Prediction of liver stiffness for hepatocellular carcinoma in chronic hepatitis C patients on interferon-based anti-viral therapy

Liver stiffness predicts hepatocellular carcinoma in chronic hepatitis C patients on interferon-based anti-viral therapy

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Short running title: Liver stiffness associated with HCC risk

Abstract

Background and Aim: The purpose of this study was to evaluate the usefulness of liver stiffness measurement (LSM) for assessing the risk of hepatocellular carcinoma (HCC) in chronic hepatitis C (CHC) patients receiving interferon (IFN) therapy.

Methods: One hundred fifty-one CHC patients who underwent LSM and received IFN therapy were included in the estimation cohort, and 56 were included in the validation study. The cumulative HCC incidences were evaluated using Kaplan-Meier plot analysis and the log-rank test. Multivariate Cox proportional hazard analyses were used to estimate the hazard ratios (HRs) of variables for HCC.

Results: In the estimation cohort, 9 of 151 patients developed HCC during the median follow-up time of 722 days. Multivariate analysis identified 3 independent risk factors for HCC: LSM (≥14.0 kPa, HR 5.58, P = 0.020), platelet count (<14.1 × 104/μL, HR 5.59, P = 0.034), and non-sustained virological response (HR 8.28, P = 0.049). The cumulative incidence of HCC development at 3 years was 59.6%, 8.2%, and 0.0% in patients with all 3 risk factors, 1–2 risk factors, and none of these risk factors, respectively. The incidence of HCC was significantly different between these groups (P < 0.001). In the validation cohort, HCC incidence was also significantly different with respect to these risk factors (P = 0.037).

Conclusion: LSM, platelet count, and IFN-therapeutic effect could be used to successfully stratify the risk of HCC in patients receiving IFN therapy and demonstrate the usefulness of LSM before IFN therapy for the management of CHC patients.

Keywords: chronic hepatitis C, liver stiffness, hepatocellular carcinoma, risk factor

Introduction

Persistent hepatitis C virus (HCV) infection is one of the major causes of chronic liver disease leading to the development of HCC, the fifth most common cancer, and the third most common cause of cancer-related death worldwide (1). HCV is responsible for 27–75% of the HCC cases in Europe and the United States and >80% of the HCC cases in Japan (2, 3). In fact, HCV-positive patients have a 20-fold higher risk of developing HCC than HCV-negative patients (4), indicating a significant carcinogenic role for persistent HCV infection. Because of this connection, many chronic hepatitis C (CHC) patients are treated with interferon (IFN)-based anti-viral therapy because it not only eradicates HCV but also reduces the rate of HCC development. IFN therapy is most effective at decreasing the risk of developing HCC in patients that achieve a sustained virological response (SVR) (5-7); however, the risk of HCC development persists after IFN therapy even in patients who do achieve SVR (8). HCC might develop immediately after IFN therapy in some cases, or during long-term IFN therapy in others (9, 10). Because assessing the risk of developing HCC is clinically important in the management of CHC patients, it is necessary to establish predictors for HCC development in patients who receive IFN therapy.

Some factors reported to predict the risk of HCC development after IFN therapy are older age, male gender, and severe fibrosis (11,12), with advanced fibrosis and cirrhosis significantly correlating with the risk of HCC development (13). To date, liver biopsy has been the gold standard for assessing the severity of liver fibrosis and cirrhosis (14), although sampling errors and intra- and inter-observer variability can lead to understaging (15, 16). In addition, it is difficult to perform liver biopsy for all patients

because of its invasiveness and rare but potentially life-threatening complications (14). As a result, liver stiffness measurement (LSM), a type of transient elastography, has become a reliable alternative for assessing hepatic fibrosis and cirrhosis mainly in patients with CHC (17, 18). LSM is noninvasive, reproducible, can be expressed numerically as continuous values, and has a wide dynamic range in the evaluation of hepatic fibrosis. These advantages over liver biopsy suggest the clinical usefulness of LSM for predicting HCC development. Here, we evaluated factors that affect the occurrence of HCC in CHC patients receiving IFN therapy, with a special focus on the predictive value of LSM.

Methods

Patients

Between October 2007 and April 2011, a total of 207 consecutive CHC patients who underwent a successful liver stiffness measurement (LSM) and then received IFN-based anti-viral therapy at the Department of Gastroenterology and Hepatology, Juntendo University Shizuoka Hospital, Shizuoka, Japan, were retrospectively enrolled in this study. CHC diagnosis was based on serum HCV-RNA positivity. Exclusion criteria were as follows: 1) hepatitis B surface antigen positivity; 2) other causes of liver disease of mixed etiologies, including autoimmune hepatitis, primary biliary cirrhosis, hemochromatosis, and Wilson's disease; 3) evidence of hepatocellular carcinoma on ultrasonography or CT; 4) previous history of liver transplantation; and 5) treatment for HCC. This study was approved by the Ethics Committee of Juntendo University Shizuoka Hospital in accordance with the Helsinki Declaration, and all patients

provided written informed consent.

