatment and at the time of SVR were risk factors for HCC, but only the pre-treatment FIB-4 index was identified as an independent risk factor of HCC by multivariate analysis. Moreover, in the log-rank test, an FIB-4 index ≥ 3 was predictive of HCC. These results suggest that classifying liver status based on PLT count and stratifying the risk of HCC incidence using the FIB-4 index enables feasible and convenient CH follow-up for surveillance of HCC developing after DAA therapy. The follow-up time interval after DAA therapy would be decided by the physicians based on their respective experience. In general, patients with CH would not need to undergo surveillance for HCC every two to three months, which is recommendable for LC patients. However, even CH patients should have a periodical inspection for HCC. The question remains, however, as to how often and which marker is most efficient. We believe that our study has provided suggestions for appropriate markers and follow-up periods relevant to HCC surveillance after DAA therapy. In other words, HCV patients before DAA treatment with a PLT count $> 10^5/\mu l$ and an FIB-4 index ≥ 3 are at a high risk of developing HCC 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 the stratification of CH and LC patients when assessing the risk of HCC

onset. More evaluations are necessary with more cases for longer observation periods.

Conclusion

A FIB-4 index ≥3 in CH patients who have obtained SVR after DAA treatment are considered to be at high risk of developing HCC, and frequent surveillance is recommended.

Acknowledgments

None

Conflicts of Interest disclosure

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**:1878–1887.
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**:1867–1877.
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**:645–653.
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**:557–565.
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**:1924–1935.
6) Vermehren J, Sarrazin C. The role of resistance in HCV treatment. *Best Pract Res Clin Gastroenterol*. 2012;**26**: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**:933–939.
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**:136–146.
9) Ioannou GN, Green PK, Berry K. HCV eradication induced by direct-acting antiviral agents reduces the risk of hepatocellular carcinoma. *J Hepatol*. 2017. (accessed 2017 Sep 5) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5837901/pdf/nihms904333.pdf
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**:411–421.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;**393**:1453–1464.
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**:476–483.
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**: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**:572–577.

15) Pradella P, Bonetto S, Turchetto S, *et al*. Platelet production and destruction in liver cirrhosis. *J Hepatol*. 2011;**54**: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**:790-796.
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**:1089-1097.
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**:1249-1255.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**:520-527.
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**:81-89.
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**: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**:32-36.
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**:518-526.
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**:550-558.
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. (accessed 2013 Jan 15) Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3545220/pdf/srep01065.pdf
26) Singh S, Facciorusso A, Loomba R, *et al*. 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**: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*. 2019;**51**:681-688.
28) Rockey DC, Caldwell SH, Goodman ZD, *et al*. Liver biopsy. *Hepatology*. 2009;**49**:1017-1044.

[Received November 25, 2019 : Accepted January 9, 2020]