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Antiplatelet Therapy and the Risk of Hepatocellular Carcinoma in Chronic Hepatitis B Patients on Antiviral Treatment

Minjong Lee,[1,2*] Goh Eun Chung,[3*] Jeong-Hoon Lee,[1] Sohee Oh,[4] Joon Yeul Nam,[1] Young Chang,[1] Hyeki Cho,[1] Hongkeun Ahn,[1] Young Youn Cho,[1] Jeong-ju Yoo,[1] Yuri Cho,[1] Dong Hyeon Lee,[1] Eun Ju Cho,[1] Su Jong Yu,[1] Dong Ho Lee,[5] Jeong Min Lee,[5] Yoon Jun Kim,[1] and Jung-Hwan Yoon[1]

Antiplatelet therapy has shown protective effects against hepatocellular carcinoma (HCC) in preclinical studies. However, it is unclear whether antiplatelet therapy lowers the risk of HCC in patients with chronic hepatitis B. A retrospective analysis was conducted of data from 1,674 chronic hepatitis B patients, enrolled between January 2002 and May 2015, whose serum hepatitis B virus DNA levels were suppressed by antivirals to <2,000 IU/mL. The primary and secondary outcomes were development of HCC and bleeding events, respectively. Risk was compared between patients with antiplatelet treatment (aspirin, clopidogrel, or both; antiplatelet group) and patients who were not treated (non-antiplatelet group) using a time-varying Cox proportional hazards model for total population and propensity score–matching analysis. The antiplatelet group included 558 patients, and the non-antiplatelet group had 1,116 patients. During the study period, 63 patients (3.8%) developed HCC. In time-varying Cox proportional analyses, the antiplatelet group showed a significantly lower risk of HCC (hazard ratio [HR], 0.44; 95% confidence interval [CI], 0.23–0.85; $P = 0.01$), regardless of antiplatelet agent. In propensity score–matched pairs, antiplatelet therapy significantly reduced the risk of HCC (HR, 0.34; 95% CI, 0.15-0.77; $P = 0.01$). However, the overall risk of bleeding was higher in the antiplatelet group (HR, 3.28; 95% CI, 1.98-5.42; $P < 0.001$), particularly for clopidogrel with or without aspirin. Treatment with aspirin alone was not associated with a higher bleeding risk (HR, 1.11; 95% CI, 0.48-2.54; $P = 0.81$). *Conclusion*: Antiplatelet therapy reduces the risk of HCC in chronic hepatitis B patients whose hepatitis B virus is effectively suppressed. However, antiplatelet therapy containing clopidogrel may increase the risk of bleeding. (HEPATOLOGY 2017;66:1556–1569)

Chronic hepatitis B virus (HBV) infection is a major global health burden. Approximately 400 million people worldwide are chronically infected with HBV.[1,2] These patients have a substantially increased risk of cirrhosis and hepatocellular carcinoma (HCC), which together are responsible for approximately 1 million deaths worldwide every year.[1,2] Despite the use of highly potent antivirals, such as entecavir and tenofovir, which may effectively reduce the risk of HCC,[3,4] long-term follow-up

Abbreviations: CD, cluster of differentiation; CHB, chronic hepatitis B; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; IQR, interquartile range; MPR, medical possession rate; NAFLD, nonalcoholic fatty liver disease; PAGE score, score based on platelets, age, and gender; TDF, tenofovir disoproxil fumarate.

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studies have shown that approximately 1%-8% of patients with cirrhosis develop HCC per year.[5,6] Beyond the preventive effects of potent antiviral agents, chemopreventive strategies aimed at decreasing the risk or delaying the onset of HCC are needed in the era of antiviral therapy.[7]