Of these 207 patients, 151 underwent ultrasonography-guided percutaneous liver biopsy within a week before treatment initiation. Liver biopsy specimens were embedded in paraffin and stained with hematoxylin-eosin, Azan-Mallory, and reticulin silver impregnation. The specimens were evaluated by an experienced pathologist who was blinded to the patients' clinical data. Histological evaluation was based on the METAVIR criteria (19). Hepatic fibrosis was defined as follows: F0, no fibrosis; F1, periportal fibrous expansion; F2, portal fibrous widening with bridging fibrosis; F3, bridging fibrosis with lobular distortion; and F4, liver cirrhosis. On the basis of the degree of lymphocyte infiltration and hepatocyte necrosis, inflammation was scored from A0 to A3, with higher scores indicating more severe inflammation. The 151 patients who underwent liver biopsy were enrolled into the estimation group for the identification of risk factors for HCC development, and the remaining 56 patients who did not undergo liver biopsy were enrolled into a group for the validation of these identified risk factors.

All laboratory tests were performed for each patient just before initiation of IFN therapy. Blood cell counts, serum alanine transaminase (ALT), gamma-glutamyl transpeptidase (γGTP), hemoglobin A1c (HbA1c), total bilirubin, albumin, prothrombin time, and alpha-fetoprotein (AFP) were measured using commercially available assays. The HCV genotype was determined using polymerase chain reaction with the HCV Genotype Primer Kit (Institute of Immunology Co., Ltd., Tokyo, Japan) and classified as genotype 1, genotype 2, or other, according to Simmonds' classification system. Serum HCV viral load was determined using quantitative reverse transcription polymerase chain reaction using the COBAS TaqMan HCV Test (Roche Diagnostics,

Branchburg, NJ).

Treatment protocol

The treatment protocol for CHC patients consisted of 1.5 μg/kg of pegylated IFN-α-2b or 180 μg of pegylated IFN-α-2a once a week, combined with ribavirin at an oral dose of 600–1000 mg/day. Duration of the treatment was 48–72 weeks for those with HCV genotype 1 and a serum HCV viral load > 5 log IU/mL. For all other patients, treatment lasted for 24 weeks. SVR was defined as undetectable serum HCV-RNA at 24 weeks after the end of treatment.

Measurement of liver stiffness

Measurement of liver stiffness by transient elastography was performed using FibroScan® (Echosens, Paris, France) within a week before treatment initiation. Technical details of the examination and procedure have been reported previously (17). Ten validated measurements were made on each patient, and results were expressed in kilopascals (kPa). Only procedures with 10 validated measurements and a success rate of at least 60% were considered reliable, and the median value was considered representative of the liver elastic modulus.

Patient follow-up and HCC diagnosis

Serum AFP was measured every month, and ultrasonography or computed tomography were performed at least every 3 to 6 months for HCC surveillance during and after treatment, with a minimum follow-up duration of 6 months after the initiation of IFN therapy. HCC was diagnosed by histological examination and/or triphasic

computerized tomography, in which hyperattenuation in the arterial phase with washout in the late phase is pathognomonic for HCC (20). The status of patients enrolled in this study was confirmed as of March 2012.

Statistical analyses

All analyses were conducted using IBM SPSS version 19 (IBM SPSS, Chicago, IL, USA), and p values less than 0.05 were considered statistically significant. Continuous variables and categorical variables were summarized as median (range) and percentage, respectively. Mann Whitney-U and chi-square tests were used when appropriate. The strength of the association between LSM and the histological fibrosis stage was estimated using the Spearman's rank correlation coefficient. Cumulative incidences of HCC development were estimated by Kaplan-Meier analysis and compared using the log-rank test. Cox logistic regression analysis was used for multivariate analysis to identify factors that were independently associated with HCC development. The cutoff value of each factor for predicting the development of HCC was determined using receiver operator characteristics analysis.

Results

Patient characteristics

A total of 229 patients received LSM followed by IFN-based anti-viral therapy at Juntendo Shizuoka Hospital during the study period. Twenty-two patients (9.6%) were excluded because of LSM failure and/or an invalid LSM. Of the remaining 207 patients, 151 underwent liver biopsy prior to IFN therapy, and together formed the risk

factor-estimation cohort. The clinical, anthropometric, and laboratory data of the estimation cohort are summarized in Table 1. The 151 patients (83 male and 68 female) had a median age of 62 years (range, 22–82 years) and a median LSM of 8.8 kPa (range, 2.8–45.7 kPa). There was a significant positive association between LSM and histological fibrosis stage ($r = 0.59$, $P < 0.001$). The prevalence of genotype 1 HCV infection was 56.3%. Following IFN-based anti-viral therapy, SVR was obtained in 83 of the 151 patients (55%). During the median follow-up period of 722 days (range, 189–1378 days), 9 patients (6.0%) developed HCC. The cumulative incidence of HCC estimated using the Kaplan-Meier method was 1.3%, 4.5%, and 9.0% at 1, 2, and 3 years, respectively (Fig. 1). Compared with patients who had not developed HCC, HCC patients were of advanced age and had a high LSM, a high fibrosis stage, a low platelet count, and a low SVR rate (Table 1).

