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ORIGINAL ARTICLE

Mac-2-binding protein glycan isomer predicts all malignancies after sustained virological response in chronic hepatitis C

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Abstract

Despite reports of hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus (HCV) infection after achieving sustained virological response (SVR), only few studies have demonstrated the incidence of other (non-HCC)

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malignancies. This study aimed to clarify the incidence, survival probability, and factors associated with malignancy, especially non-HCC malignancies, in patients with chronic HCV infection after achieving SVR. In this retrospective study, records of 3580 patients with chronic HCV infection who achieved SVR following direct-acting antiviral (DAA) treatment were analyzed. The cumulative post-SVR incidence of non-HCC malignancies was 0.9%, 3.1%, and 6.8% at 1, 3, and 5 years, respectively. The survival probability for patients with non-HCC malignancies was 99.1%, 78.8%, and 60.2% at 1, 3, and 5 years, respectively, and the rate was significantly lower than that for patients with HCC. The Cox proportional hazards regression model identified Mac-2-binding protein glycan isomer (M2BPGi) cutoff index (COI) ≥ 1.90 at baseline and ≥ 1.50 at 12 weeks following DAA treatment as significant and independent factors associated with the post-SVR incidence of non-HCC malignancies. Furthermore, patients with either M2BPGi COI ≥ 1.90 at baseline or M2BPGi COI ≥ 1.50 at SVR12 had a significantly higher risk of post-SVR incidence of non-HCC malignancies than of HCC. *Conclusion:* M2BPGi measurements at baseline and SVR12 may help predict the post-SVR incidence of non-HCC malignancies in patients with chronic HCV infection who achieved SVR following DAA treatment. Early identification of these patients is critical to prolong patient survival.

INTRODUCTION

Chronic hepatitis C virus (HCV) infection is associated with the induction of both hepatic and extrahepatic manifestations[1–4] and is responsible for a significantly large number of deaths. Various solid and hematological malignancies are also related to chronic HCV infection.[4–12] Using interferon (IFN)–based anti-HCV therapy, achieving sustained virological response (SVR) reportedly reduces the incidence of non-Hodgkin's lymphoma.[3,13] Recently, treatment to eradicate HCV has rapidly evolved from IFN-based therapy to IFN-free therapy, which involves direct-acting antiviral agents (DAAs). This change has drastically improved both SVR rates (to approximately 100%) and treatment tolerance, even in patients with cirrhosis.[14–17] As with IFN-based therapy, DAA treatment can reduce the occurrence of hepatocellular carcinoma (HCC) by achieving SVR,[18,19] thereby improving the survival probability for patients with chronic HCV infection, including those with cirrhosis or HCC.[20–22] Meanwhile, a recent study has shown that the 5-year cumulative incidence of non-liver-related events and malignancies were 13.3% and 6.2%, respectively, in patients with HCV-related cirrhosis who achieved SVR following DAA treatment. Notably, in patients with Child-Pugh class A without any previous liver-related events who achieved SVR following DAA treatment, there was no difference in the 5-year cumulative incidence of liver-related and non-liver-related events.[23] Furthermore, in the United States, liver-related mortality decreased in patients with chronic HCV infection who achieved SVR following DAA treatment, whereas mortality associated with non-liver-related malignancies increased.[4] A nationwide study in Taiwan, which focused on all types of malignancies other than HCC (non-HCC malignancies), found that SVR achieved through DAA treatment significantly reduced the risk of gastric cancers and non-Hodgkin's lymphoma in patients aged <65 years.[24] However, in France, the post-SVR incidence of extrahepatic malignancies was higher in patients receiving either IFN-based or DAA treatment than in the general population.[25] Although the post-SVR incidence and survival probability of non-HCC malignancies has not yet been fully clarified, early detection of these malignancies is expected to improve the survival of these patients further.

Therefore, this study aimed to clarify the incidence and survival probability of HCC and non-HCC malignancies and identify factors associated with malignancy, especially non-HCC malignancies, after achieving SVR following DAA treatment in patients with chronic HCV infection.

METHODS

Patient population

A total of 4427 patients with chronic HCV infection who achieved SVR following DAA treatment between February 2012 and April 2020 at 17 different institutions in Japan (Hamamatsu University Hospital, Nippon

Medical School Hospital, Nippon Medical School Chiba Hokusoh Hospital, Ogaki Municipal Hospital, Ehime Prefectural Central Hospital, St. Marianna University Hospital, Kitasato University Hospital, Yokohama City University Medical Center, Kagawa University Hospital, Hamamatsu Medical Center, Iwata City Hospital, Seirei Hamamatsu General Hospital, Shimada Municipal Hospital, Kikkoman General Hospital, Ibaraki Medical Center, Shinmatsudo Central General Hospital, and Nagoya City University Hospital) were retrospectively enrolled in this study. Patients with chronic HCV infection were diagnosed by persistent detection of serum HCV RNA. SVR was defined as the disappearance of serum HCV RNA at 12 weeks following DAA treatment (SVR12). Each attending physician determined the surveillance methods and interval periods, but patients with chronic hepatitis underwent a medical examination at least once a year and patients with cirrhosis every 6 months. Diagnoses of non-HCC malignancies and HCC were determined by physical examination, biochemical tests including tumor markers, radiology, endoscopy, and/or pathology reports. Of the 4427 enrolled patients, 847 were excluded for the following reasons: (1) a history of HCC (n = 450) or non-HCC malignancy (n = 301) before DAA treatment; and (2) incidence of HCC (n = 86) or non-HCC malignancy (n = 10) between the treatment initiation and SVR achievement. Therefore, the records of the remaining 3580 patients were included in this analysis (Figure 1). There were 2634, 929, 8, 4, and 5 patients with HCV genotypes 1, 2, 3, other, and unknown, respectively. The number of patients who received each DAA treatment regimen was as follows: daclatasvir/asunaprevir (n = 980), sofosbuvir/ledipasvir (n = 1005), ombitasvir/paritaprevir/ritonavir (n = 274), daclatasvir/asunaprevir/beclabuvir (n = 17), elbasvir/grazoprevir (n = 178), glecaprevir/pibrentasvir (n = 412), sofosbuvir + ribavirin (n = 642), ombitasvir/paritaprevir/ritonavir + ribavirin (n = 65), sofosbuvir/ledipasvir + ribavirin (n = 4), sofosbuvir/velpatasvir (n = 2), and sofosbuvir/velpatasvir + ribavirin (n = 1).

Laboratory tests

Hematological and biochemical parameters, including white blood cell counts, hemoglobin (Hb) concentration, platelet (PLT) counts, total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (GGT), total protein, albumin (Alb), total cholesterol, low-density lipoprotein–cholesterol, blood urea nitrogen, creatinine (Cre), glycosylated hemoglobin (HbA1c), Mac-2-binding protein glycan isomer (M2BPGi), and alpha-fetoprotein (AFP) levels were measured using standard laboratory methods. Serum HCV-RNA concentrations were measured with reverse-transcription polymerase chain reaction using commercial kits at the respective institutions. The fibrosis-4 (Fib-4) index and estimated glomerular filtration rate (eGFR; ml/min/1.73 m^2) were calculated as follows: (1) Fib-4 index = age (year) × (AST [U/l]/PLT count [$\times 10^9$/l]) × (ALT [U/l])$^{1/2}$ and (2) eGFR = 194 × (Cre [mg/dl])$^{-1.094}$ × (age [year])$^{-0.287}$ × 0.739 (if female).

