FIB-4 index and serum α-fetoprotein are useful predictors of hepatocellular carcinoma occurrence in hepatitis B patients with nucleos(t)ide analogs therapy

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Abstract. Current antiviral therapies cannot achieve eradication of hepatitis B virus (HBV) and can reduce but not eliminate the risk of hepatocellular carcinoma (HCC) in patients with chronic HBV infection. The present study aimed to identify the risk factors for HCC development by analyzing nucleoside analogue (NA)-treated patients as a retrospective cohort using fibrosis-4 index (FIB-4 index) as a non-invasive fibrosis marker. A total of 260 patients with HBV receiving NAs without a history of HCC between January 2001 and January 2021 were included in the present study. The incidence of HCC in patients with HBV during NA therapy and the factors contributing to HCC occurrence were identified using clinical characteristics and blood test results. Among the 260 patients, 40 patients (15.4%) developed HCC. Univariate and multivariate analysis showed that age [hazard ratio (HR), 1.03; P=0.045], male sex (HR, 3.14; P<0.01) and FIB-4 index at 6 months after NA treatment <1.95 (HR, 4.35; P<0.01) correlated with the incidence of HCC. The cumulative incidence of HCC in patients with FIB-4 index at 6 months after NA treatment >1.95 was significantly higher compared with that in patients with FIB-4 index ≤1.95 (P<0.01). Multivariate analysis in patients in which serum α-fetoprotein (AFP) level at 6 months after NA treatment was measured showed that FIB-4 index >1.95 (HR, 8.27; P=0.014) and serum AFP level >4 ng/ml (HR, 4.26; P=0.033) contributed to HCC occurrence. FIB-4 index at 6 months after NA treatment and serum AFP levels at 6 months after NA treatment were predictors for the development of HCC in patients with HBV during NA treatment. Further study of hepatocarcinogenesis during NA with a longer follow-up period and larger numbers of participants is required.

Introduction

Hepatocellular carcinoma (HCC) accounted for 80% of the 826,000 global cases of primary liver cancer and was the third most common cause of cancer mortality in 2018 (1). Chronic hepatitis and liver cirrhosis (LC) caused by hepatitis B virus (HBV) and hepatitis C virus are the major preneoplastic conditions of HCC (1).

HBV is a small, partially double-stranded DNA virus that causes acute and chronic hepatitis in humans. HBV is one of the most hazardous viral pathogens for humans, and 2 billion individuals have been infected and >350 million are chronic carriers of the virus (2). Despite the improvement in the management of chronic HBV infection by antiviral therapy and universal vaccine, HCC remains the fourth most common cause of cancer-associated mortalities overall worldwide (3). Patients with untreated HBV infection are at a 5- to 100-fold higher risk for developing HCC compared with healthy individuals (4).

Nucleotide and nucleoside analogues (NAs) function as competitive inhibitors of the HBV reverse transcriptase, as their incorporation into the DNA strand provokes chain termination (5). The NAs lamivudine (LAM), adefovir (ADF), entecavir (ETV), tenofovir disoproxil fumarate (TDF) and tenofovir alafenamide (TAF) result in virological remission in almost all compliant patients with chronic HBV infection and are associated with significant improvement of liver inflammation and fibrosis (6-8). However, while the current antiviral agents decrease HCC risk in patients with HBV (9-11), they do not eliminate the risk of HCC because HBV is not

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Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; LC, liver cirrhosis; NA, nucleotide analogs; AFP, α-fetoprotein; FIB-4 index, fibrosis-4 index; AST, aspartate aminotransferase; PLT, platelet count; ALT, alanine aminotransferase; ROC, receiver operating characteristic; AUC, area under ROC curve; HR, hazard ratio; LAM, lamivudine; ADF, adefovir; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; SOT, start of treatment

Key words: hepatocellular carcinoma, hepatitis B virus, nucleotide analogs, FIB4-index, AFP

eradicated (12,13). Several groups have developed scores for the prediction of HCC in NA-treated patients (14,15). Liver inflammation and fibrosis caused by alcohol abuse and obesity are established risk factors for HCC (16). However, previous prediction models have not included these factors. A simple and non-invasive test that reflects factors other than HBV is needed to predict carcinogenesis. Previous studies have identified age, sex, fibrosis-4 index (FIB-4 index), serum α-fetoprotein (AFP) level, HBV-DNA level and HBV core promotor mutations as risk factors of HCC occurrence in patients with HBV during NA therapy. However, the early occurrence of HCC during NA therapy in previous studies may have included HCC not detected by imaging before NA therapy (17-21). The present study aimed to identify definite predictors of HCC occurrence by analyzing HCC occurrence ≥1 year following NA treatment.