Risk analyses

Univariate analysis revealed that age ($P = 0.029$), LSM ($P = 0.005$), platelet count ($P = 0.002$), AFP ($P = 0.003$), and non-SVR ($P = 0.011$) were associated with HCC development (Table 2). Multivariate Cox logistic regression analysis identified 3 independent risk factors: LSM ≥ 14.0 kPa (HR 5.58, 95% CI 1.32–23.64, $P = 0.02$), non-SVR (HR 8.28, 95% CI 1.01–68.05, $P = 0.049$), and platelet count $< 14.1 \times 10^4/\mu L$ (HR 5.59, 95% CI 1.14–27.53, $P = 0.034$). The 1-, 2-, and 3-year cumulative incidence rates of HCC development in patients with LSM < 14.0 kPa were 0.8%, 2.3%, and 4.6%, respectively, whereas those with LSM ≥ 14.0 kPa were 3.2%, 12.0%, and 22.2%, respectively ($P = 0.005$) (Fig. 2A). The cumulative incidence rates of HCC development in patients with SVR were 0.0%, 2.0%, and 2.0%, respectively, whereas those without

SVR were 3.0%, 7.4%, and 17.1%, respectively (P = 0.011) (Fig. 2B). The cumulative incidence rates of HCC development in patients with a platelet count ≥ 14.1 × 10^4/μL were 0.0%, 0.0%, and 4.2%, respectively, whereas those with a platelet count < 14.1 × 10^4/μL were 4.0%, 13.4%, and 19.1%, respectively (P = 0.002) (Fig. 2C).

Number of risk factors and HCC development

The number of risk factors varied between patients: 12 patients (7.9%) had all 3 risk factors; 32 patients (21.2%) had 2; 50 patients (33.1%) had 1, and 57 patients (37.7%) had none of these risk factors (Fig. 3). Patients without these risk factors did not develop HCC during the study period. In patients with 1 or 2 risk factors, the cumulative incidence rates at 1, 2, and 3 years were 1.2%, 3.1%, and 8.2%, respectively, whereas patients with all 3 risk factors had significantly higher cumulative incidence rates (9.1%, 39.4%, and 59.6% at 1, 2, and 3 years, respectively; log-rank test, P < 0.001) (Fig. 4).

The relationship between the number of risk factors and HCC development in the validation cohort

Fifty-six patients who received IFN therapy without liver biopsy were enrolled into the validation group for analysis of these 3 risk factors. The 56 patients (33 male and 23 female) had a median age of 65 years (range, 35–79 years) and a median LSM of 8.0 kPa (range, 2.6–32.0 kPa). There were no significant differences in clinical, anthropometric, and laboratory findings between the validation and estimation cohorts (data not shown). In the validation cohort, 7 patients (12.5%) had all 3 risk factors, 25 patients (44.6%) had 1 or 2 risk factors, and 24 patients (42.9%) had none of these risk

factors. Patients without these risk factors did not develop HCC during the study period. In patients with 1 or 2 risk factors and patients with all 3 risk factors, the cumulative incidence rates at 3 years were 12.7% and 28.6%, respectively. There was also a significant difference in the cumulative incidences of HCC development according to the number of risk factors (P = 0.037, Fig. 5).

Discussion

Patients with liver cirrhosis or pre-existing severe hepatic fibrosis have a higher risk of developing HCC (2), even after IFN-based therapy with SVR (9, 10). Clinical diagnosis of liver cirrhosis can be easily made in cases showing stigmata of end-stage liver disease, such as ascites, jaundice, variceal bleeding, and hepatic encephalopathy; however, diagnosis becomes difficult if the liver shows compensation and normal or near normal laboratory findings. Liver biopsy has been considered the only diagnostic method for the assessment of early compensated cirrhosis, although several studies have pointed out sampling variability as a potential limitation of biopsy to diagnose cirrhosis (21, 22). Given the importance of assessing the HCC risk factors in managing CHC patients, we evaluated factors that affect the occurrence of HCC in CHC patients receiving IFN therapy, with a special focus on the predictive value of LSM as an alternative to liver biopsy.

Our data identified 3 risk factors for developing HCC after IFN therapy. Consistent with previous reports (5-7), we found that failure to achieve SVR was a significant predictor of HCC development among patients receiving IFN therapy. Although it is possible that IFN therapy itself reduces the risk of HCC (6, 7), non-SVR

patients had an approximately 8-fold higher risk of developing HCC than SVR patients. In addition, we identified both high LSM and low platelet count as significant predictors of HCC development independently of non-SVR. The LSM threshold ≥ 14.0 kPa identified here as a risk factor for HCC is in agreement with previously reported cutoff values for liver cirrhosis (15, 16), further supporting the idea that pre-existing liver cirrhosis increases the risk of HCC development. Similar to LSM, the platelet count reflects the severity of CHC (21) and is used to estimate the degree of fibrosis (23-25). Previous reports have also shown low platelet counts to represent a risk of HCC (23, 24). Our cohort showed that LSM was sometimes high even in patients without a low platelet count, whereas other patients had a low platelet count without LSM elevation. Such patients are nevertheless at risk of HCC, suggesting that LSM and platelet count indicate advanced fibrosis or compensated cirrhosis in a complementary manner.