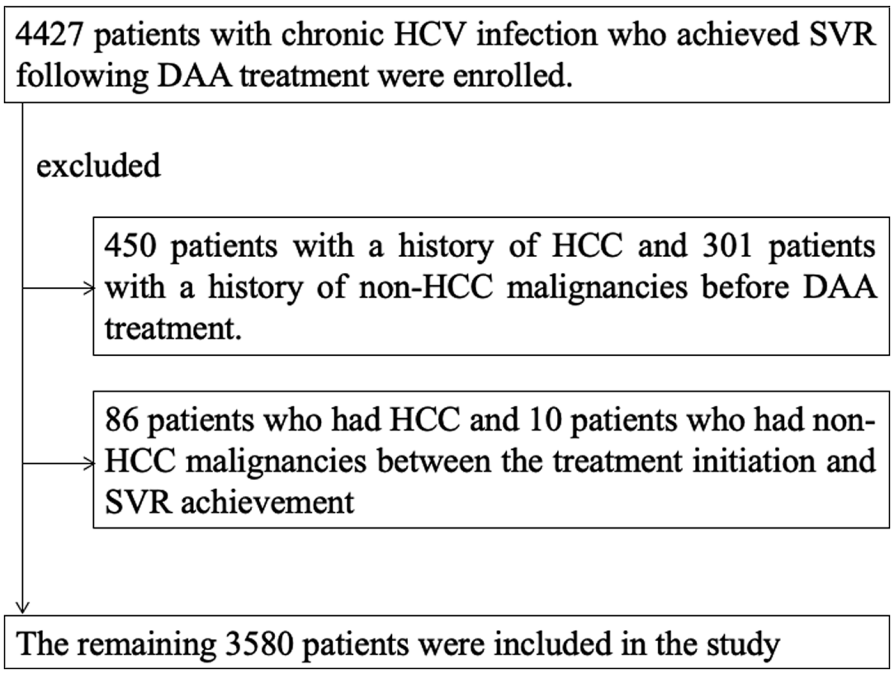

FIGURE 1 Flowchart of the patient selection process. Abbreviations: DAA, direct-acting antiviral agents; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; SVR, sustained virological response

Statistical analyses

Statistical analyses were performed using GraphPad Prism, version 7.0 (GraphPad Software, San Diego, CA, USA) and IBM SPSS Statistics for Macintosh, version 26 (IBM Corp., Armonk, NY, USA). Data of patient characteristics are presented as numbers for categorical data and mean ± SDs or medians (interquartile ranges) for continuous variables. The age-standardized incidence rate of post-SVR non-HCC malignancies was calculated using the 1985 population model of Japan as the standard population. As appropriate, categorical data were evaluated to identify the differences between two groups using Fisher's exact or chi-square test. Continuous variables were evaluated using the Mann–Whitney U test. One-way analysis of variance was performed for differences among three or more groups, followed by the Kruskal-Wallis test. The cumulative incidence and probability of survival associated with HCC or non-HCC malignancies were assessed using the Kaplan–Meier method. Differences among the cumulative rates were assessed using the log-rank test. Youden's index was used to determine the optimal cutoff values of receiver operator characteristic curves. Backward stepwise selection for Cox proportional hazards models was used to identify significant and independent factors associated with the post-SVR incidence of non-HCC malignancies. Male sex, Hb concentration, Alb level, HbA1c level, Fib-4 index, M2BPGi level, and AFP level, which have been reported as predictive factors of the incidence or progression of HCC[26–31] or other cancers[32–34] and the post-SVR incidence of HCC, were set as variables. Then, the variables were removed one by one, with the least significant one being removed first. Finally, we reported only variables that were significant at $p < 0.05$ in the final model.

RESULTS

Patient characteristics

The mean follow-up period, defined as the period between the end of DAA treatment and the final survival confirmation by each attending physician, was 2.77 ± 1.39 years. A total of 124 non-HCC malignancies developed in 121 patients. HCC developed in 155 patients after achieving SVR. Both HCC and non-HCC malignancies developed in 10 patients. The following types of non-HCC malignancies were observed: lung cancer (n = 22), gastric cancer (n = 21), colorectal cancer (n = 17), breast cancer (n = 10), pancreatic cancer (n = 9), prostate cancer (n = 7), malignant lymphoma (n = 6), bladder cancer (n = 5), intrahepatic cholangiocarcinoma (n = 4), uterine cancer (n = 4), leukemia (n = 3), renal cancer (n = 2), ovarian cancer (n = 2), extrahepatic cholangiocarcinoma (n = 2), thyroid cancer (n = 1), multiple myeloma (n = 1), esophageal cancer (n = 1), myelodysplastic syndrome (n = 1), ureter cancer (n = 1), cholangiolocellular carcinoma (n = 1), duodenal carcinoma (n = 1), acoustic neuroma (n = 1), tongue cancer (n = 1), and unknown primary cancer (n = 1). Post-SVR gastric cancer was diagnosed earlier than other non-HCC malignancies (Figure S1A). Overall, 90 patients died during the follow-up period. The causes of death are given in Table 1. The survival probabilities of patients with post-SVR pancreatic cancer and lung cancer were lower than that of patients with other non-HCC malignancies. The median survival duration was 2.69 years for post-SVR pancreatic cancer, 4.37 years for post-SVR lung cancer, and undefined for other post-SVR non-HCC malignancies (Figure S1B).

The clinical characteristics of patients with and without non-HCC malignancies at baseline and SVR12 are given in Table 2. The age at baseline and the post-SVR incidence of HCC were significantly higher in patients with non-HCC malignancies than in patients without any non-HCC malignancies. At baseline, Hb concentration, ALT level, GGT level, and Alb level were significantly lower and Fib-4 index and M2BPGi level were significantly higher in patients with non-HCC malignancies. Additionally, significantly lower Hb concentration

TABLE 1 Causes of death in patients with chronic HCV infection who achieved SVR following DAA treatment

Types	Number
Cerebral stroke	9
Bacterial pneumonia	8
Lung cancer	8
Pancreatic cancer	7
Heart disease	7
HCC	6
Gastric cancer	5
Chronic renal failure	5
Liver failure	5
Interstitial pneumonia	4
Senile decay	2
Sepsis	2
Myelodysplastic syndrome	2
Colorectal cancer	1
Malignant lymphoma	1
Intrahepatic cholangiocarcinoma	1
Unknown primary cancer	1
Suicide	1
Choking	1
Fall accident	1
Multiple organ failure	1
Rupture of esophageal varices	1
Drowning	1
Unknown	10