Recent preclinical studies have suggested potential therapeutic applications of antiplatelet therapy in hepatitis B models. Platelets are key facilitators of this immune-mediated injury as they promote accumulation of cluster of differentiation 8–positive ($CD8^+$) T cells.[8] In an HBV transgenic mouse model of chronic immune-mediated liver disease that rapidly progresses to HCC, aspirin and/or clopidogrel reportedly decreased T cell–mediated inflammation, fibrosis severity, and progression to HCC.[9] In epidemiological studies, however, the effect of aspirin on HCC prevention is controversial. A large population-based study in the National Institutes of Health's Association of American Retired Persons Diet and Health Study cohort showed that aspirin use was associated with a 41% lower risk of HCC compared to nonuse.[10] By contrast, other population-based case–control and cohort studies have not observed an association between aspirin use and risk of HCC due to any cause, although these studies were not specifically designed to focus on aspirin use and HCC.[11,12] In addition, epidemiological studies have had critical limitations to draw concrete conclusions given that important confounders, such as age, gender, cirrhosis status, and antiviral treatments in chronic hepatitis B (CHB) patients, were not adequately controlled.

We investigated whether antiplatelet therapy is associated with a reduction in HCC incidence in patients chronically infected with HBV, which is one of the most important risk factors in developing HCC,[13] effectively suppressed by nucleos(t)ide analogues.

Materials and Methods

STUDY DESIGN

The study population was obtained from inpatient and outpatient database files between November 1, 2002, and May 31, 2015, at Seoul National University Hospital (Seoul, Korea) and consisted of a cohort of 14,392 consecutive adult CHB patients with suppressed HBV (serum HBV DNA levels <2,000 IU/mL) by antiviral treatment (Fig. 1). Patients were excluded if they met any of the following criteria: younger than age 18 or older than age 85; HCC development within 6 months from the index date; diagnosis with HCC before study enrollment; hepatitis B surface antigen seroclearance within 6 months from the index date; coinfection with other hepatotrophic viruses or human immunodeficiency virus; duration of antiplatelet or antiviral therapy <6 months; previous or current treatment with any other potentially confounding drugs, such as statins, metformin, sulfonylurea, insulin, or nonsteroidal anti-inflammatory drugs except aspirin for more than 1 month; serum HBV DNA levels ≥2,000 IU/mL at enrollment; or active alcoholism. To minimize the potential confounding effects of an antiviral regimen on HCC development, any patients who had been treated with antiviral agents that were never used in the antiplatelet group, such as pegylated interferon-α or clevudine-containing or tenofovir disoproxil fumarate (TDF)–containing regimens, were excluded from the non-antiplatelet group. None of the patients in the antiplatelet group were treated with pegylated interferon-α or clevudine-containing or TDF-containing regimens.

The entire cohort was divided according to antiplatelet therapy: the non-antiplatelet group consisted of patients treated with various antiviral agents, and the antiplatelet group was patients who were treated

ARTICLE INFORMATION:

From the [1]Department of Internal Medicine and Liver Research Institute, Seoul National University College of Medicine, Seoul, Korea; [2]Department of Internal Medicine, Kangwon National University Hospital, Chuncheon, Korea; [3]Department of Internal Medicine, Healthcare Research Institute, Gangnam Healthcare Center, Seoul National University Hospital, Seoul, Korea; [4]Department of Biostatistics, Seoul Metropolitan Government Seoul National University Boramae Medical Center, Seoul, Korea; [5]Department of Radiology, Seoul National University College of Medicine, Seoul, Republic of Korea.

ADDRESS CORRESPONDENCE AND REPRINT REQUESTS TO:

Jeong-Hoon Lee, M.D., Ph.D.
Department of Internal Medicine, Seoul National University Hospital
101 Daehak-ro, Jongno-gu
Seoul 03080, Korea
E-mail: pindra@empal.com or JHLeeMD@gmail.com
Tel: + 82-2-2072-2228

