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【 ORIGINAL ARTICLE 】

The Effect of MAFLD on Hepatocarcinogenesis in HBeAg-negative Patients with Undetectable HBV-DNA under NA Therapy: A Multicenter Study

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Abstract:

Objective The progression of liver fibrosis and a male sex are risk factors for hepatocarcinogenesis under nucleos(t)ide analog (NA) therapy. Metabolic dysfunction-associated fatty liver disease (MAFLD) is a risk factor for hepatocarcinogenesis. This study aimed to investigate the factors involved in hepatocarcinogenesis during NAs therapy, including MAFLD.

Methods This study is a retrospective study [observation period: median 9.4 years (2.1-19.6 years)]. The subjects were 164 patients taking NAs for more than 2 years and were hepatitis B envelope antigen (HBeAg)-negative with undetectable hepatitis B virus (HBV)-DNA. The patient had no history of hepatocellular carcinoma (HCC). We investigated the profile of HCC onset after NAs therapy using a decision tree analysis

Results HCC developed in 20.7% (34/164) of the patients during the observation period. The prevalence of MAFLD was significantly higher in the HCC group than in the non-HCC group (64.7% vs. 43.9%, p=0.03). In particular, in the low-medium risk group classified by PAGE-B, MAFLD increased the risk of HCC development. According to a multivariate analysis, fibrosis-4 (FIB-4) index≥2.67, a male sex, and MAFLD (OR 2.4, 95%CI 1.0-6.0, p=0.04) were independent factors associated with the onset of HCC. In a decision tree analysis, MAFLD was the second classifier for the onset of HCC, next to the FIB-4 index (MAFLD 62.5%, non-MAFLD 28.5%).

Conclusions We found that MAFLD was an independent risk factor for HCC in HBeAg-negative patients with undetectable HBV-DNA after NAs therapy. We further revealed that MAFLD was the second-best classifier for hepatocarcinogenesis, next to the FIB-4 index. MAFLD therefore appears to have a synergistic effect on hepatocarcinogenesis with hepatic fibrosis.

Key words: chronic hepatitis B, HCC, MAFLD

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Introduction

In patients with chronic hepatitis B (CHB), Hepatocellular carcinoma (HCC) is the main cause of mortality (1). Nucleos(t)ide analog (NA) therapy significantly reduces the incidence of HCC in CHB patients; however, liver carcinogenesis remains an important issue (2). An older age, male

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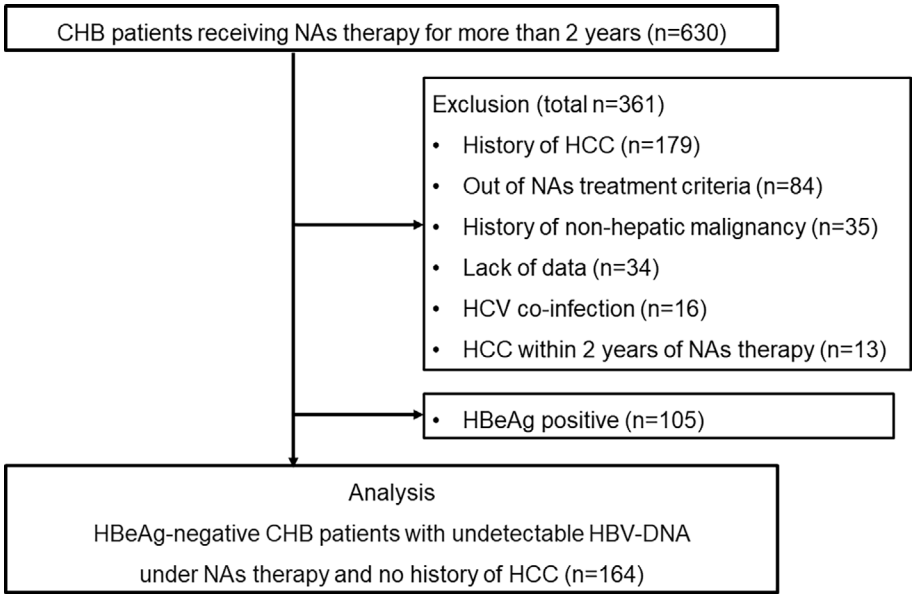

Figure 1. Flow chart for patient selection.

sex, cirrhosis, and thrombocytopenia at baseline are risk factors for HCC in CHB patients receiving NAs (3). PAGE-B is a scoring system that uses baseline patient age, sex, and platelets to predict the HCC risk in CHB patients under NAs (4). Although several risk scores have been developed to predict HCC in CHB patients, the PAGE-B score has been externally validated in independent cohorts of Caucasian, mixed ethnicity, or Asian patients, usually offering at least relatively good predictability in the low-risk group (5). Moreover, alcohol consumption has been reported as a risk factor for HCC (6). Thus, various non-viral factors have been associated with an increased risk of HCC in CHB patients receiving NAs (6).