Materials and methods

Patients. A total of 675 patients with HBV were treated with NA at Aso Iizuka Hospital (Iizuka, Japan) between January 2001 and January 2021. The exclusion criteria were as follows: i) follow-up of <1 year; ii) development of HCC before administration or within 1 year of administration of NA; iii) acute hepatitis and acute-on-chronic hepatitis; iv) *de novo* HBV; v) prevention of HBV reactivation; vi) co-infection with HCV; and vii) continuation from other hospitals (Fig. 1). Finally, the present study included 260 patients in this study. The present study was conducted in accordance with the guidelines of the Declaration of Helsinki and was approved by the Ethics Committee of Aso Iizuka Hospital (approval no. 21141). Informed consent of individual patients was not obtained because of the retrospective nature of this study.

Serum levels of HBV DNA were quantified by a fully automated system. A quantitative polymerase chain reaction (PCR) assay (Cobas TaqMan HBV Test v2.0; Roche Diagnostics) was used before 2017, according to the manufacturer's instructions. HBV DNA was processed from 650 μl of sample using the cobas AmpliPrep and cobas TaqMan 96 analyzer for automated sample extraction and real-time PCR amplification and detection, according to the manufacturer's instructions. The assay primers and probes target the HBV pre-Core/Core region (preC-C) gene. Another PCR assay (Cobas 6800/8800 system HBV; Roche Diagnostics) was used after 2017, according to the manufacturer's instructions. HBV DNA was extracted, purified, and amplified from 500 μl of samples on the cobas 6800/8800 analyzer system according to the manufacturer's instructions. The viral region targeted by this assay primers and probes is the preC-C gene.

FIB-4 index. The FIB-4 index was used to evaluate fibrosis clinically before and after NA treatment. The FIB-4 index was calculated using the following formula: FIB-4 index=[aspartate aminotransferase (AST, IU/l) x age (years)/platelet count (10^9/l) x alanine aminotransferase (ALT, IU/l)1/2] (22).

HBV DNA after 6 months often shows negative readings and the FIB-4 index after 6 months reflects other factors such as alcohol abuse and obesity instead of HBV. Therefore, the present study used the value of FIB-4 index at 6 months after NA treatment.

Follow-up definitions. Patients with chronic hepatitis and cirrhosis caused by HBV were treated using NA. Chronic hepatitis B was diagnosed as positive HBsAg for >6 months, elevated ALT ≥31 IU/l and serum HBV DNA ≥3.3 log IU/ml (23). LC was diagnosed on the basis of liver histology or transient elastography or the presence of gastro-esophageal varices. HCC surveillance was performed by blood tests and imaging examinations including ultrasonography, computed tomography and magnetic resonance imaging, each 3-6 months.

Statistical analysis. Results are shown as the median (inter-quartile range). Significant differences between groups were examined by χ^2, Fisher's Exact test or Mann-Whitney U-test. Statistical analyses were performed using the Kaplan-Meier method, log-rank test, receiver operating characteristic (ROC) analysis and Cox hazard analysis using JMP statistical software (version 11.0 for Windows; SAS Institute, Inc.). All P-values were derived from two-tailed tests, and P<0.05 was considered to indicate a statistically significant difference. ROC and area under the curve (AUC) values were calculated to define cut-off values for risk factors of HCC occurrence.

Results

Characteristics of patients at the start of NA and cumulative occurrence of HCC. Table I shows the clinical characteristics of the 260 patients with HBV in the present study. The median age of the overall patient group was 53.0 (range, 44.0-61.1) years, and 59.2% of patients were male. Serum HBV-DNA concentration was 6.2 log IU/ml. The numbers of patients that received specific NAs are as follows: LAM (n=77), ETV (n=163), TDF (n=8), and TAF (n=12). The median follow-up duration was 8.18 (4.73-13.07) years, and 40 (15.4%) patients developed HCC during the follow-up period (Fig. 2).