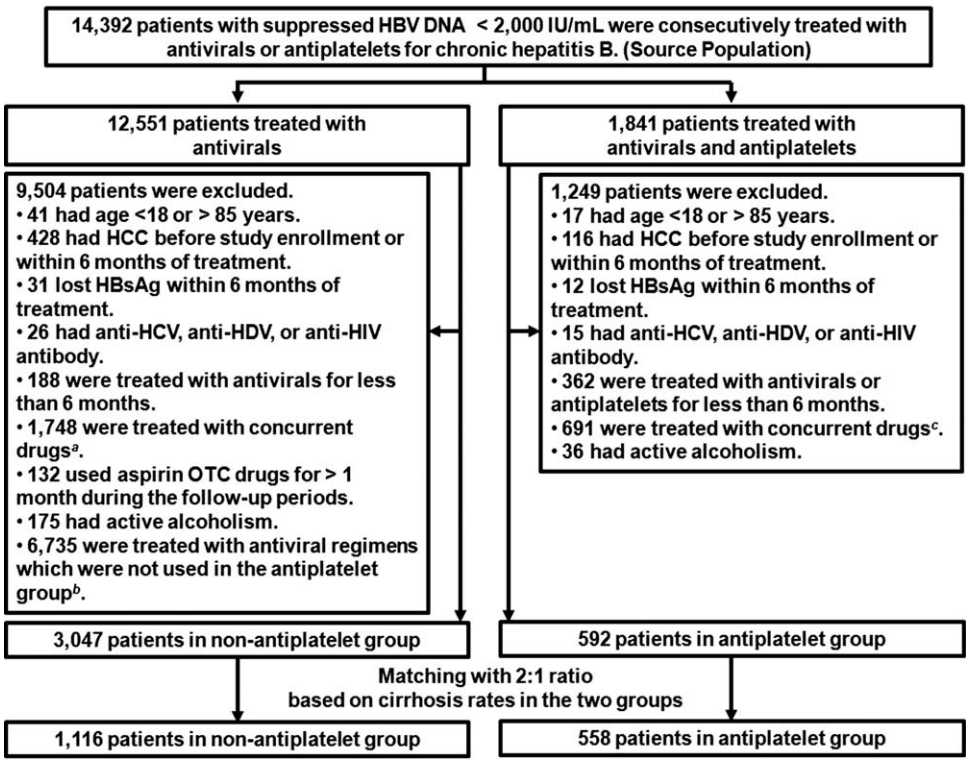

FIG. 1. Patient flow diagram. Patients excluded when treated with other potential chemopreventive drugs such as stains, metformin, sulfonylurea, insulin, and nonsteroidal anti-inflammatory drugs, and with two or more of the above drugs. [a]Concurrent drugs (n = 1,748) of statins (n = 309), metformin (n = 243), sulfonylurea (n = 109), insulin (n = 103), nonsteroidal anti-inflammatory drugs (n = 341), and two or more of the above drugs (n = 643) in the non-antiplatelet group. [b]Pegylated interferon-α (n = 112) and clevudine (nucleoside analogue)-containing (n = 2,591) or TDF-containing (n = 4,032) regimens were excluded in the non-antiplatelet group. None of the patients in the antiplatelet group were treated with pegylated interferon-α or with a clevudine-containing or TDF-containing regimen. [c]Concurrent drugs (n = 691) of statins (n = 132), metformin (n = 91), sulfonylurea (n = 34), insulin (n = 37), nonsteroidal anti-inflammatory drugs (n = 133), and two or more of the above drugs (n = 264) in the antiplatelet group. Abbreviations: HBsAg, surface antigen of the hepatitis B virus; HCV, hepatitis C virus; HDV, hepatitis D virus; HIV, human immunodeficiency virus; OTC, over-the-counter.

with antiplatelets (aspirin 100 mg/day, clopidogrel 75 mg/day, or both) and antiviral agents. All antiplatelet agents were prescribed by the physicians at Seoul National University Hospital for the patients visiting the outpatient clinic on a regular basis. We excluded patients in the non-antiplatelet group who had taken aspirin, including over-the-counter drugs containing aspirin, for more than 1 month before the index date, based on both medical history taken in the outpatient clinic and data in the questionnaire on medication history in the patient's file in the outpatient or inpatient clinic. During the study period, patients in the non-antiplatelet group who had taken aspirin or over-the-counter drugs containing aspirin for more than 1 month were also excluded, based on the questionnaire used to screen for medications with high bleeding risk (e.g., antiplatelets and anticoagulants) before endoscopic surveillance every 1-2 years for esophageal varix or gastric cancer.

The patients in the non-antiplatelet group were randomly selected from the database file with matching to the antiplatelet group (2:1 ratio) by the presence of cirrhosis. This study was approved by the institutional review board of Seoul National University Hospital, and the requirement for informed consent from patients was waived.