Fatty liver is frequently observed in patients with CHB and it is reported to be associated with an increased risk of HCC (7). In addition, a meta-analysis demonstrated that DM was associated with a >25% increase in the risk of HCC in patients with CHB (8). A large Korean population-based cohort study demonstrated that the risk of HCC was positively associated with body mass index (BMI) in a dose-response manner in patients with CHB (9). Moreover, patients with CHB receiving NAs treatment for metabolic syndrome have been shown to have a significantly higher cumulative incidence of HCC and a shorter overall survival than patients with no metabolic syndrome (10). Thus, various metabolic dysfunctions are associated with HCC onset in patients receiving NAs.

Recently, a new concept of fatty liver disease has been proposed: metabolic dysfunction-associated fatty liver disease (MAFLD), defined as hepatic steatosis with metabolic dysfunction (11). The concept of MAFLD is considered to be useful for identifying patients with hepatic fibrosis in health check-up examinees and at a high risk of HCC in a nationwide population-based cohort study (12). In addition, MAFLD diagnosed at baseline is closely associated with hepatic fibrosis rather than viral factors in patients with HBV infection (13). A multicenter retrospective cohort study further demonstrated that a MAFLD diagnosis at baseline is associated with hepatocarcinogenesis in patients with HBV infection (14). However, the effect of MAFLD during NAs treatment on hepatocarcinogenesis in patients with CHB remains unclear.

This study aimed to investigate the factors under NAs therapy associated with hepatocarcinogenesis in patients with CHB, including MAFLD.

Materials and Methods

Study design and subjects

We performed a retrospective study to assess the risk factors for HCC development in hepatitis B envelope antigen (HBeAg)-negative patients with undetectable hepatitis B virus (HBV)-DNA under nucleos(t)ide treatment for more than 2 years. The inclusion criteria were HBsAg positivity and hepatitis C virus antibody negativity. Patients with autoimmune hepatitis, primary biliary cirrhosis, or hemochromatosis were also excluded.

We enrolled 630 CHB patients who had received NAs therapy for more than 2 years between 2000 and 2019. We excluded patients who met any of the following criteria: 1) history of HCC before NAs treatment, 2) out of NAs treatment criteria, 3) history of non-hepatic malignancy, 4) lack of data, 5) hepatitis C virus co-infection, 6) HCC within 2 years of NAs therapy, and 7) HBeAg positive. Finally, we analyzed HBeAg-negative CHB patients with undetectable HBV-DNA under NAs therapy and no history of HCC (n=164) (Fig. 1).

These patients were routinely screened for HCC, according to the HBV guidelines established by the Japan Society

of Hepatology (JSH). Of these, 34 patients developed HCC (HCC group) and 130 patients did not develop HCC (non-HCC group) in the state of HBeAg-negative and undetectable HBV-DNA under NAs treatment.

In this study, we analyzed the cumulative HCC incidence rates classified by PAGE-B and compared the characteristics of the two groups that obtained sufficient control by NAs treatment. We used clinical data from the initial treatment of NAs and at the time of the final observation until the last visit before December 2019. In the HCC group, the data at the time of the final observation were defined as the data at the onset of HCC.

Nucleos(t)ide analogs

The treatment goal of antiviral therapy in patients with persistent HBV infection is to prevent liver failure and inhibit HCC by suppressing the activity of hepatitis and the progression of liver fibrosis, thereby improving the patient's life expectancy and overall quality of life. The long-term goal of antiviral therapy is to eliminate HBsAg; however, it is difficult to use only NAs treatment. Therefore, the three short-term goals of NAs treatment are the persistent normalization of alanine aminotransferase (ALT), HBeAg-negative and-positive anti-HBe antibody, and the suppression of HBV-DNA replication (15).

In Japan, nucleos(t)ide analog was approved in 2000, and the use of nucleos(t)ide analog has markedly improved the natural course of CHB patients. NAs currently approved by the medical insurance system in Japan include five agents: lamivudine (LAM), adefovir (ADV), entecavir (ETV), tenofovir disoproxil fumarate (TDF), and tenofovir alafenamide (TAF). LAM, the first NAs, was approved by medical insurance in 2000, followed by ADV in 2004, ETV in 2006, TDF in 2014, and TAF in 2017. In Japan, the current 1st line drugs are ETV, TDF, and TAF because they have a lower incidence of viral resistance than LAM and ADV. However, in clinical practice, there are many cases of LAM resistance and treatment with a combination of two drugs.

As a general rule in this study, NAs were used for patients with chronic hepatitis and cirrhosis who needed treatment; cases of chronic hepatitis with ALT ≥ 31 U/L or more and HBV-DNA levels ≥ 2,000 IU/mL were indicated for treatment. Cases of cirrhosis patients with detectable HBV-DNA irrespective of the HBeAg status, ALT levels or HBV-DNA levels.