TABLE 2 Clinical characteristics of the enrolled patients

	Total		Non-HCC malignancies (−)		Non-HCC malignancies (+)		
	n		n		n		*p*
Sex (male/female)	3580	1675/1905	3459	1619/1840	121	66/55	N.S.
Age at baseline (years)	3580	67 (58–74)	3459	67 (58–74)	121	69 (64–77)	<0.001
Body mass index at baseline	2641	22.5 (20.4–24.8)	2544	22.5 (20.4–24.8)	97	22.4 (20.0–24.6)	N.S.
Post-SVR incidence of HCC (yes/no)	3580	155 / 3425	3459	145 / 3314	121	10 / 111	<0.05
WBC (/μl)							
At baseline	3309	4800 (3900–5900)	3188	4800 (3900–5900)	121	4800 (3700–5965)	N.S.
At SVR12	2943	5110 (4120–6240)	2834	5100 (4118–6213)	109	5300 (4110–6315)	N.S.
Hb (g/dl)							
At baseline	3298	13.6 (12.5–14.7)	3177	13.6 (12.6–14.7)	121	13.2 (11.6–14.6)	<0.01
At SVR12	3167	13.5 (12.4–14.6)	3053	13.5 (12.4–14.6)	114	12.9 (11.6–14.2)	<0.001
PLT (10^4/μl)							
At baseline	3580	16.0 (12.0–20.1)	3459	16.1 (12.0–20.1)	121	15.2 (11.3–19.0)	N.S.
At SVR12	3550	16.8 (12.7–20.9)	3430	16.8 (12.8–20.9)	120	16.9 (12.6–21.1)	N.S.
Total bilirubin (mg/dl)							
At baseline	3577	0.7 (0.5–0.9)	3456	0.7 (0.5–0.9)	121	0.7 (0.5–0.9)	N.S.
At SVR12	3352	0.7 (0.5–0.9)	3239	0.7 (0.5–0.9)	113	0.7 (0.5–0.9)	N.S.
AST (U/l)							
At baseline	3580	38 (27–59)	3459	38 (27–59)	121	38 (27–54)	N.S.
At SVR12	3548	22 (18–27)	3428	22 (18–27)	120	23 (19–28)	N.S.
ALT (U/l)							
At baseline	3580	36 (23–62)	3459	36 (23–62)	121	32 (21–54)	<0.05
At SVR12	3550	15 (11–21)	3430	15 (11–21)	120	15 (10–20)	N.S.
GGT (U/l)							
At baseline	3073	30 (19–53)	2961	30 (19–53)	112	26 (17–42)	<0.05
At SVR12	2973	19 (14–29)	2868	20 (14–29)	105	19 (14–29)	N.S.
Total protein (g/dl)							
At baseline	2424	7.5 (7.2–7.8)	2332	7.5 (7.2–7.8)	92	7.6 (7.1–8.0)	N.S.
At SVR12	2667	7.4 (7.1–7.7)	2568	7.4 (7.1–7.7)	99	7.5 (7.2–7.8)	N.S.
Alb (g/dl)							
At baseline	3549	4.2 (3.9–4.4)	3429	4.2 (3.9–4.4)	120	4.0 (3.7–4.2)	<0.001
At SVR12	3429	4.3 (4.0–4.5)	3312	4.3 (4.0–4.5)	117	4.2 (3.8–4.3)	<0.001
Total cholesterol (mg/dl)							
At baseline	2598	168 (148–193)	2507	169 (148–193)	91	164 (138–183)	N.S.
At SVR12	2320	186 (162–211)	2243	186 (162–211)	77	187 (162–208)	N.S.
LDL-cholesterol (mg/dl)							
At baseline	1466	93 (76–114)	1405	94 (76–115)	61	88 (69–112)	N.S.
At SVR12	1265	110 (90–131)	1216	110 (90–131)	49	107 (83–126)	N.S.
BUN (mg/dl)							
At baseline	2697	14.7 (12.0–18.0)	2598	14.7 (12.0–18.0)	99	15.0 (12.1–17.3)	N.S.
At SVR12	2774	15.2 (12.5–18.8)	2671	15.2 (12.5–18.8)	103	14.5 (12.1–17.9)	N.S.
Cre (mg/dl)							
At baseline	3558	0.71 (0.60–0.85)	3438	0.71 (0.61–0.85)	120	0.70 (0.58–0.86)	N.S.
At SVR12	3478	0.74 (0.62–0.88)	3361	0.74 (0.62–0.88)	117	0.74 (0.60–0.86)	N.S.

(Continues)

TABLE 2 (Continued)

	Total		Non-HCC malignancies (−)		Non-HCC malignancies (+)		
	n		n		n		p
eGFR (ml/min/1.73 m^2)							
At baseline	3291	74.0 (62.0–85.0)	3172	73.8 (62.0–85.0)	119	75.0 (58.9–84.6)	N.S.
At SVR12	3252	71.0 (60.0–82.0)	3133	71.0 (60.0–82.0)	119	70.6 (57.9–81.5)	N.S.
HbA1c (%)							
At the start of DAAs	1837	5.5 (5.2–6.0)	1760	5.5 (5.2–6.0)	77	5.6 (5.3–6.2)	N.S.
At SVR12	1371	5.6 (5.2–6.0)	1320	5.6 (5.2–6.0)	51	5.8 (5.4–6.3)	<0.05
Fib-4							
At baseline	3580	2.77 (1.80–4.44)	3459	2.76 (1.80–4.40)	121	3.32 (2.21–5.56)	<0.01
At SVR12	3542	2.32 (1.57–3.38)	3422	2.31 (1.57–3.35)	120	2.65 (1.84–4.01)	<0.05
M2BPGi (COI)							
At baseline	1334	1.90 (1.10–3.53)	1281	1.87 (1.08–3.51)	53	2.44 (1.56–4.24)	<0.05
At SVR12	1309	1.09 (0.67–1.72)	1258	1.07 (0.67–1.66)	51	1.47 (0.84–2.58)	<0.01
AFP (ng/ml)							
At baseline	3443	4.2 (2.7–8.1)	3331	4.2 (2.7–8.2)	112	4.7 (2.4–7.9)	N.S.
At SVR12	3230	3.0 (2.0–4.8)	3122	3.0 (2.0–4.8)	108	3.0 (2.0–5.0)	N.S.

Abbreviations: AFP, alpha-fetoprotein; Alb, Albumin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; Cre, creatinine; eGFR, estimated glomerular filtration rate; GGT, γ-glutamyltransferase; Hb, hemoglobin; HbA1c, glycosylated hemoglobin; LDL, low-density lipoprotein; N.S., not significant; PLT, platelet; WBC, white blood cell.

and Alb level and significantly higher HbA1c level, Fib-4 index, and M2BPGi level were noted at SVR12 in patients with non-HCC malignancies.

Cumulative post-SVR incidence and survival of non-HCC malignancies or HCC

After the end of DAA treatment, the survival probability for all of the patients was 99.6% at 1 year, 97.9% at 3 years, and 94.0% at 5 years (Figure 2A). The age-standardized incidence rate of post-SVR non-HCC malignancies (per 100,000 population) was 270.7 at 1 year, 401.7 at 2 years, and 262.5 at 3 years. The cumulative post-SVR incidence of non-HCC malignancies was significantly lower than that of HCC (0.9% vs. 1.4% at 1 year, 3.1% vs. 4.3% at 3 years, and 6.8% vs. 8.0% at 5 years; $p < 0.05$) (Figure 2B). On comparing patients with and without malignancies after achieving SVR, the survival probabilities for patients with HCC + non-HCC malignancies, HCC only, non-HCC malignancies only, and without any malignancy were 100%, 100%, 99.1%, and 99.6% at 1 year; 90.0%, 98.3%, 78.8%, and 98.7% at 3 years; and 90.0%, 85.1%, 60.2%, and 96.9% at 5 years, respectively. Notably, the survival probability for patients with non-HCC malignancies only was lower than that for patients with HCC only (Figure 2C). Similarly, the survival probability after the diagnosis of non-HCC malignancies was significantly lower than that after the diagnosis of HCC ($p < 0.001$) (Figure 2D).