ENDPOINTS AND FOLLOW-UP EVALUATION

The primary endpoint of interest in this study was HCC development. Secondary endpoints evaluated

included bleeding events, bleeding-related mortality, and the development of malignancy other than HCC, end-stage kidney disease, and cardiovascular events. The index date was defined as the first date that the patient on antiviral treatment achieved a serum level of HBV DNA <2,000 IU/mL. The censored date was defined as the date of patient's death, last date of follow-up, or data cutoff date (May 31, 2015).

Patients regularly underwent clinical examinations, liver function tests, and measurement of serum HBV DNA levels every 6 months. The main modality for HCC surveillance in this study was ultrasonography with serum alpha-fetoprotein levels according to the current guidelines of this country.[14] Compliance with taking antiviral agents was confirmed by both meticulous reviews of medical records written by physicians who prescribed the agents and serial follow-up of HBV DNA titer every 6 months.[15] When results of surveillance tests were equivocal, 3-month interval screenings were permitted. Compliance with antiplatelet agents in the antiplatelet group was assessed when the patients visited the outpatient clinic where physicians had first prescribed antiplatelet agents, such as the departments of cardiology, endocrinology, neurology, family medicine, and neurosurgery. To evaluate patient compliance with antiplatelet agents, we calculated the medical possession rates (MPRs) of the patients in the antiplatelet group stratified by specific antiplatelet therapy and determined the median MPR values. MPR was calculated by the total days of medication supply divided by time interval. We used an MPR cutoff value for good compliance of >80%.[16] Scores based on platelets, age, and gender (PAGE-B),[17] developed for HCC risk assessment in CHB patients under effective antiviral therapy, were used to stratify the risk of developing HCC at baseline in both groups.

DEFINITIONS

Diagnosis of liver cirrhosis, HCC, and bleeding events were defined in the Supporting Information. Viral breakthrough during follow-up was defined as an increase in HBV DNA by >1 log compared to nadir or HBV DNA 100 IU/mL in patients on antiviral therapy with previously undetectable levels.[18] Serum assays were also described in the Supporting Information.

STATISTICAL ANALYSES

A time-varying Cox proportional hazards regression model, propensity score-matching, and competing risk analyses were performed. Detailed statistical analyses were described in the Supporting Information.

Results

BASELINE CHARACTERISTICS

The total study population was comprised of 1,674 CHB patients: 1,116 patients were on antivirals only without antiplatelets (non-antiplatelet group), and 558 patients were taking antiplatelet therapy in addition to antiviral treatment (antiplatelet group) (Fig. 1).

The two groups differed significantly in terms of baseline characteristics (Table 1). Patients in the non-antiplatelet group were significantly younger (mean, 50 versus 55 years), had a higher hepatitis B e antigen–positivity rate (24.4% versus 14.5%), and had higher serum HBV DNA levels (mean, 1.5 versus 1.4 $\log_{10}$ IU/mL) compared to those in the antiplatelet group. The Model for End-Stage Liver Disease scores in the antiplatelet group were significantly higher than those in the non-antiplatelet group. There were no significant differences between the numbers of patients taking each type of antiviral treatment (Supporting Table S1). In the antiplatelet group, the median duration of exposure to antiplatelet therapy was 27.6 months (interquartile range [IQR], 7.2-60.5 months): the median duration of exposure to antiplatelets among patients treated with aspirin, clopidogrel, or dual antiplatelets of aspirin plus clopidogrel was 38.5 months (IQR, 10.4-68.7 months), 17.3 months (IQR, 6.0-36.7 months), and 18.0 months (IQR, 6.0-47.1 months), respectively. The main purpose of taking antiplatelet agents was the prevention of cardiovascular disease (42.1%). In particular, patients mainly took aspirin for preventive purposes (56.9%). Clopidogrel alone or dual antiplatelet treatment with both aspirin and clopidogrel was prescribed mainly after percutaneous coronary intervention for ischemic heart disease (32.2% and 59.2%, respectively). The median MPRs for the patients treated with aspirin, clopidogrel, or dual antiplatelets were 88.3% (IQR 72.2%-99.1%), 82.7% (IQR 71.6%-95.3%), and 81.5% (IQR 70.5%-96.2%), respectively.