Biochemical examinations

Venous blood samples were collected in the morning after a 12-hour overnight fast. The following biochemical examinations were performed using standard clinical methods, as previously described: blood platelet count, prothrombin time, plasma glucose levels, hemoglobin A1c (HbA1c) levels, and serum levels of aspartate aminotransferase (AST), ALT, gamma-glutamyl transpeptidase (GGT), albumin, total bilirubin, alpha-fetoprotein (AFP), and des-gamma-carboxy prothrombin (DCP).

Diagnosis of HCC

The diagnosis of HCC was based on the criteria of the clinical practice manual proposed by JSH using serum AFP and DCP levels and imaging techniques, including ultrasonography, computed tomography, magnetic resonance imaging, hepatic angiography, and/or a tumor biopsy (16).

Measurement of HBsAg, HBcrAg, and HBV DNA

The serum HBsAg levels were measured using LUMIPULSE HQ-HBsAg (Fujirebio, Tokyo, Japan), which has a dynamic range of 0.005-150,000 IU/mL. Serum HBcrAg levels were measured using the LUMIPULSE HBcrAg (Fujirebio), which has a range of 3.0-6.7 LogU/mL. Serum HBV-DNA levels were measured using the COBAS TaqMan® HBV Auto v2.0 Kit (Roche Diagnostics, Tokyo, Japan), which has a lower detection limit of 20 IU/mL.

Measurement of FIB-4 index

We evaluated fibrosis markers during NAs treatment. Fibrosis-4 (FIB-4) is often reported to be a non-invasive fibrosis marker (17). FIB-4 index ≥2.67 was defined as advanced fibrosis, according to previous reports. (17).

Diagnosis of diabetes mellitus

Diabetes mellitus was diagnosed if fasting blood glucose levels were >126 mg/dL and HbA1c levels were >6.5%, according to the diagnostic criteria for diabetes mellitus or the documented use of antidiabetic agents (18).

Definition of hepatic steatosis

Hepatic steatosis was evaluated using the hepatic steatosis index (HSI): HSI =8× ALT/AST + BMI (+2 if type 2 diabetes mellitus was present, +2 if female) (19). A cutoff value of 30 was used, as previously described (19).

PAGE-B

PAGE-B is a scoring system that predicts the 5-year cumulative incidence of HCC in Caucasian chronic hepatitis B patients under NAs (4). Age, gender, and platelets in PAGE-B are evaluated with the following scores; Age (years): score, 16-29: 0, 30-39: 2, 40-49: 4, 50-59: 6, 60-69: 8, ≥70: 10, gender: score, female: 0, male: 6, platelets (/mm^3): score, ≥200,000: 0, 100,000-199,999: 6, <100,000: 9. Scores range from 0 to 25 and are categorized as low (≤9), medium (10-17), or high (≥18) risk.

Definition of MAFLD

MAFLD was defined according to the criteria proposed by an international expert panel (11). The criteria included evidence of fatty liver in addition to one of the following: overweight/obesity, presence of type 2 diabetes mellitus, or lean/normal weight with evidence of metabolic risk abnormalities (11). Overweight was defined as a BMI ≥23 kg/m^2, and type 2 diabetes mellitus was defined as an HbA1c level ≥6.5% or receipt of specific drug treatment. Metabolic dys-

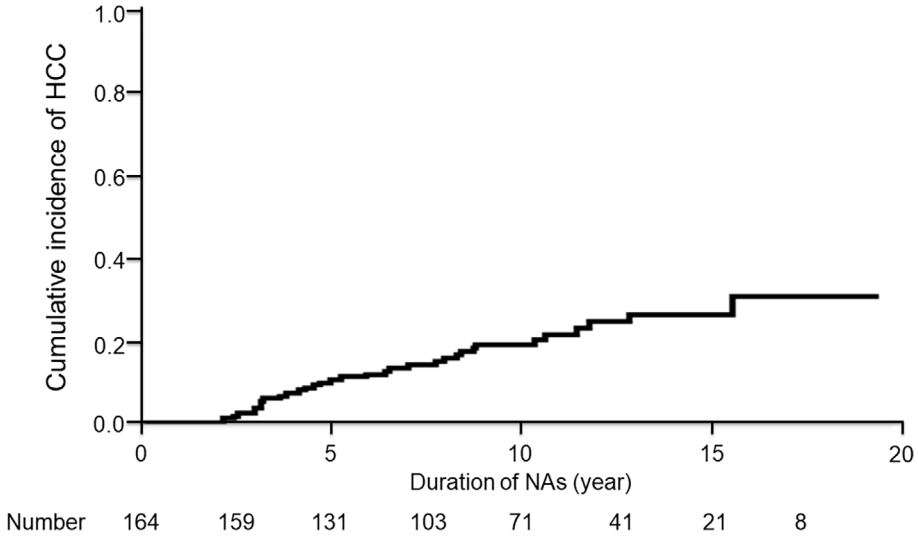

Figure 2. The cumulative incidence of HCC in patients with undetectable HBV-DNA under NAs treatment.