The present study detected significant differences between patients with and without HCC occurrence, including age (P=0.017), sex (P=0.014), type of NA (P<0.01), platelets at start of treatment (SOT) (P<0.01) and 6 months after NA treatment (P<0.01) and FIB-4 index at SOT (P<0.01) and 6 months after NA treatment (P<0.01). There was no significant difference in HBV DNA between the two groups (P=0.669) (Table I).

Differences of HCC occurrence between patients treated with the older and newer NAs. Several studies reported that the new NAs, including ETV, TDF and TAF, reduce HCC risk to a greater extent compared with the old NAs (LAM and ADF) (24,25). It has been reported that LAM-resistant mutations arise in ~23% of patients after 12 months of therapy and in up to 80% after 5 years of treatment, and patients with LAM-resistant mutations have a higher risk of HCC (26). In the present study, drug resistance developed in 41 of 77 patients (53.2%) treated with LAM and the patients with NA resistance had higher incidence of HCC compared with the patients without NAs resistance (P<0.01) (data not shown). In the current study, the rate of HCC occurrence in patients treated with old NAs was higher compared with that of patients treated with new NAs (P<0.01; Table II). HBV DNA (P<0.01), platelets (P<0.01), and FIB-4 index at SOT (P<0.01) were higher in patients treated with old NAs compared with the values in patients treated with

Table I. Characteristics of patients with or without HCC during NA treatment.

Characteristic	All	HCC occurrence	No HCC occurrence	P-value
n	260	40	220	
Age, years	53 (44-61)	56 (48-72)	52 (43-60)	0.017
Male/Female, n	154/106	31/9	123/97	0.014
NA treatment, n				<0.01
LAM	77	21	56	
ETV	163	19	144	
TDF	8	0	8	
TAF	12	0	12	
HBV-DNA, log IU/ml	6.2 (4.5-7.2)	6.25 (5.33-7.05)	6.2 (4.38-7.2)	0.669
Blood test at SOT				
PLT, $x10^4/mm^3$	17.15 (11.9-22.48)	11.05 (7.56-16.73)	18.15 (13.58-23.5)	<0.01
TB, mg/dl	0.8 (0.6-1.2)	1.05 (0.7-1.85)	0.8 (0.6-1.1)	0.077
AST, IU/l	49 (31-94.75)	62 (36.75-100)	46.5 (30.75-83.5)	0.950
ALT, IU/l	50.5 (35-113)	57 (34.5-101.25)	50 (34.75-122)	0.628
Alb, g/dl	4.0 (3.7-4.3)	3.7 (2.98-4.1)	4.0 (3.8-4.35)	<0.01
FIB-4 index	2.19 (1.21-4.16)	5.44 (2.04-7.5)	2.03 (1.18-3.41)	<0.01
Blood test at 6 months after treatment				
PLT, $x10^4/mm^3$	18.4 (3.7-52.5)	10.7 (6.65-17.53)	18.45 (14-23.4)	<0.01
AST, IU/l	27 (22-36)	33 (25.25-43.25)	26 (21-34)	0.018
ALT, IU/l	26 (18.25-37)	27 (21.25-35.75)	26 (18-37)	0.523
FIB-4 index	1.71 (1.1-2.84)	3.73 (1.96-5.76)	1.54 (1.01-2.49)	<0.01
Follow up duration, years	8.18 (4.73-13.07)			

Data are expressed as median (inter-quartile range) unless otherwise specified. HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogs; HBV, hepatitis B virus; PLT, platelet count; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alb, albumin; FIB-4 index, fibrosis-4 index; LAM, lamivudine; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide.