The clinical characteristics of patients stratified by Fib-4 index at baseline or SVR12 are provided in Tables 1 and 2. Body mass index at baseline and SVR12 was significantly higher in patients with Fib-4 index < 1.45 than in patients with 1.45 ≤ Fib-4 index ≤ 3.25 and Fib-4 index > 3.25. HbA1c level at baseline was significantly higher in patients with 1.45 ≤ Fib-4 index ≤ 3.25 than in patients with Fib-4 index < 1.45 and Fib-4 index > 3.25, and HbA1c level at SVR12 was significantly higher in patients with 1.45 ≤ Fib-4 index ≤ 3.25 than in patients with Fib-4 index > 3.25. Although there was a significant difference in the cumulative post-SVR incidences of HCC among patients stratified by Fib-4 index at baseline or SVR12 (baseline, $p < 0.001$; SVR12, $p < 0.001$), these incidences of non-HCC malignancies showed no significant difference (baseline, $p = 0.17$; SVR12, $p = 0.25$) (Figure 3A–D). Furthermore, there was no significant difference in the survival probability for patients with non-HCC malignancies stratified by Fib-4 index at baseline or SVR12 (Figure S2A,B).

Risk factors for post-SVR incidence of non-HCC malignancies at baseline

The results of our analysis of risk factors at baseline associated with the post-SVR incidence of non-HCC malignancies are given in Table 3. Univariate analysis identified the following as significant risk factors: male sex, Hb concentration < 13.0 g/dl, Alb level < 4.1 g/dl, Fib-4 index ≥ 2.90, and M2BPGi cutoff index (COI)

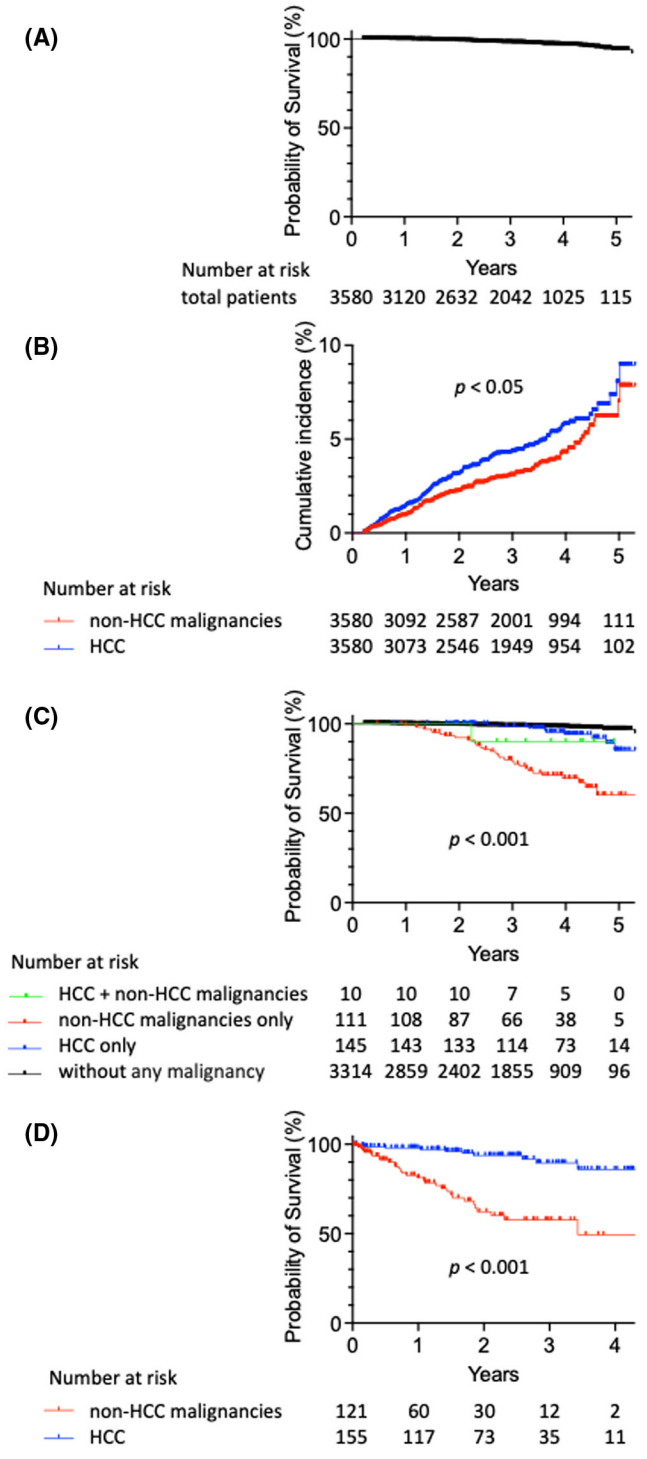

FIGURE 2 Cumulative post-SVR incidence of malignancies and associated survival probability. (A) Post-DAA treatment survival probability for all enrolled patients achieving SVR. (B) Post-DAA treatment cumulative incidence of non-HCC malignancies and HCC. (C) Post-DAA treatment survival probability for patients with HCC + non-HCC malignancies, non-HCC malignancies only, HCC only, and without any malignancy. (D) Survival probability after the diagnosis of non-HCC malignancy or HCC. The *p* values were determined using the log-rank test

≥ 1.90. Multivariate analysis revealed that M2BPGi COI ≥ 1.90 at baseline (hazard ratio [HR] 2.736, 95% confidence interval [CI] 1.233–6.072; $p < 0.05$) was significantly and independently associated with the post-SVR incidence of non-HCC malignancies. The cumulative post-SVR incidence of non-HCC malignancies in patients with M2BPGi COI ≥ 1.90 and M2BPGi COI < 1.90 at baseline was 0.8% and 0.6% at 1 year, 3.1% and 1.6% at 2 years, 4.6% and 2.7% at 3 years, and 6.9% and 3.3% at 4 years, respectively (Figure 4A). There was no difference in M2BPGi levels at baseline among patients with post-SVR non-HCC malignancies (median M2BPGi COI of HCC, 5.56; lung cancer, 2.90; gastric cancer, 2.43; colorectal cancer, 2.19; breast cancer, 1.33; pancreatic cancer, 2.81; others, 2.15) (Figure S3A).

Risk factors for post-SVR incidence of non-HCC malignancies at SVR12

The results of our analysis of risk factors at SVR12 associated with the post-SVR incidence of non-HCC malignancies are provided in Table 4. Univariate analysis identified the following as significant risk factors: male sex, Hb concentration < 13.0 g/dl, Alb level < 4.3 g/dl, HbA1c level ≥ 5.8%, and M2BPGi COI ≥ 1.50. Multivariate analysis revealed that M2BPGi COI ≥ 1.50 at SVR12 (HR 2.695, 95% CI 1.044–6.958; $p < 0.05$) was significantly and independently associated with the post-SVR incidence of non-HCC malignancies. The cumulative post-SVR incidence of non-HCC malignancies in patients with M2BPGi COI ≥ 1.50 and M2BPGi COI < 1.50 at SVR12 was 2.4% and 1.3% at 1 year, 5.2% and 1.9% at 2 years, 6.7% and 3.1% at 3 years, and 7.9% and 4.6% at 4 years, respectively (Figure 4B). As noted at baseline, there was no difference in M2BPGi levels at SVR12 among patients with the post-SVR non-HCC malignancies (median M2BPGi COI of HCC, 2.08; lung cancer, 1.75; gastric cancer, 1.18; colorectal cancer, 1.93; breast cancer, 0.65; pancreatic cancer, 1.26; others, 1.41) (Figure S3B).