In agreement with a previous report, our findings indicate that LSM could be used to stratify the risk of HCC development in CHC patients (26). Moreover, combination of LSM with platelet count and the IFN-therapeutic effect could be used to stratify the risk of HCC in patients receiving IFN therapy. Patients without all 3 risk factors had a very low risk of HCC development, and patients with 1 or 2 risk factors had a moderate risk. Conversely, patients with all 3 risks had an extremely high risk. In clinical practice, frequency of HCC surveillance should be decided based on HCC risk. Indeed, each of these 3 factors has previously been shown to be associated with the risk of developing HCC. However, here we have proposed a new, non-invasive risk assessment based on the combination of LSM and 2 other factors. In the present study, we did not identify advanced histological fibrosis stage F3－4 as a risk factor for HCC likely due to liver biopsy sampling variability, because patients were not excluded based

on the length of liver biopsy samples, an important factor affecting variability in histological assessment of liver fibrosis (15). Taken together, these findings suggest that LSM would be more useful than liver biopsy for diagnosis of patients with liver cirrhosis who are at high risk of HCC, especially those with compensated cirrhosis.

Our data indicate patients with all of the 3 risk factors require the most intensive HCC surveillance; however, this study does have a few limitations. One drawback is that LSM failure and unreliable results occur in some patients. In our cohort, 9.0% of patients who received LSM did not yield reliable results. Because subcutaneous fat attenuates the transmission of share waves and the ultrasonic signals into the liver used to determine LSM, obesity is the principal reason for LSM failure (27). In addition, it is likely that obesity itself is associated with an increased risk of HCC (28). As a result, our findings might not reflect the risk of HCC in obese patients. Another recent report demonstrated that a new FibroScan® XL probe, designated for use in obese patients, could reduce LSM failure and facilitate reliable results (29). A study using this new probe will more accurately evaluate the predictive value of LSM for the risk of HCC development.

In conclusion, our findings indicate that LSM, platelet count, and IFN-therapeutic effect could be used to successfully stratify the risk for HCC development in patients receiving IFN-based antiviral therapy and demonstrate the usefulness of LSM before IFN therapy for the management of CHC patients.

Acknowledgement

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Disclosure

The authors declare no conflict of interest.

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Figure legends

Fig. 1: Incidence of hepatocellular carcinoma (HCC) in 151 patients with chronic hepatitis C receiving interferon-based anti-viral therapy estimated using the Kaplan-Meier method.

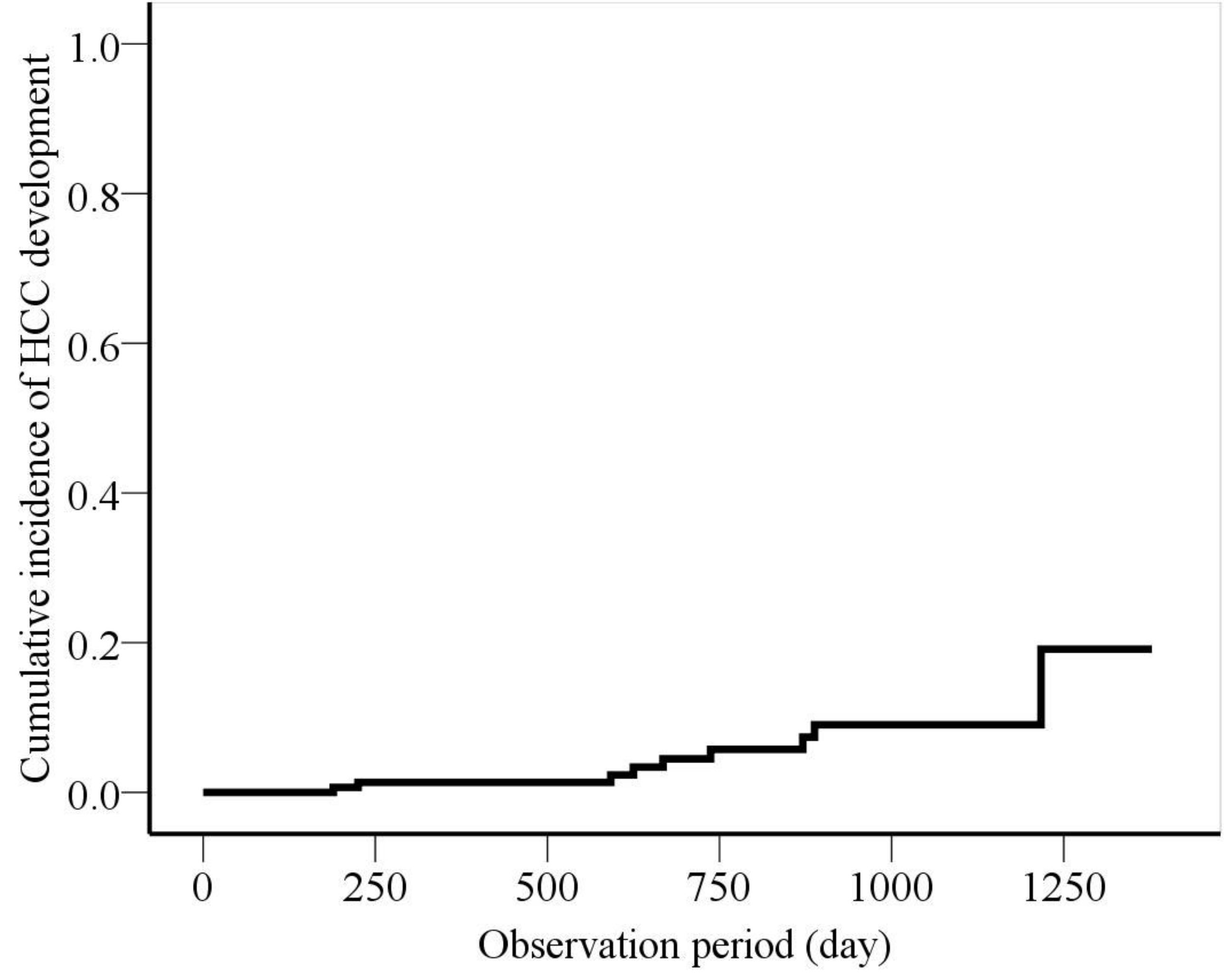
Fig. 2: Kaplan–Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development. Patients were stratified according to liver stiffness