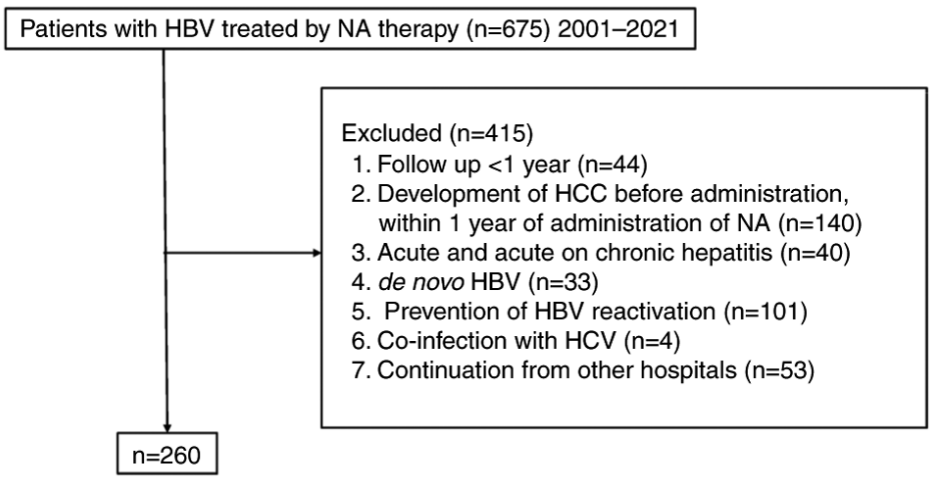

Figure 1. Recruitment flow chart. HBV, hepatitis B virus; NA, nucleos(t)ide analogs; HCV, hepatitis C virus.

new NAs (Table II). Fibrosis in patients treated with old NAs was more advanced compared with that observed in patients treated with the newer NAs (Table II).

Univariate and multivariate analysis for risk factors of HCC. Using the ROC value for HCC occurrence, the cut-off value of FIB-4 index at 6 months after NA treatment was 1.95 (AUC, 0.757; 1-specificity, 0.350; sensitivity, 0.775) (data not shown). From univariate analysis, age, male sex, old NA and FIB-4 index at 6 months were identified as risk factors of HCC. HBV DNA level showed no association. Multivariate analysis showed that age [hazard ratio (HR),

Table II. Characteristics of patients treated with old (LAM) and new NAs (ETV, TDF and TAF).

Characteristics	Old (LAM)	New (ETV, TDF, TAF)	P-value
n	77	183	
Age, years	49.5 (45-56.5)	55 (43-61.25)	0.012
Male/Female, n	54/23	100/83	<0.01
HCC occurrence, n	22	20	<0.01
HBV-DNA, log IU/ml	6.6 (5.5-7.4)	6.1 (4.25-7.1)	<0.01
Blood test at SOT			
PLT, $x10^4/mm^3$	14.7 (8.45-17.4)	18.95 (13.08-23.8)	<0.01
TB, mg/dl	1.0 (0.7-1.63)	0.8 (0.6-1.1)	<0.01
AST, IU/l	67 (39.75-130)	43 (29-74.25)	<0.01
ALT, IU/l	64 (42.75-157.75)	46.5 (24.75-88.25)	0.057
Alb, g/dl	3.9 (3.4-4.2)	4.1 (3.8-4.4)	<0.01
FIB-4 index	3.29 (1.79-6.67)	1.89 (1.09-3.32)	<0.01
FIB-4 index at 6 months after treatment	2.04 (1.39-4.07)	1.5 (1.02-2.52)	0.056
FIB-4 inverse index	1.96 (1.42-2.17)	2.18 (1.87-2.31)	0.028

Data are expressed as median (inter-quartile range) unless otherwise specified. NA, nucleos(t)ide analogs; LAM, lamivudine; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; PLT, platelet count; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alb, albumin; FIB-4 index, fibrosis-4 index

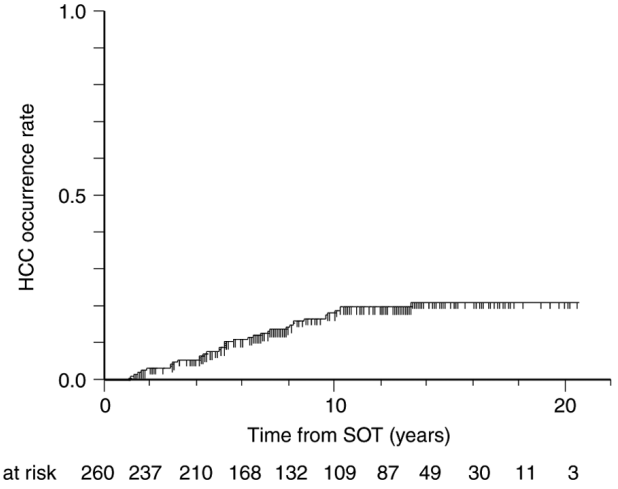

Figure 2. Cumulative incidence rate of HCC during NA treatment. HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogs; SOT, start of treatment.