regulation was defined as the presence of at least two metabolic risk abnormalities: (a) waist circumference ≥90 and ≥ 80 cm in men and women, respectively; (b) blood pressure ≥130 mmHg or receiving specific drug treatment; (c) plasma triglycerides ≥150 mg/dL (≥1.70 mmol/L) or receiving specific drug treatment; (d) plasma highdensity lipoprotein-cholesterol levels <40 mg/dL (<1.0 mmol/L) in men and < 50 mg/dL (<1.3 mmol/L) in women or receiving specific drug treatment; and (e) prediabetes [fasting glucose levels of 100-125 mg/dL (5.6-6.9 mmol/L), 2-h post-load glucose levels of 140-199 mg/dL (7.8-11.0 mmol/L), or an HbA1c level of 5.7-6.4% (39-47 mmol/L)] (11).

Statistical analysis

An χ^2-test was used to analyze the association between categorical variables, and the Mann-Whitney *U*-test and Wilcoxon test were used for continuous variables. Univariate and multivariate analyses were conducted using the Cox proportional hazards model to identify risk factors associated with HCC development at the initial treatment of NAs. Odds ratios and their 95% confidence intervals, along with the corresponding p values, are presented.

Risk factors for HCC development in HBeAg-negative patients with undetectable HBV-DNA under NAs treatment were statistically evaluated using univariate logistic regression models. Multivariable logistic regression models were used to identify independent risk factors for HCC development. Hazard ratios and their 95% confidence intervals, along with the corresponding p values, are presented.

The cumulative HCC incidence rates were analyzed using the Kaplan-Meier method. The quoted p values were 2-sided, and $p<0.05$ was considered to be statistically significant.

Results

Cumulative incidence of HCC under NAs treatment

Of 164 patients, 34 (20.7%) developed HCC after NAs treatment. Fig. 2 shows the cumulative incidence of HCC in patients with undetectable HBV-DNA levels after NAs treatment. The 3-, 5-, 10-, and 15-year cumulative incidence rates of HCC were 3.7, 10.8, 19.8, and 27.2%, respectively.

Patients' Characteristics

Table 1 shows a comparison of the patient characteristics between the HCC development group (n=34) and the non-HCC group (n=130) at the initial treatment of NAs. Univariate analysis revealed that the male sex ratio, GGT, albumin, total bilirubin, prothrombin time, platelet count, LAM case, and high-risk group of PAGE-B were significantly associated with HCC development. According to a multivariate analysis, PAGE-B was an independent factor associated with HCC development (Table 2).

Table 3 shows the characteristics of HBeAg-negative patients with undetectable HBV-DNA under NAs treatment at final observation. There were no significant differences in age between the HCC and non-HCC groups. The male to female ratio and BMI were significantly higher in the HCC group than in the non-HCC group. The prevalence of obesity and diabetes was significantly higher in the HCC group than that in the non-HCC group. The hepatic steatosis index and prevalence of MAFLD were significantly higher in the HCC group than those in the non-HCC group.

There was no significant difference in the serum HBsAg levels between the HCC and non-HCC groups. The male sex ratio, serum ALT level, serum GGT level, and FIB-4 index were significantly higher in the HCC group than in the non-HCC group. The serum albumin levels and platelet counts

Table 1. Comparison of Patients' Characteristics between HCC and Non-HCC at the Initial Treatment of NAs.

	HCC (n=34)		non-HCC (n=130)		
	Median (IQR)	Range (min-max)	Median (IQR)	Range (min-max)	p
Age (years)	52.0 (45.8-59.3)	30-71	48.0 (41.5-55.0)	22-70	0.0521
Sex (female/male)	17.7%/82.3% (6/28)	N/A	39.2%/60.8% (51/79)	N/A	0.0186
AST (U/L)	70 (49-104)	30-355	59 (41-119)	21-1,020	0.2549
ALT (U/L)	84 (57-148)	34-282	78 (42-159)	31-1,690	0.7901
GGT (U/L)	77 (42-90)	26-220	38 (22-90)	11-384	0.0197
Albumin (g/dL)	3.9 (3.2-4.3)	1.3-4.8	4.1 (3.8-4.3)	2.2-5.2	0.0250
Total bilirubin (mg/dL)	1.0 (0.7-1.6)	0.5-4.7	0.8 (0.7-1.2)	0.3-2.1	0.0476
Prothrombin time (%)	73 (61-86)	44-102	88 (65-97)	52-120	0.0005
Platelet count ($\times 10^4/\mu L$)	10.4 (7.1-14.6)	4.5-23.5	16.4 (13.1-19.7)	6.2-35.2	<.0001
FIB-4 index	3.9 (2.5-6.9)	0.7-15.5	2.0 (1.3-3.3)	0.1-8.3	<.0001
Pre-existing cirrhosis (%) (No/Yes)	61.8% (13/21)	N/A	14.6% (111/19)	N/A	<.0001
HBeAg positivity (%) (No/Yes/Unknown)	44.1% (17/15/2)	N/A	41.5% (68/54/8)	N/A	0.7914
HBV-DNA (Log IU/mL)	5.6 (3.9-6.4)	2.2-7.9	6.0 (5.1-6.8)	2.4-8.3	0.0956
HBV genotype (A: B: C: Unknown)	0: 0: 31: 3	N/A	1: 5: 105: 19	N/A	0.4169
Nucleos(t)ide analog					
LAM	23		57		
ETV	11	N/A	68	N/A	0.0357
TDF	0		5		
PAGE-B (Low: Medium: High)	2: 12: 20	N/A	23: 75: 32	N/A	0.0006