Association between the cumulative post-SVR incidence and survival of all malignancies and M2BPGi levels

Our results showed associations between the post-SVR incidence of non-HCC malignancies or HCC and the M2BPGi levels at baseline and SVR12 (Figures 5 and 6). In the present study, M2BPGi COI ≥ 1.90 at baseline and M2BPGi COI ≥ 1.50 at SVR12 were defined as high levels. The cumulative post-SVR incidences of non-HCC malignancies in patients with both or either high

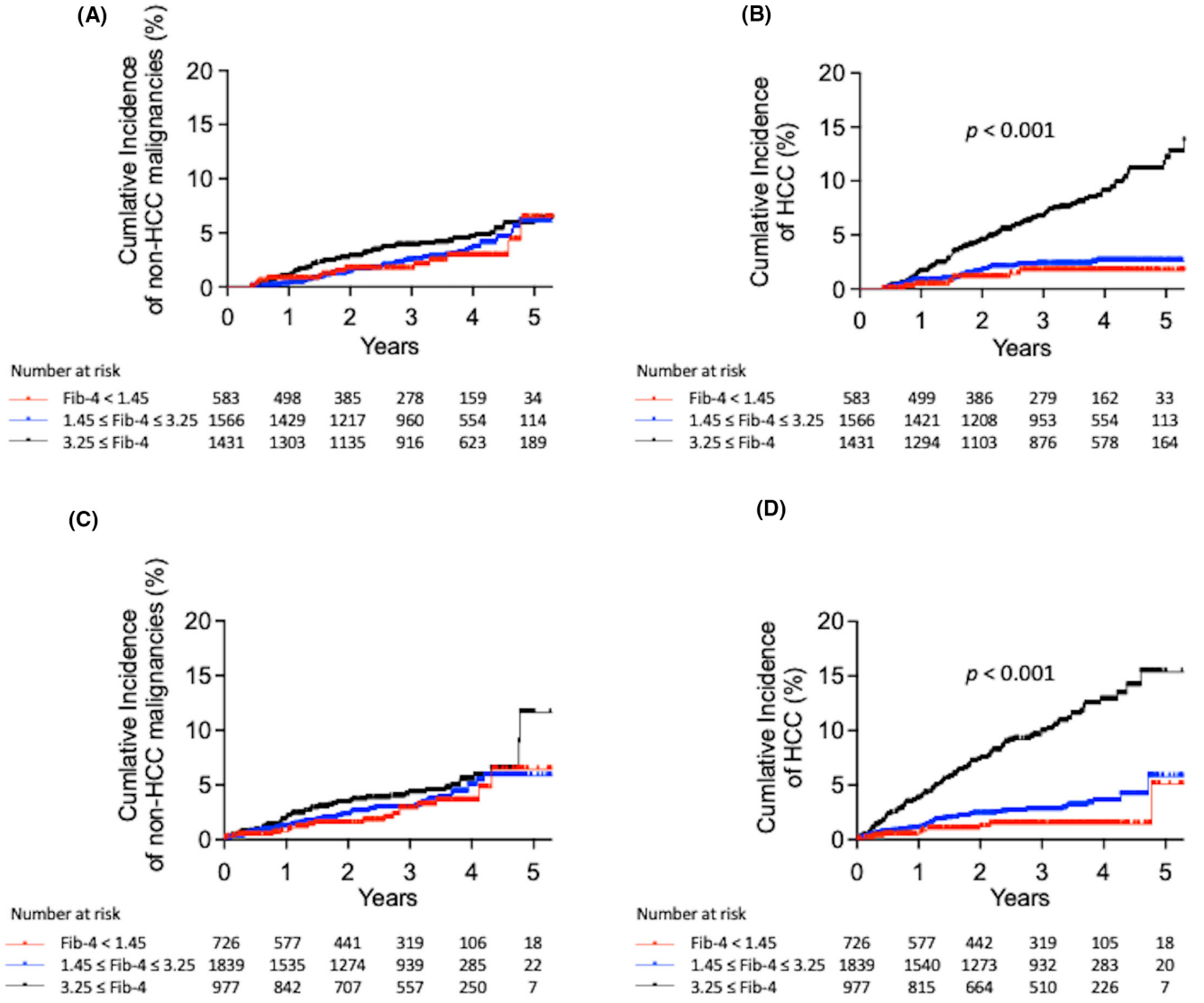

FIGURE 3 Cumulative post-SVR incidence of malignancies for patients stratified by Fibrosis-4 index (Fib-4). (A–D) Cumulative incidence of non-HCC malignancies and HCC after achieving SVR according to stratification based on Fib-4 at baseline (A,B) and 12 weeks following DAA treatment (SVR12) (C,D). One case was excluded from the analysis at SVR12 due to missing Fib-4 data. The *p* values were determined using the log-rank test

TABLE 3 Univariate and multivariate analysis to identify independent factors associated with incidence of non-HCC malignancies after achievement of SVR at baseline

	Univariate			Multivariate		
Category	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Male sex	1.498	1.047–2.142	<0.05			
Hb < 13.0 g/dl	1.768	1.237–2.526	<0.01			
Alb < 4.1 g/dl	2.421	1.680–3.489	<0.001			
HbA1c ≥ 5.4%	1.541	0.932–2.549	0.092			
Fib-4 ≥ 2.90	1.476	1.026–2.124	<0.05			
M2BPGi COI ≥ 1.90	1.790	1.013–3.164	<0.05	2.736	1.233–6.072	<0.05
AFP ≥ 4.0 ng/ml	1.152	0.787–1.688	0.467			

Abbreviations: CI, confidence interval; HR, hazard ratio.