measurement (LSM) (A), sustained virological response (SVR) (B), and platelet count (C).

Fig. 3: Patient distribution at each risk factor. LSM, liver stiffness measurement; Plt, platelet count; SVR, sustained virological response

Fig. 4: Kaplan-Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development. Patients were stratified according to the number of risk factors.

Fig. 5: Kaplan-Meier curves comparing the cumulative incidence of hepatocellular carcinoma (HCC) development in the validation cohort. Patients were stratified according to the number of risk factors they had.


Cumulative incidence of HCC development
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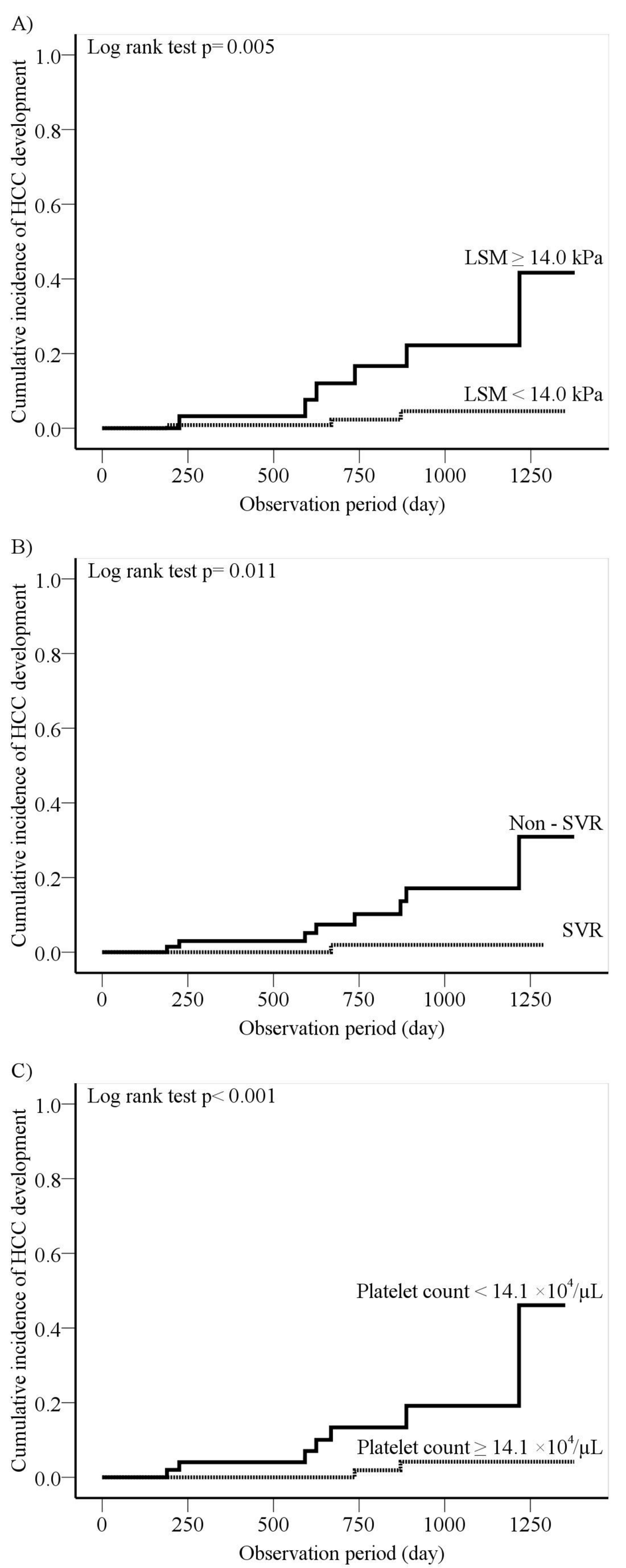
0.8
0.6
0.4
0.2
0.0
0
250
500
750
1000
1250
Observation period (day)