Data are expressed as the median [interquartile range (IQR)], range, or number.

N/A: not applicable, NAs: nucleos(t)ide analogs, LAM: lamivudine, ETV: entecavir, TDF: tenofovir disoproxil fumarate, HDL cholesterol: high-density lipoprotein cholesterol, DM: diabetes mellitus, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, HBsAg: HB surface antigen, FIB-4: fibrosis-4.

Table 2. Cox Proportional Hazards Model to Identify the Independent Risk Factors Associated with HCC Development at the Initial Treatment of NAs.

Variable	Reference	HR	95% CI	p value
PAGE-B High risk	Low	1.83	1.06-3.16	0.0306
PAGE-B Medium risk	Low	1.93	1.19-3.13	0.0080

HR: hazard ratios, CI: confidence interval

were significantly lower in the HCC group than in the non-HCC group.

Logistic regression analysis for risk factors of HCC development

In the stepwise procedure, the following three factors were selected as explanatory variables: FIB-4 Index, male sex, and MAFLD. In the logistic analysis, the FIB-4 index and male sex were identified as independent factors associated with HCC development (Table 4). MAFLD was identified as an independent factor associated with the onset of HCC.

Cumulative incidence of HCC in the MAFLD group

The cumulative incidence of HCC was significantly higher in the MAFLD group than in the non-MAFLD group (Fig. 3). In the MAFLD/non-MAFLD groups, the 3-, 5-, 10-, and 15-year cumulative HCC incidence rates were 3.8%/3.6%, 13.2%/8.5%, 26.8%/13.2%, and 36.4%/18.7%, respectively.

Cumulative incidence of HCC classified by PAGE-B

The cumulative incidence of HCC classified by PAGE-B/MAFLD significantly increased with a higher risk in the initial treatment of NAs (p=0.0048) (Fig. 4). In cases classified as low risk by PAGE-B, the cumulative HCC incidence rates at 3-, 5-, and 10-year with MAFLD/non-MAFLD were 0%/0%, 0%/0%, and 21.3%/0%, respectively. In cases classified as medium-risk by PAGE-B, the cumulative HCC incidence rates at 3-, 5-, 10-, and 15-year with MAFLD/non-MAFLD were 2.1%/5.1%, 8.7%/7.8%, 16.8%/7.8%, and 25.4%/7.8%, respectively. In cases classified as high-risk by the PAGE-B, the cumulative HCC incidence rates at 3-, 5-, 10-, and 15-year with MAFLD/non-MAFLD were 6.2%/5.0%, 18.9%/20.0%, 41.9%/32.1%, and 50.2%/49.1%, respectively.

Decision-tree analysis for the onset of HCC

We investigated the risk factors for HCC in CHB patients receiving NAs treatment using decision-tree analysis (Fig. 5). The impact on the onset of HCC was determined using the FIB-4 index. Among patients with an FIB-4 index <2.67, 14.1% developed HCC. In contrast, 43.2% developed HCC in patients with an FIB-4 index ≥2.67. Among these patients, the second most affected factor was MAFLD, and 62.5% developed HCC in patients with FIB-4 index ≥2.67 and MAFLD.

Table 3. Comparison of the Patients' Characteristics between HCC and Non-HCC at the Final Observation.