The probable etiologies for serum alanine aminotransferase levels exceeding 80 U/L were as follows: nonalcoholic fatty liver disease (NAFLD; n = 81), drug-induced liver injury (n = 25), ischemic hepatitis (n = 2), and congestive heart failure (n = 6).

The interval between each surveillance by ultrasonography with serum alpha-fetoprotein levels in the non-antiplatelet group was 6.1 ± 1.9 months, which

TABLE 1. Baseline Characteristics

	Non-Antiplatelet Group (n = 1,116)	Antiplatelet Group				$P^{\dagger}$ (Non-AP versus AP)	$P^{\ddagger}$ (Non-AP versus A versus C versus D)
		All (n = 558)	Aspirin (n = 343)	Clopidogrel (n = 90)	Dual* (n = 125)		
Age (years)	50.3 ± 10.8	55.2 ± 11.0	54.2 ± 11.1	57.3 ± 11.5	56.5 ± 10.0	<0.001	<0.001
Male, n (%)	716 (64.2%)	339 (60.8%)	197 (57.4%)	57 (63.3%)	85 (68.0%)	0.09	0.17
DM, n (%)	93 (8.3%)	184 (33.0%)	125 (36.4%)	24 (26.7%)	35 (28.0%)	<0.001	<0.001
Cirrhosis, n (%)	136 (12.2%)	68 (12.2%)	42 (12.2%)	13 (14.4%)	13 (10.4%)	1.000	0.85
Cause of antiplatelet therapy, n (%)						<0.001	<0.001
Preventive		235 (42.1%)	195 (56.9%)	18 (20.0%)	22 (17.6%)		
Coronary heart disease		169 (30.3%)	66 (19.2%)	29 (32.2%)	74 (59.2%)		
Arrhythmia		41 (7.3%)	31 (9.0%)	4 (4.4%)	6 (4.8%)		
Brain infarct		40 (7.2%)	11 (3.2%)	19 (21.1%)	10 (8.0%)		
Others[§]		73 (13.1%)	40 (11.7%)	20 (22.2%)	13 (10.4%)		
CTP class						<0.001	<0.001
A	1,095 (99.1%)	514 (92.1%)	309 (90.1%)	87 (96.7%)	118 (94.4%)		
B	21 (1.9%)	44 (7.6%)	34 (9.9%)	3 (3.3%)	7 (5.6%)		
C	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)		
MELD score	8.1 ± 2.9	9.1 ± 3.7	8.9 ± 3.5	9.8 ± 4.5	9.2 ± 3.5	<0.001	<0.001
HBeAg positivity, n (%)	272 (24.4%)	81 (14.5%)	55 (16.0%)	4 (4.4%)	22 (17.6%)	<0.001	<0.001
HBV DNA ($\log_{10}$ IU/mL)	1.5 ± 0.6	1.4 ± 0.6	1.4 ± 0.7	1.4 ± 0.7	1.3 ± 0.6	0.03	0.01
ALT (IU/L)						<0.001	<0.001
<80	1,044 (93.5%)	514 (92.1%)	311 (90.7%)	85 (94.4%)	118 (94.4%)		
80-200	72 (6.5%)	30 (5.4%)	22 (6.4%)	4 (4.4%)	4 (3.2%)		
>200	0 (0.0%)	12 (2.2%)	8 (2.3%)	1 (1.1%)	3 (2.4%)		
Albumin (g/dL)	4.3 (4.1, 4.5)	4.2 (3.8, 4.4)	4.2 (3.8, 4.4)	4.3 (3.8, 4.5)	4.1 (3.7, 4.4)	<0.001	<0.001
Total bilirubin (mg/dL)	0.8 (0.6, 1.1)	0.9 (0.6, 1.2)	0.9 (0.7, 1.1)	0.8 (0.6, 1.2)	0.8 (0.6, 1.2)	0.16	0.09
Creatinine (mg/dL)	0.9 (0.8, 1.0)	1.0 (0.8, 1.1)	0.9 (0.8, 1.1)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)	<0.001	<0.001
PT INR	1.0 (1.0, 1.1)	1.0 (1.0, 1.1)	1.0 (1.0, 1.1)	1.0 (1.0, 1.1)	1.0 (1.0, 1.1)	0.77	0.77
Platelet ($\times 10^3/\mu$L)	194 (169, 229)	191 (146, 234)	195 (151, 238)	184 (127, 224)	184 (150, 226)	<0.001	<0.001