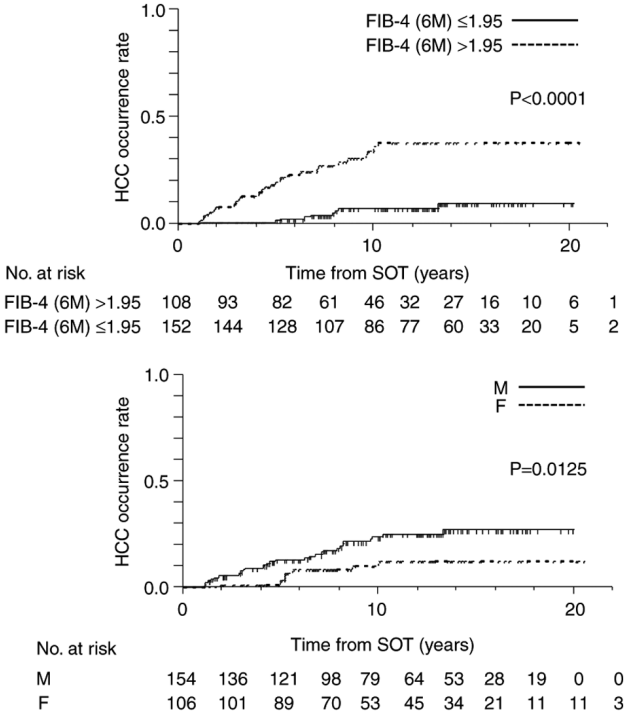

Figure 3. Kaplan-Meier analysis of the cumulative HCC occurrence stratified based on FIB-4 inverse index and sex during NA treatment. HCC, hepatocellular carcinoma; FIB-4 index, fibrosis-4 index; SOT, start of treatment.

1.31; P=0.0453], male sex (HR, 3.14; P<0.01) and FIB-4 index at 6 months >1.95 (HR, 4.35; P<0.01) were associated with the occurrence of HCC (Table III). Fig. 3 shows the cumulative incidence of HCC based on male sex and FIB-4 index at 6 months. There were significant differences with regard to male sex (P=0.0125) and FIB-4 index at 6 months (P<0.0001).

Multivariate analysis for risk factors of HCC in patients with serum AFP level at 6 months after NA treatment. Additional analysis was performed in the group in which serum AFP level at 6 months after NA treatment was measured. Table IV shows the clinical characteristics of these patients. There were significant differences in sex (P=0.029), content of NA (P<0.01), platelets at SOT (P<0.01) and 6 months after NA treatment (P<0.01) and FIB-4 index at SOT (P<0.01) and 6 months after NA treatment (P<0.01) between patients with and without HCC occurrence. Serum AFP level at SOT (P=0.011) and 6 months after NA treatment (P<0.01) were higher in patients with HCC occurrence

Table III. Factors associated with HCC occurrence during NA treatment.

	Univariate			Multivariate		
Factor	HR	95% CI	P-value	HR	95% CI	P-value
Age, years	1.04	1.02-1.07	<0.01	1.03	1.02-1.06	0.0453
Male sex	2.49	1.19-5.23	0.016	3.14	1.46-6.75	<0.01
HBV-DNA, log IU/ml	0.99	0.81-1.23	0.966			
FIB-4 index >1.95	5.93	2.82-12.47	<0.01	4.35	1.92-9.87	<0.01
Old NA	2.164	1.16-4.04	0.016	1.72	0.89-3.33	0.108

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogs; FIB-4 index, fibrosis-4 index.

Table IV. Characteristics of patients with or without HCC during NA treatment with measured serum AFP levels.

Characteristic	HCC occurrence	No HCC occurrence	P-value
n	21	110	
Age, years	56 (53-61)	55 (42.8-62.3)	0.217
Male/Female, n	17/4	60/50	0.029
NA Treatment, n			<0.01
LAM	9	5	
ETV	12	86	
TDF	0	7	
TAF	0	12	
HBV-DNA, log IU/ml	6.0 (4.63-7)	6.1 (4.1-7.1)	0.984
Blood test at SOT			
PLT, $x10^4/mm^3$	9.4 (6.7-12.6)	18.85 (12.78-23.58)	<0.01
AST, IU/l	62 (40-102)	42.5 (28-72.5)	0.914
ALT, IU/l	61 (33-98)	44 (31.75-82.25)	0.433
FIB-4 index	6.18 (3.52-7.76)	1.99 (1.08-3.8)	<0.01
AFP, ng/ml	31.2 (7.33-72.3)	3.5 (2.6-6.4)	0.011
Blood test at 6 months after treatment			
PLT, $x10^4/mm^3$	9.4 (6.4-13.0)	19.1 (14.08-23.48)	<0.01
AST, IU/l	33 (27-40.5)	26 (20-33.25)	<0.01
ALT, IU/l	28 (21.5-40)	25.5 (18-34.5)	0.202
FIB-4 index	4.09 (2.22-5.61)	1.46 (0.9-2.51)	<0.01
AFP, ng/ml	7.6 (4.8-16.05)	3.35 (2.4-4.325)	<0.01