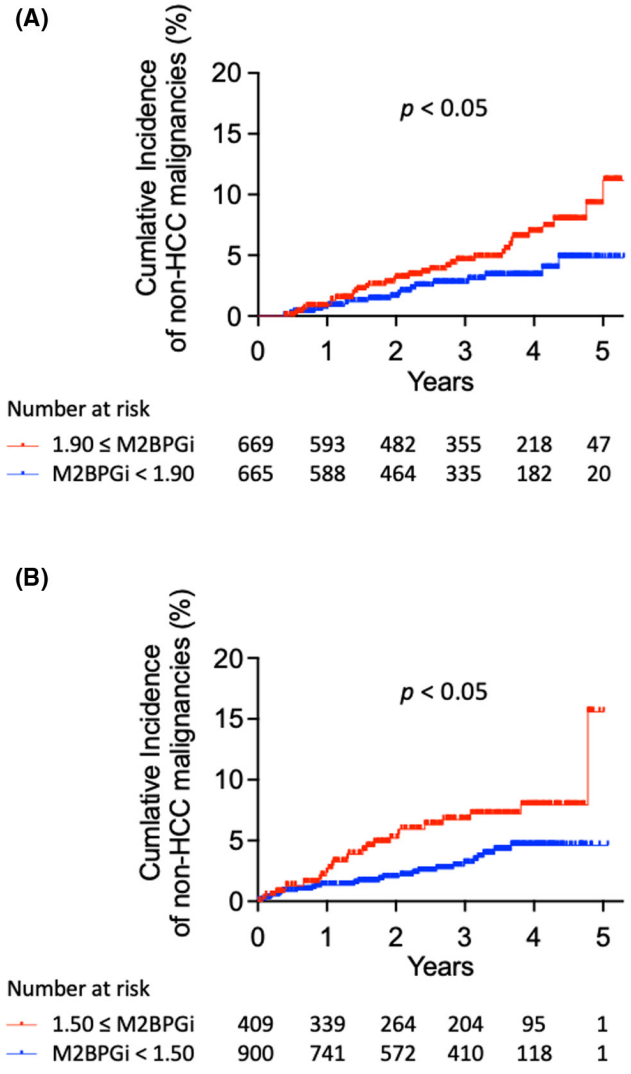

FIGURE 4 Association between the cumulative post-SVR incidence of non-HCC malignancies and Mac-2 binding protein glycan isomer (M2BPGi) levels. (A,B) Cumulative incidence of non-HCC malignancies after achieving SVR according to stratification based on the M2BPGi levels at baseline (A) and at SVR12 (B). The *p* values were determined using the log-rank test

M2BPGi levels at baseline and SVR12 and patients with both low M2BPGi levels were 1.7%, 2.8%, and 1.0% at 1 year; 4.2%, 3.8%, and 1.8% at 2 years; 5.7%, 5.9% and 2.6% at 3 years; and 7.4%, 9.8%, and 3.3% at 4 years, respectively ($p < 0.05$) (Figure 5A). No differences in the types of malignancies were identified among the three groups. The cumulative post-SVR incidences of HCC in patients with both or either high M2BPGi levels at baseline and SVR12 and patients with both low M2BPGi levels were 4.4%, 0.0%, and 0.2% at 1 year; 8.9%, 1.0%, and 0.9% at 2 years; 11.0%, 1.7%, and 0.9% at 3 years; and 12.8%, 2.9%, and 1.5% at 4 years, respectively ($p < 0.001$) (Figure 5B). Furthermore, the survival probabilities were 99.7%, 99.6%, and 99.8% at 1 year; 98.0%, 99.0%, and 99.1% at 2 years; 95.9%, 97.7%, and 99.1% at 3 years; and 93.5%, 93.9%, and 98.5% at 4 years, respectively ($p < 0.05$) (Figure 5C). Among patients with both high M2BPGi levels at baseline and SVR12, the cumulative post-SVR incidence of non-HCC malignancies was significantly lower than that of HCC (1.7% vs. 4.4% at 1 year, 4.2% vs. 8.9% at 2 years, 5.7% vs. 11.0% at 3 years, and 7.4% vs. 12.8% at 4 years; $p < 0.05$) (Figure 6A). Interestingly, the cumulative post-SVR incidence of non-HCC malignancies was significantly higher than that of HCC in patients with either high M2BPGi levels at baseline or SVR12 (2.5% vs. 0.0% at 1 year, 4.0% vs. 1.0% at 2 years, 5.3% vs. 1.7% at 3 years, and 10.9% vs. 2.9% at 4 years; $p < 0.01$) (Figure 6B). No significant differences were noted in the cumulative post-SVR incidence of non-HCC malignancies and HCC in patients with both low M2BPGi levels at baseline and SVR12 (1.0% vs. 0.2% at 1 year, 1.8% vs. 0.9% at 2 years, 2.6% vs. 0.9% at 3 years, and 3.3% vs. 1.5% at 4 years) (Figure 6C).

DISCUSSION

In this multicenter retrospective observational study, we focused on the incidence of non-HCC malignancies

TABLE 4 Univariate and multivariate analysis to identify independent factors associated with incidence of non-HCC malignancies after achievement of SVR at SVR12

	Univariate			Multivariate		
Category	**HR**	**95% CI**	***p***	**HR**	**95% CI**	***p***
Male sex	1.502	1.050–2.148	<0.05			
Post-SVR incidence of HCC	1.464	0.765–2.803	0.250			
Hb < 13.0 g/dl	1.820	1.260–2.628	<0.01			
Alb < 4.3 g/dl	1.900	1.302–2.772	<0.01			
HbA1c ≥ 5.8%	2.253	1.295–3.919	<0.01			
Fib-4 ≥ 2.45	1.343	0.935–1.930	0.110			
M2BPGi COI ≥ 1.50	1.990	1.147–3.455	<0.05	2.695	1.044–6.958	<0.05
AFP ≥ 2.8 ng/ml	1.275	0.857–1.899	0.231			