A)
Log rank test p= 0.005
Cumulative incidence of HCC development
1.0
0.8
0.6
0.4
0.2
0.0
LSM ≥ 14.0 kPa
LSM < 14.0 kPa
0
250
500
750
1000
1250
Observation period (day)
B)
Log rank test p= 0.011
Cumulative incidence of HCC development
1.0
0.8
0.6
0.4
0.2
0.0
Non - SVR
SVR
0
250
500
750
1000
1250
Observation period (day)
C)
Log rank test p< 0.001
Cumulative incidence of HCC development
1.0
0.8
0.6
0.4
0.2
0.0
Platelet count < 14.1 ×10^4/μL
Platelet count ≥ 14.1 ×10^4/μL
0
250
500
750
1000
1250
Observation period (day)

LSM < 14.0kPa
SVR
Plt ≥ 14.1x10⁴/μL
n=57

LSM ≥ 14.0 kPa
n=5

n=7

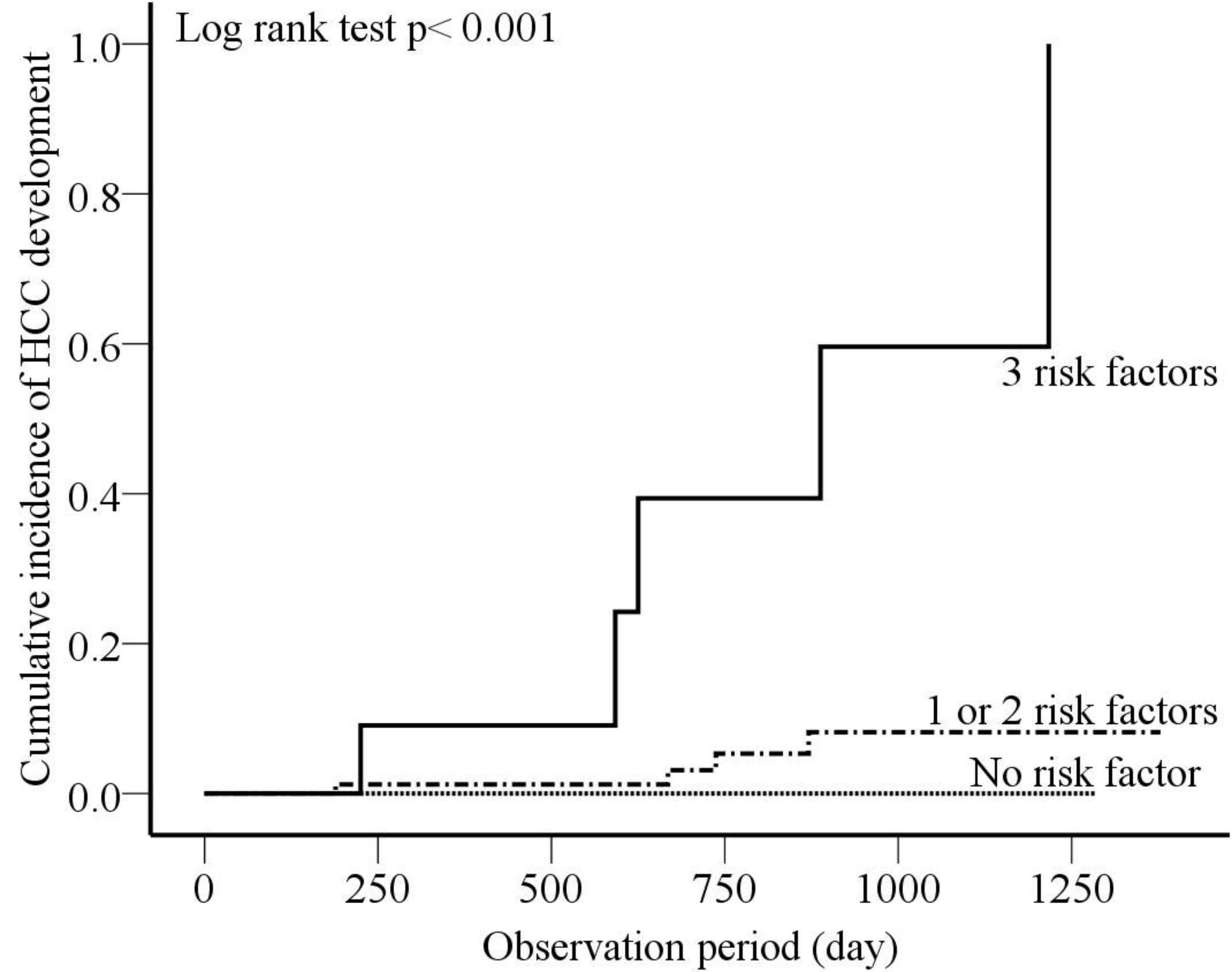
Non-SVR
n=32

n=12

n=8

n=17

Plt>14.1x10⁴/μL
n=13


Log rank test p< 0.001
Cumulative incidence of HCC development
1.0
0.8
0.6
0.4
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3 risk factors
1 or 2 risk factors
No risk factor
0
250
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1000
1250
Observation period (day)

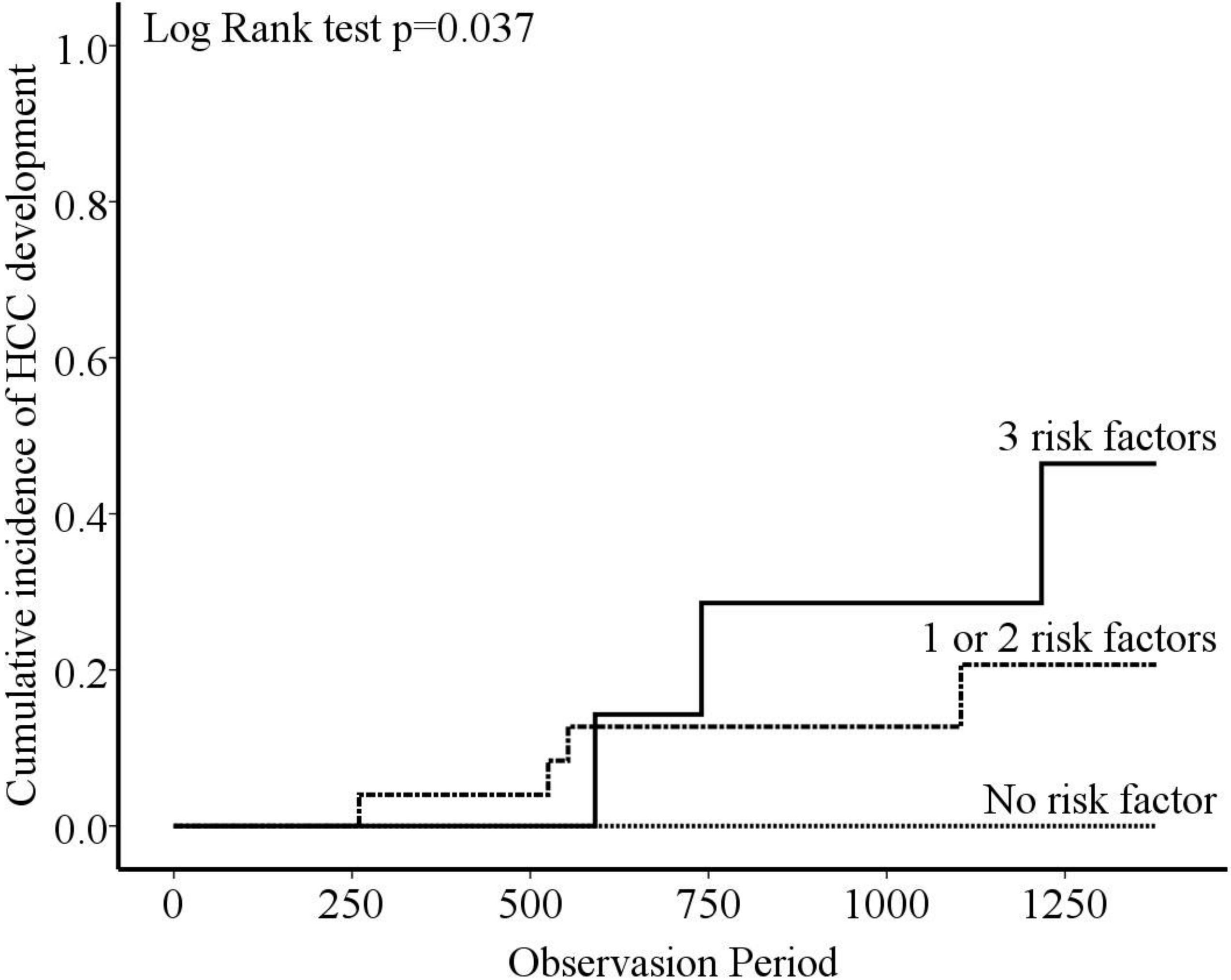
Log Rank test p=0.037
Cumulative incidence of HCC development
1.0
0.8
0.6
0.4
0.2
0.0
3 risk factors
1 or 2 risk factors
No risk factor
0
250
500
750
1000
1250
Observasion Period