	HCC (n=34)		non-HCC (n=130)		
	Median (IQR)	Range (min-max)	Median (IQR)	Range (min-max)	p
Duration of NAs (year)	5.1 (3.1-8.5)	2.1-15.7	9.9 (7.0-13.2)	2.1-19.6	<.0001
Age (years)	57.0 (51.8-66.3)	32-76	59.0 (50-66)	34-80	0.9628
Sex (female/male)	17.7%/82.3% (6/28)	N/A	39.2%/60.8% (51/79)	N/A	0.0186
Body mass index (kg/m^2)	24.1 (22.0-27.0)	17.6-33.0	22.6 (20.4-24.9)	17.1-33.1	0.0086
Obesity (No/Yes)	29.4%/70.6% (10/24)	N/A	56.9%/43.1% (74/56)	N/A	0.0043
Alcohol drinking ≥ 30 g/day (No/Yes)	82.3%/17.7% (28/6)	N/A	89.2%/10.8% (116/14)	N/A	0.2752
Hypertension (No/Yes)	76.5%/23.5% (26/8)	N/A	69.2%/30.8% (90/40)	N/A	0.4088
Hypertriglyceridemia (No/Yes)	85.3%/14.7% (29/5)	N/A	73.8%/26.2% (96/34)	N/A	0.1627
Depressed HDL-cholesterol (No/Yes)	97.1%/2.9% (33/1)	N/A	96.9%/3.1% (126/4)	N/A	0.9673
Diabetes (No/Yes)	73.5%/26.5% (25/9)	N/A	87.7%/12.3% (114/16)	N/A	0.0408
Hepatic steatosis index	33.4 (28.9-38.7)	24.9-41.6	30.3 (27.5-34.2)	21.4-44.6	<.0001
MAFLD (No/Yes)	35.3%/64.7% (12/22)	N/A	56.1%/43.9% (73/57)	N/A	0.0302
Obese-MAFLD (No/Yes)	41.2%/58.8% (14/20)	N/A	59.2%/40.8% (77/53)	N/A	0.0593
Lean-MAFLD (No/Yes)	94.1%/5.9% (32/2)	N/A	96.1%/3.9% (125/5)	N/A	0.6010
DM-MAFLD (No/Yes)	85.3%/14.7% (29/5)	N/A	89.2%/10.8% (116/14)	N/A	0.5231
Biochemical examinations					
AST (U/L)	26 (23-40)	11-60	22 (19.8-27.3)	14-118	0.0029
ALT (U/L)	26.5 (21-34.5)	5-76	19 (14-28.3)	8-159	0.0021
GGT (U/L)	37 (22-60.5)	16-124	21 (14-40.3)	6-330	0.0004
Albumin (g/dL)	4.2 (3.9-4.5)	2.7-5.0	4.3 (4.1-4.5)	3.4-5.2	0.0451
Total bilirubin (mg/dL)	0.9 (0.6-1.3)	0.4-3.5	0.8 (0.7-1.0)	0.3-1.9	0.4570
Prothrombin time (%)	88 (75-102)	58-114	105 (93-113)	74-130	<.0001
Platelet count (×10^4/μL)	11.2 (7.7-17.2)	3.3-26.1	18.6 (15.2-21.8)	8.9-31.6	<.0001
HBsAg (IU/mL)	602 (103-1,777)	0.1-3,240	923 (167-2,608)	0-20,300	0.1448
HBcrAg ≥ 3.0 LogU/mL (No/Yes)	14.7%/85.3% (5/29)	N/A	35.4%/64.6% (46/84)	N/A	0.0204
FIB-4 index	2.4 (1.7-4.9)	0.8-13.2	1.6 (1.2-2.3)	0.6-5.0	0.0002
Nucleos(t)ide analogs		N/A		N/A	0.0143
LAM		5		4	
ETV		17		83	
TAF		1		14	
LAM+TAF		11		25	
ETV+TAF		0		4	

Data are expressed as the median (interquartile range [IQR]), range, or number.

N/A: not applicable, NAs: nucleos(t)ide analogs, LAM: lamivudine, ADV: adefovir, ETV: entecavir, TDF: tenofovir disoproxil fumarate, TAF: tenofovir alafenamide, MAFLD: metabolic-associated fatty liver disease, HDL cholesterol: high-density lipoprotein cholesterol, DM: diabetes mellitus, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: gamma-glutamyl transpeptidase, HBsAg: HB surface antigen, HBcrAg: HB core-related antigen, FIB-4: fibrosis-4

Table 4. Logistic Regression Analysis for Risk Factors of HCC Development Used by Stepwise Method.

Variable	Reference	OR	95% CI	p value
FIB4-index≥2.67	<2.67	7.5	2.8 - 19.8	<.0001
Male	Female	3.9	1.3 - 11.7	0.0087
Presence of MAFLD	Absence	2.4	1.0 - 6.0	0.0460

FIB-4: fibrosis-4, MAFLD: metabolic dysfunction-associated fatty liver disease, OR: odds ratio, CI: confidence interval

Discussion

This study demonstrated that, in addition to advanced fibrosis and male sex, MAFLD was an independent risk factor for HCC development in HBeAg-negative patients with undetectable HBV-DNA under NAs therapy. In particular, MAFLD increased the risk of HCC development in the low-medium risk groups classified by PAGE-B. We further demonstrated that MAFLD was the second classifier for hepatocarcinogenesis next to the FIB-4 index in CHB patients with NAs treatment in the decision tree analysis.