Data are expressed as median (inter-quartile range) unless otherwise specified. HCC, hepatocellular carcinoma; NA, nucleos(t)ide analogs; HBV, hepatitis B virus; PLT, platelet count; TB, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; Alb, albumin; FIB-4 index, fibrosis-4 index; LAM, lamivudine; ETV, entecavir; TDF, tenofovir disoproxil fumarate; TAF, tenofovir alafenamide; AFP, α-fetoprotein.

compared with those in patients with no HCC. The cut-off value of serum AFP level at 6 months after treatment was 4 ng/ml (AUC, 0.842; 1-specificity, 0.3; sensitivity, 0.905) (data not shown). FIB-4 index at 6 months >1.95 (HR, 8.27; P=0.014) and serum AFP level at 6 months >4 ng/ml (HR, 4.26; P=0.033) were identified as risk factors in multivariate analysis (Table V). Fig. 4 shows Kaplan-Meier analysis of the cumulative HCC occurrence stratified on the basis of cut-off values of serum AFP level at 6 months after treatment (P<0.0001).

Discussion

Current antiviral therapies cannot achieve complete eradication of HBV; therefore, these treatments decrease HCC risk but do not eliminate the risk of HCC in patients with chronic HBV infection (12,13,27). Thus, HCC represents the main challenge in the management of patients with HBV, as HCC may occur even under effective long-term antiviral therapy and is the only factor that currently affects liver-related mortality in diagnosed and treated patients with HBV (12).

Table V. Factors associated with HCC occurrence during NA treatment with the addition of serum AFP level.

	Multivariate		
Parameter	HR	95% CI	P-value
Age, years	1.01	0.95-1.06	0.786
Male sex	2.34	0.72-7.59	0.157
FIB-4 index at 6 months >1.95	8.27	1.51-45.09	0.014
Old NA	1.71	0.61-4.75	0.310
AFP at 6 months >4 ng/ml	4.26	1.13-16.13	0.033

HCC, hepatocellular carcinoma; HBV, hepatitis B virus; NA, nucleos(t)ide analogs; FIB-4 index, fibrosis-4 index; AFP, α-fetoprotein.

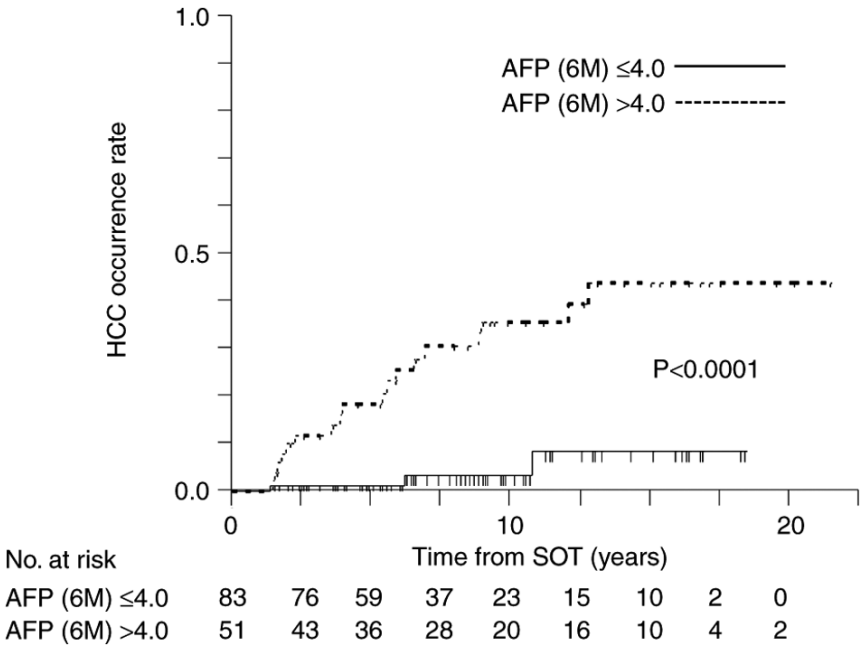

Figure 4. Kaplan-Meier analysis of the cumulative HCC occurrence stratified based on serum AFP levels at 6 months after NA treatment. HCC, hepatocellular carcinoma; AFP, α-fetoprotein; SOT, start of treatment.