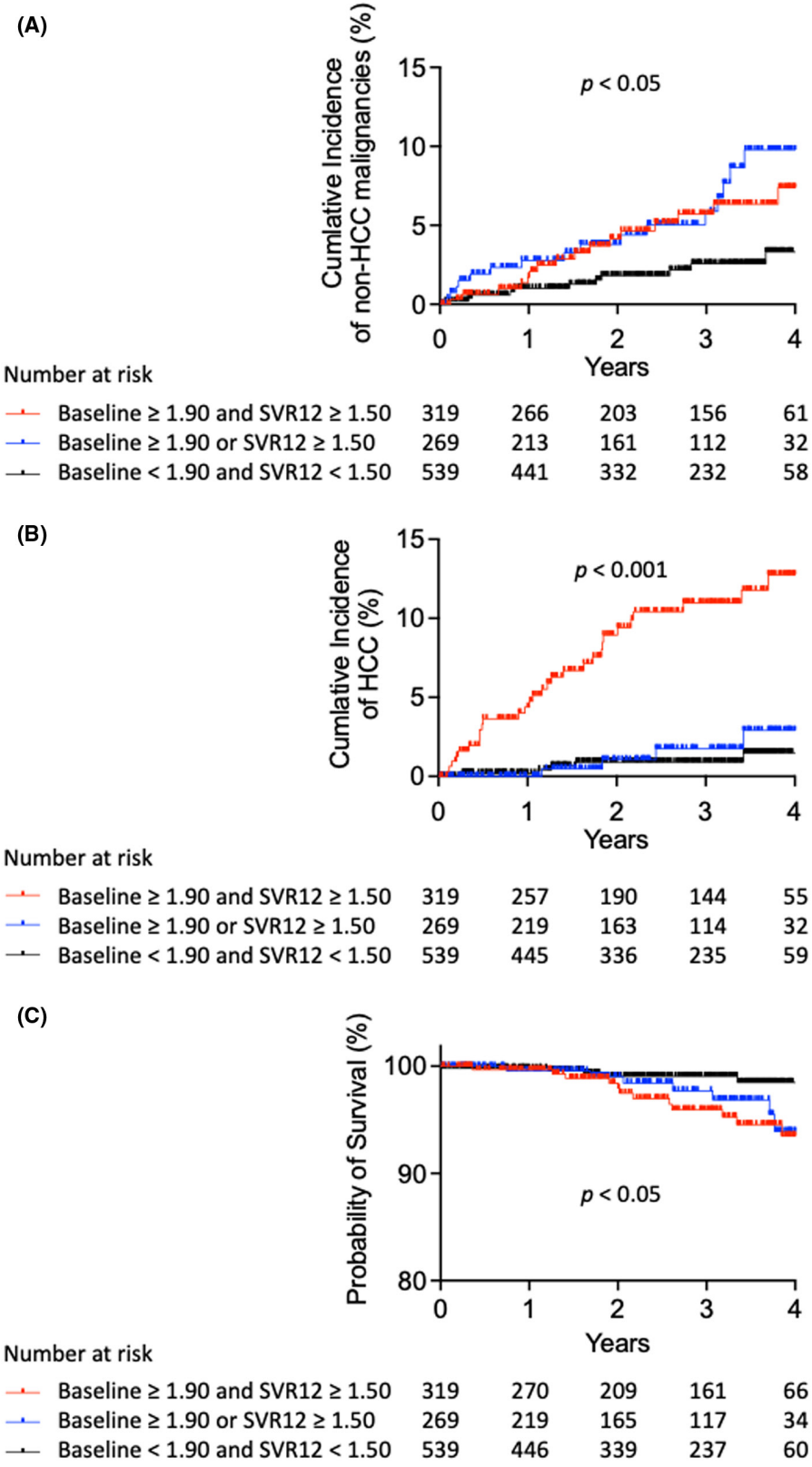

FIGURE 5 Association between the cumulative post-SVR incidence of malignancies or probability of survival and M2BPGi levels at both baseline and SVR12. (A–C) Cumulative incidence of non-HCC malignancies (A) and HCC (B), and the probability of survival (C) after achieving SVR according to stratification based on the M2BPGi levels at baseline and at SVR12. The *p* values were determined using the log-rank test

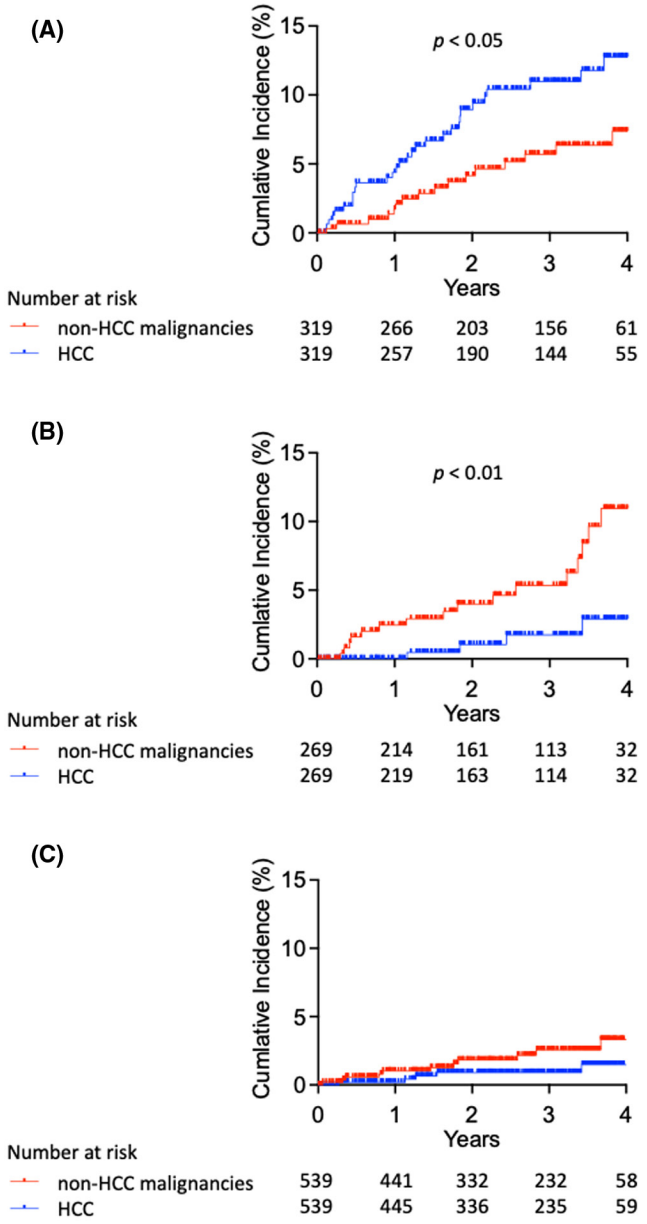

FIGURE 6 Cumulative post-SVR incidence of malignancies in patients stratified by M2BPGi levels at both baseline and SVR12. (A–C) Cumulative incidence of non-HCC malignancies and HCC in patients with both (A) or either (B) M2BPGi cutoff index (COI) ≥ 1.90 at baseline and M2BPGi COI ≥ 1.50 at SVR12, and both M2BPGi COI < 1.90 at baseline and M2BPGi COI < 1.50 at SVR12 (C). The *p* values were determined using the log-rank test

after achieving SVR following DAA treatment in patients with chronic HCV infection. We identified several trends in the evaluated data sets. First, the survival probability for patients with post-SVR non-HCC malignancies was significantly lower than that for patients with post-SVR HCC. Second, M2BPGi COI ≥ 1.90 at baseline and COI ≥ 1.50 at SVR12 were significant and independent factors associated with the post-SVR incidence of non-HCC malignancies. Third, patients with either high M2BPGi levels at baseline or SVR12 had a significantly higher risk of the post-SVR incidence of non-HCC malignancies than of HCC. In contrast, patients with both high M2BPGi levels at baseline and SVR12 had a significantly higher risk of the post-SVR incidence of HCC.

Achieving SVR drastically reduces the incidence of HCC and improves the prognosis of patients. However, it cannot entirely prevent the development of HCC. Therefore, proper surveillance based on predictive risk factors for HCC is required. Previous studies have identified several predictive factors that can be measured noninvasively, including seromarkers,[26,27,35] noninvasive indirect liver stiffness measurements,[36,37] and genetic factors.[38] Meanwhile, a large retrospective cohort study in the United States demonstrated that the age-adjusted mortalities of oral cavity cancer, rectal cancer, non-Hodgkin's lymphoma, and pancreatic cancer were significantly higher in patients with chronic HCV infection than in the general population. Furthermore, ages at diagnosis and death for several non-HCC malignancies, including oral cavity cancer, non-Hodgkin's lymphoma, and pancreatic cancer, were significantly lower in patients with chronic HCV infection than in the general population.[5] In France, the post-SVR incidence of extrahepatic malignancies was still higher in patients with chronic HCV infection receiving antiviral treatment than in the general population.[25] In our study, the 5-year post-SVR cumulative incidence of non-hepatocellular malignancies after SVR was 6.8%, which was comparable to a previous report.[23] According to the National Cancer Registry in Japan (2016–2018),[39] the age-standardized incidence rate of all cancers except liver cancer (per 100,000 population) was 387.3 in 2016, 375.6 in 2017, and 372.5 in 2018. These rates were not different from the rates of post-SVR non-HCC malignancies observed in our study, although the difference in the incidence of non-HCC malignancies before and after SVR in patients with chronic HCV infection in Japan is still unknown. Furthermore, the incidence risk of liver and non-liver-related events is dependent on medical history prior to DAA treatment.[23] In fact, in the DAA treatment era, mortality associated with non-liver-related malignancies has increased in patients with chronic HCV infection who achieved SVR.[4] However, the development of non-HCC malignancies after achieving SVR has received little attention. The survival probability for patients with HCC was generally lower than that for patients with non-HCC malignancies other than pancreatic cancer.[40,41] Although the survival probability of all malignancies for patients with chronic HCV infection is still unknown, the survival probability for SVR patients with non-HCC malignancy was lower than that of SVR patients with HCC in our study. Unlike HCC screening, the examination intervals have varied significantly and are not constant, as they depend on the attending physicians. Non-HCC malignancies could have been examined and diagnosed after the onset of

symptoms. This low interest and inconsistent follow-up might have led to low survival among SVR patients with non-HCC malignancies. However, it is impossible to test for non-HCC malignancies in all SVR patients regularly. Accordingly, it is crucial to establish the predictive factors of non-HCC malignancies after achieving SVR in patients with HCV infection.