In this study, 20.3% of HBeAg-negative patients with undetectable HBV-DNA under NAs treatment developed HCC during a mean follow-up period of 9.4 years. The 3-, 5-, 10-, and 15-year cumulative HCC incidence rates were 3.7, 10.8, 19.8, and 27.2%, respectively. In this study, the FIB-4 index was the most significant risk factor for hepatocarcinogenesis. A retrospective cohort of 986 Korean patients with no history of HCC demonstrated that a high FIB-4 index

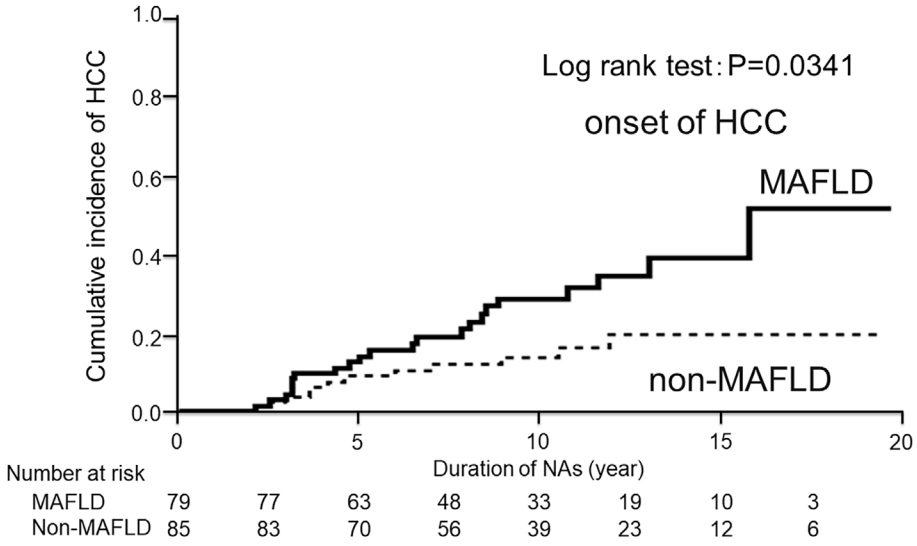

Figure 3. **The cumulative incidence of HCC with and without MAFLD.**

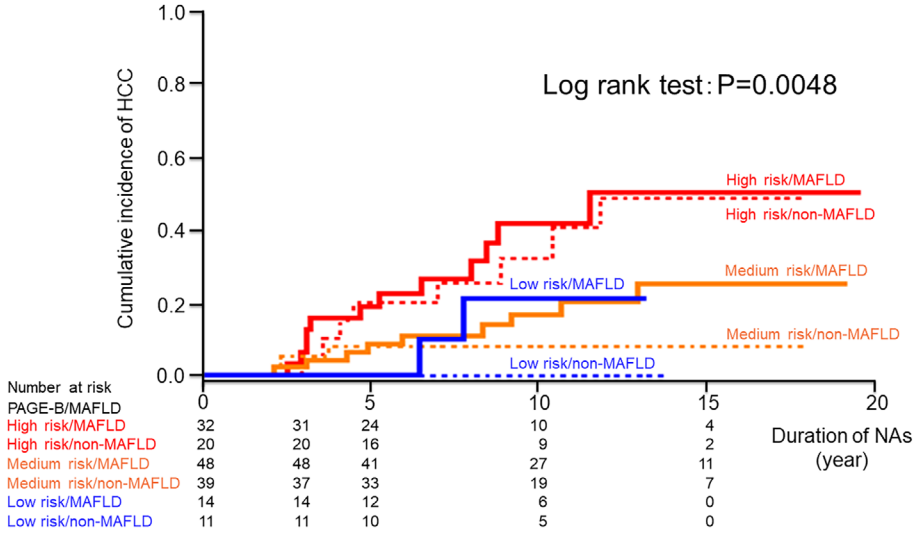

Figure 4. **The cumulative incidence of HCC with and without MAFLD in CHB patients classified by PAGE-B.**

was a highly predictive risk factor for HCC (20). In Japan, Tada et al. also reported that a high FIB-4 index is a risk factor for developing HCC, and that the 3-, 5-, and 10-year cumulative HCC incidence rates were 6.5%, 11.9%, and 21.8%, respectively, in patients with CHB receiving NA therapy (21). Thus, the results of our study are in good agreement with those of previous studies. Our database seems to have the general characteristics of HBeAg-negative patients with undetectable HBV-DNA under NAs treatment.

PAGE-B is an independent risk factor for HCC development, based on data from the initial treatment of NAs (4). In addition, MAFLD was an independent risk factor for HCC development in HBeAg-negative patients with undetectable HBV-DNA under NAs treatment. Van Kleef et al. reported that the presence of MAFLD in CHB patients increases the risk of HCC development (14), supporting our results. We further showed that the presence of MAFLD has a synergistic effect even when classified by PAGE-B, and caution is required, especially in the low- and medium-risk groups.