HCC risk is the highest among untreated chronic patients with HBV with LC (1,28,29). The FIB-4 index has been used as a non-invasive and surrogate marker for advanced hepatic fibrosis (22) and shows high predictive values for the development of HCC risk in patients with chronic HBV infection (30,31). Previous studies have reported FIB-4 index as a risk factor for HCC occurrence in patients with HBV during NA therapy but the early occurrence of HCC during NA therapy in previous studies may have included HCC not detected by imaging before NA therapy (17,18). The present study analyzed HCC occurrence ≥1 year following NA treatment and identified FIB-4 index at 6 months after NA treatment >1.95 as a predictor of HCC occurrence during long-term NA therapy. The follow up duration in the current study was longer compared with previous studies and accurate cut off value of FIB-4 index was obtained.

In the present study, baseline HBV DNA level was not associated with HCC risk, which was consistent with some previous studies (32,33). However, these findings were in contrast to studies assessing HCC risk in untreated patients (34,35). Several studies have demonstrated that the new NAs, including ETV, TDF and TAF, reduce HCC risk to a greater extent compared with older NAs (LAM and ADF) (24,25). In the present study, HCC occurrence in patients treated by old NAs was more frequent compared with that in patients treated by new NAs, and old NAs were one of the contributing factors for HCC occurrence as determined by univariate analysis. This result might be because of the advanced fibrosis in patients treated with old NAs compared with that in patients treated with new NAs. Overall, the present study showed the importance of the new NAs to reduce HCC risk.

The current results identified four factors, sex, age, FIB-4 index at 6 months after NA treatment and serum AFP level at 6 months after NA treatment, that were associated with HCC risk during NA therapy. Older age is a well-known risk factor for HCC (36). Elevated AFP serum level is one of the common risk factors for HCC occurrence in patients with HBV and hepatitis C virus (16,37-39). In the group where serum AFP levels were measured, the present study showed that FIB-4

index >1.95 and serum AFP level >4 ng/ml at 6 months after NA treatment may be useful markers to select patients at higher risk of carcinogenesis. Previous prediction models such as GAG-HCC, CU-HCC and REACH-B (18-21) have identified these factors. The limitations of these studies were that the patients were enrolled regardless of receiving antiviral therapy and the difficulty of diagnosing LC. Only patients receiving antiviral therapy were enrolled in the current study.

The PAGE-B score was recently developed and validated in mostly Caucasian European patients under ETV or TDF therapy. In this score, which includes age, sex and platelet counts, probably reflecting the severity of liver disease, the addition of cirrhosis does not substantially improve the discrimination (14). While PAGE-B is useful, FIB-4 index >1.95 and serum AFP level >4 ng/ml at 6 months after NA treatment are simpler predictors.

The present study has several limitations. The study group was from a single center, which limited the numbers of HCC events during NA. Additionally, the present study did not consider the involvement of HBV core promoter mutations, BMI, alcohol consumption and diabetes due to insufficient information.

In conclusion, FIB-4 index >1.95 and serum AFP level >4 ng/ml at 6 months were predictors for the development of HCC in patients with HBV during NA treatment. Further study of hepatocarcinogenesis during NA is required with a longer follow-up period and larger numbers of participants. Regular careful examinations are required if the FIB-4 index and serum AFP at 6 months after NA treatment are high.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

AK, MM, MY, KT, AM and KM designed the study. AK, MM, KT and YK assisted with data analyses. AK wrote the initial draft of the manuscript. MY and KM contributed to analysis and interpretation of data. MY and KM assisted in the preparation and critical review of the manuscript. AK and MY confirm the authenticity of all the raw data. All authors agreed to be accountable for all aspects of the work. All authors read and approved the final version of the manuscript

Ethics approval and consent to participate

The research was conducted in accordance with the principles of the Declaration of Helsinki. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki. This study was approved by the ethics committee of Aso Iizuka Hospital (approval no. 21141; Iizuka, Japan). Informed consent of individual patients was not obtained because of the retrospective nature of this study.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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