Table 1: Baseline characteristics of the estimation cohort

Variables	All	HCC development (+)	HCC development (-)	P value
Number of patients	151	9	142	
Age (years)	62 (22–82)	67 (60–82)	61 (22–80)	0.010†
Male (%)	55	55.6	54.9	1.000‡
BMI (kg/m^2)	23.5 (18.1–36.8)	23.8 (23.3–25.7)	23.4 (18.1–36.8)	0.217†
Habitual drinker (%)	10.6	11.1	10.6	1.000‡
Fibrosis stage (F0–2/F3–4)	115/36	5/4	110/32	0.048‡
Inflammatory grade (A0–1/A2–3)	33/118	0/9	33/109	0.101‡
LSM (kPa)	8.8 (2.8–45.7)	14.8 (9.8–45.7)	8.7 (2.8–34.8)	0.002†
Observation period (days)	722 (189–1378)	688 (189–1217)	733 (190–1378)	0.467†
Genotype 1 (%)	56.3	100	53.5	0.065‡
HCV-RNA (log IU/mL)	6.4 (0.0–7.7)	6.5 (2.9–7.2)	6.3 (0.0–7.7)	0.168†
Albumin (g/dL)	4.1 (3.4–4.8)	4.1 (3.5–4.6)	4.1 (3.4–4.8)	0.390†
ALT (IU/L)	59 (10–410)	75 (27–181)	57 (10–410)	0.467†
Total bilirubin (mg/dL)	0.7 (0.3–1.8)	0.8 (0.5–1.3)	0.7 (0.3–1.8)	0.070†
γGTP (IU/L)	44 (4–517)	75 (31–129)	41 (4–517)	0.120†
Hemoglobin A1c (%)	5.1 (3.7–8.2)	5.1 (3.7–6.1)	5.1 (4.2–8.2)	0.561†
Ferritin (ng/mL)	134 (8–2096)	215 (8–1026)	134 (9–2096)	0.675†
White blood cell count (×10^3/μL)	4.9 (2.0–10.3)	4.3 (3.0–7.3)	4.9 (2.0–10.3)	0.496†
Hemoglobin (g/dL)	13.8 (8.9–17.5)	13.3 (9.9–17.5)	13.8 (8.9–17.1)	0.376†
Platelet count (×10^4/μL)	16.3 (5.2–37.0)	9.6 (5.2–19.4)	16.5 (5.8–37.0)	0.004†
Prothrombin time (%)	100 (70–157)	93 (79–120)	102 (70–157)	0.185†
AFP (ng/mL)	6 (1–306)	14 (4–109)	6 (1–306)	0.004†
SVR rate (%)	55	11.1	57.7	0.011‡

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; γGTP, γ-glutamyl

transpeptidase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LSM, liver stiffness measurement; SVR, sustained virological response

Scale data are shown as median (range).

P values are for comparisons between patients with and without HCC development.

† Mann-Whitney U test, ‡ chi-square test

Table 2: **Univariate analysis of factors associated with hepatocellular carcinoma development**

Variables	n	Cumulative incidence of HCC (%)		P value
		1 year	3 years	
Age (years)				
<60	63	0.0	0.0	0.029
≥60	88	2.3	13.6	
Sex				
Female	68	1.5	12.1	0.910
Male	83	1.2	6.7	
BMI* (kg/m^2)				
<23.8	50	0.0	5.3	0.250
≥23.8	42	2.4	6.0	
Habitual drinker				
No	135	0.8	9.6	0.905
Yes	16	6.2	6.2	
Fibrosis stage				
F0-2	115	0.9	6.7	0.228
F3-4	36	2.9	15.0	
LSM (kPa)				
<14	119	0.8	4.6	0.005
≥14	32	3.2	22.2	
ALT (IU/L)				
<55	71	0.0	4.9	0.123
≥55	80	2.5	12.9	
γGTP* (IU/L)				
<55	83	0.0	5.2	0.057
≥55	67	3.0	13.5	
Hemoglobin A1c* (%)				
<5.5	109	0.9	6.8	0.219
≥5.5	25	0.0	18.8	
Ferritin* (ng/mL)				
<210	74	1.4	10.0	0.175
≥210	43	2.3	16.3	
Platelet count ($\times 10^4/\mu L$)				
≥14.1	101	0.0	4.2	0.002

<14.1	50	4.0	19.1	
AFP * (ng/mL)				
<10	95	0.0	5.6	0.003
≥10	38	4.9	22.3	
SVR				
Yes	83	0.0	2.0	0.011
No	68	3.0	17.1	

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; BMI, body mass index; γGTP, γ-glutamyl transpeptidase; HCC, hepatocellular carcinoma; LSM, liver stiffness measurement; SVR, sustained virological response

*Data not available for all patients.