While M2BPGi level and Fib-4 index are well-known and reliable markers for assessing liver fibrosis in patients with chronic liver diseases, only M2BPGi level was identified as a contributing factor associated with the post-SVR incidence of non-HCC malignancies. M2BPGi is detected using a lectin-antibody sandwich immunoassay for *Wisteria floribunda* agglutinin–positive Mac-2-binding protein, a unique fibrosis-related glyco-alteration of α1-acid glycoprotein.[42] M2BPGi levels differ among various etiologies of chronic liver diseases, even at the same fibrosis stage. In chronic HCV infection, the mean (±SD) COI was 1.3 ± 0.1 for F0–F1, 2.2 ± 0.1 for F2, 3.3 ± 0.2 for F3, and 5.2 ± 0.3 for F4.[28,43] Importantly, elevated M2BPGi levels could be attributed to the high probability of developing HCC in patients with chronic HCV infection, regardless of the treatment outcome (SVR or treatment failure).[26,28,29] Despite having the same histopathological fibrosis stages, a study has reported that patients with high M2BPGi levels had a higher HCC occurrence rate than those with low M2BPGi levels, suggesting that M2BPGi could be a reliable surrogate marker for assessing the risk of HCC.[28] In terms of molecular pathology, M2BPGi has been reported to enhance the progression of HCC via the activation of mammalian target of rapamycin signaling, although its mechanism has not yet been fully clarified.[44] Alternatively, M2BPGi levels could reflect the fibrotic progression of other organs, such as the heart, lungs, and pancreas.[45–47] Elevated M2BPGi levels have been reported in patients with pancreatic ductal adenocarcinoma.[33] M2BPGi is secreted from hepatic stellate cells (HSCs) and induces Mac-2 protein expression in Kupffer cells. In turn, Kupffer cells with expressed Mac-2 activate HSCs to be fibrogenic.[48] M2BPGi levels may be indicative not only of the degree of hepatic fibrotic progression but also of the activation and molecular biological roles of HSCs and cancer-associated stellate cells in extrahepatic fibrotic disease progression.[43] Interestingly, the previous study demonstrated that M2BPGi COI ≥ 1.80 at SVR could predict survival of patients with chronic HCV infection who achieved SVR following DAA treatment. Notably, 4 of 16 participants died of non-HCC malignancies after achieving SVR in that study. Therefore, although further investigations are needed, M2BPGi levels are potentially associated with the occurrence of non-HCC malignancies.[49] In the present study, we found that either or both M2BPGi COI ≥ 1.90 at baseline and COI ≥ 1.50 at SVR12 were significantly and independently associated with the post-SVR incidence of non-HCC malignancies. Moreover, patients with either M2BPGi COI ≥ 1.90 at baseline or M2BPGi COI ≥ 1.50 at SVR12 had a significantly higher risk of the post-SVR incidence of non-HCC malignancies than of HCC. Meanwhile, the cumulative post-SVR incidence of HCC in patients with continuously high M2BPGi levels at the two time points was significantly higher than that in patients with either high M2BPGi levels or both low M2BPGi levels at the two time points. Moreover, patients with continuously high M2BPGi levels at the two time points had a significantly higher risk of the post-SVR incidence of HCC than of non-HCC malignancies. M2BPGi levels might indicate not only liver fibrosis but also severe fibrosis and reflect activation of cancer-associated stellate cells in other organs, thus suggesting a higher risk of non-HCC malignancies. Although the significant relationship between M2BPGi levels and the occurrence of non-HCC malignancies cannot be elucidated, our findings highlight the importance of monitoring M2BPGi levels for early detection of non-HCC malignancies as well as HCC even after achieving SVR.

The strength of the current study was that the cumulative post-SVR incidence and survival of HCC and non-HCC malignancies and the predictive factors for each of these cases were clarified using a large cohort of patients from a real-world, multicenter database. However, this study also had several limitations. First, patients with hepatitis B virus (HBV) coinfection were not excluded. In addition, we did not consider data on alcohol and tobacco consumption due to the lack of access to this information at several institutions that participated in this study. Consequently, the influence of HBV coinfection, alcohol consumption, and tobacco use on the post-SVR incidence of non-HCC malignancies remains unclear. Second, we evaluated the patients' HbA1c levels without considering their diabetes status. The influence of antidiabetic drugs and insulin treatment on carcinogenesis was not investigated, because the details of patients' diabetes treatment and course were unknown. Third, the attending physicians arbitrarily determined the examination methods and interval periods for patients with HCC and non-HCC malignancies. Therefore, these variations might have influenced the malignancy detection rate. Fourth, all 17 institutions were tertiary referral hospitals, and some of the patients were transferred to clinics closer to their homes, thus interrupting the post-SVR follow-ups. Hence, the follow-up period was short. Finally, we could not fully compare M2BPGi levels among different non-HCC malignancies due to the small number of patients. Each of the non-HCC malignancies is heterogeneous and should be studied separately to make it easier to detect certain post-SVR non-HCC malignancies. Further studies are required to address these limitations.

In conclusion, our findings suggest that M2BPGi levels at baseline and at SVR12 should be closely

monitored in patients with chronic HCV infection in whom SVR has been achieved through DAA treatment. M2BPGi level can be considered a surrogate marker for predicting the development of HCC and non-HCC malignancies in these patients. Although future comparisons of the incidence of malignancies in these patients with that in the general population are needed, non-HCC malignancies have a significant impact on the prognosis of patients with chronic HCV infection who achieved SVR following DAA treatment. Early identification of such high-risk patients may help diagnose and treat all malignancies early, thereby prolonging survival.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Study concept and design: Kazuhito Kawata. *Data curation:* Masanori Atsukawa, Satoshi Yasuda, Hidenori Toyoda, Kazuyoshi Ohta, Takeshi Chida, Hidenao Noritake, Taeang Arai, Katsuhiko Iwakiri, Tomomi Okubo, Atsushi Hiraoka, Tsunamasa Watanabe, Haruki Uojima, Akito Nozaki, Joji Tani, Asahiro Morishita, Fujito Kageyama, Yuzo Sasada, Masamichi Nagasawa, Masahiro Matsushita, Tatsuki Oyaizu, Shigeru Mikami, Tadashi Ikegami, Hiroshi Abe, Kentaro Matsuura, and Yasuhito Tanaka. *Statistical analyses and data interpretation:* Kazuhito Kawata and Hidenao Noritake. *Manuscript draft:* Kazuhito Kawata and Akihito Tsubota. All authors have read and approved the final version of the manuscript.

ETHICS STATEMENT

This study was approved by the Ethics Committee of Hamamatsu University School of Medicine (internal review board approval number: EG19-297). The study protocols conformed to the ethical guidelines of the Declaration of Helsinki. Informed consent was obtained from the enrolled patients through the opt-out method on the website of each participating institution.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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