We also found that MAFLD was the second risk factor for hepatocarcinogenesis. In contrast, Chen et al. reported that diabetes mellitus, metabolic syndrome, and obesity are not significant risk factors for hepatocellular carcinoma in an HBV-endemic area of Southern Taiwan (22). The participants in the previous study were recruited during health checks conducted between April and November 2004 and were considered subjects who had not received NAs treatment for HBV. These results suggest that viral factors, such as HBV-DNA and HBcrAg, were more strongly involved than metabolic abnormalities in a previous study by Chen et al. Similar to our results, van Kleef et al. reported that the presence of MAFLD in patients with CHB was associated with an increased risk of liver-related clinical events and death (14). MAFLD should be recognized as a risk factor

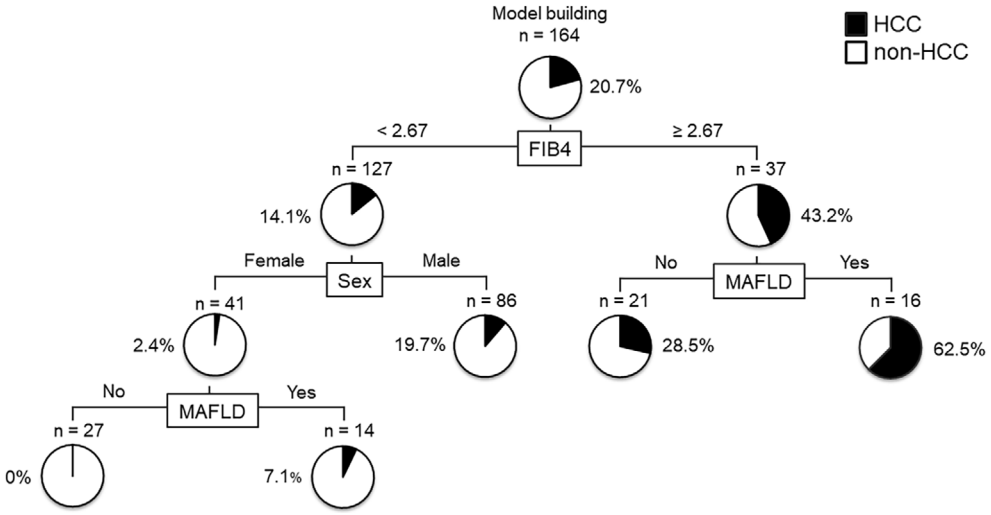

Figure 5. The profile of hepatocarcinogenesis using a decision tree analysis. The patients were classified according to the indicated cutoff values of the variables. The pie graphs indicate the percentage of patients with non-HCC (white) /patients with HCC (black) in each group.

for hepatocarcinogenesis in CHB patients receiving NAs treatment.

In patients with CHB, the following are the possible mechanisms of MAFLD-associated hepatocarcinogenesis. The pathogenic mechanism underlying the relationship between fatty liver and cancer risk is that hepatic fat causes both systemic and local inflammation and may promote carcinogenesis (23). Recent evidence suggests that DM, obesity, and metabolic factors are risk factors for hepatocellular carcinoma. A meta-analysis and systematic review of seven studies with a total of 21,842 CHB patients revealed that diabetes mellitus was significantly associated with an increased risk of hepatocellular carcinoma (24). A multicenter prospective cohort study involving 5,754 HBV patients receiving NAs therapy showed that obesity increases the risk of HCC (25). A large cohort study of 1,690 male HBV patients in Taiwan found that having three or more metabolic risk factors, compared to no factors, significantly increased the risk of HCC (26). Thus, our results are in good agreement with those of the previous studies.

The present study is associated with some limitations. First, it was a retrospective study. Second, this study was conducted in a Japanese cohort. Third, the present study used various NAs, including monotherapy and combination therapy. However, the strongest point of this study was that we evaluated the risk factors for HCC measured under NAs treatment, whereas most studies have examined pre-HBV treatment parameters (27, 28). In addition, all subjects in this study were HBeAg-negative with undetectable HBV -DNA under long-term NAs treatment.

In conclusion, we herein demonstrated that. in addition to advanced fibrosis and a male sex, MAFLD was an independent risk factor for HCC development in HBeAg-negative patients with undetectable HBV-DNA under NAs therapy. In particular, in the low-medium risk group classified by PAGE-B, MAFLD increased the risk of HCC development. We further demonstrated that MAFLD was the second classifier for hepatocarcinogenesis next to the FIB-4 index in CHB patients with NAs treatment in the decision tree analysis. Thus, MAFLD may be an important pathogenic factor for hepatocarcinogenesis in CHB patients under sufficient control of HBV infection.

This study was approved by the Clinical Research Ethics Committee of Kurume University Hospital, which integrated ethical reviews from all participating institutions (Kurume University School of Medicine, Public Yame General Hospital, Kumamoto Central Hospital, and Omuta City Hospital; approval number: 17209).

An opt-out approach was used to obtain informed consent from patients, and personal information was protected during data collection.

Author's disclosure of potential Conflicts of Interest (COI).
Takumi Kawaguchi: Honoraria, Janssen Pharmaceutical, Taisho Pharmaceutical, Kowa, Otsuka Pharmaceutical, Eisai, ASKA Pharmaceutical, AbbVie, and EA Pharma.

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