Values are expressed as the mean with standard deviation or median with IQR for continuous variables and frequency with proportion for categorical variables.
*Combination treatment of aspirin and clopidogrel.
†*P* value as comparison of the antiplatelet and non-antiplatelet groups.
‡*P* value as comparison of the non-antiplatelet, aspirin, clopidogrel, and dual antiplatelet therapy groups. *P* values estimated by χ^2 test or Fisher's exact test for categorical variables and Mann-Whitney *U* test or Kruskal-Wallis test for continuous variables.
§Other causes included peripheral vessel disease, transient ischemic attack, small-vessel disease in the brain, valvular heart disease, cardiomyopathy, and brain aneurysm coiling.
Abbreviations: A, aspirin group; ALT, alanine aminotransferase; AP, antiplatelet group; C, clopidogrel group; CTP, Child-Turcotte-Pugh; D, dual antiplatelet therapy group; DM, diabetes mellitus; HBeAg, hepatitis B virus e antigen; MELD, Model for End-Stage Liver Disease; non-AP, non-antiplatelet group; PT INR, international normalized ratio for prothrombin time.

was not significantly different from 6.2 ± 1.3 months in the antiplatelet group ($P = 0.51$; Supporting Table S2). When hepatic nodules that were suspicious for HCC were detected during ultrasonography, serum alpha-fetoprotein levels were rising, or the results of the ultrasonography exam were incomplete due to cirrhotic liver parenchyma, contrast-enhanced liver computed tomography and/or liver-specific contrast-enhanced magnetic resonance imaging were performed. The proportion of patients examined by contrast-enhanced liver computed tomography plus liver-specific contrast-enhanced magnetic resonance imaging was not significantly different between the non-antiplatelet and antiplatelet groups.

CLINICAL EVENTS DURING THE STUDY PERIOD

During the study period (median, 57 months; IQR, 31-93 months), a total of 63 (3.8%) patients developed HCC. For patients with HCC in this study, a significant difference in HCC stages according to the Barcelona Clinic Liver Cancer staging system and treatment modalities between the antiplatelet and non-antiplatelet groups was not found (Supporting Tables S3 and S4). Among 1,611 patients without HCC development, 90 (5.6%) expired and 17 (1.1%) underwent liver transplantation. Among 63 patients with HCC development, 4 (6.3%) expired and 1 (1.6%) underwent liver transplantation.

Viral breakthrough occurred in 126 (11.3%) of the 1,116 non-antiplatelet patients and 50 (9.0%) of the 558 antiplatelet patients ($P = 0.14$) (not shown as a table). Among the 1,116 non-antiplatelet patients, 35 (3.1%) of the patients with viral breakthrough showed poor compliance and 91 (8.2%) showed drug resistance; among the 558 antiplatelet patients, 25 (4.5%) of the patients with viral breakthrough showed poor compliance and 25 (4.5%) showed drug resistance. The patients with antiviral resistance achieved complete virologic suppression after their antiviral regimen was changed to rescue therapy with nucleos(t)ide analogues.

ASSOCIATION BETWEEN ANTIPLATELET THERAPY AND HCC DEVELOPMENT

Multivariable Analyses

To minimize the immortal time bias, time-varying Cox regression analyses to identify factors predictive of HCC development were performed for the entire cohort (n = 1,674) (Table 2). Antiplatelet therapy overall was independently associated with a significantly lower risk of HCC development (hazard ratios [HR], 0.44; 95% confidence interval [CI], 0.23-0.85; $P = 0.01$) after adjustment for age, platelet counts, and viral breakthrough (Table 2). The cumulative incidence rates of HCC were 1.6% at 5 years for patients in the antiplatelet group and 5.2% in the non-antiplatelet group (Fig. 2A). Regarding respective antiplatelet agents, the aspirin treatment showed an independent association with a significantly lower risk of HCC development (HR, 0.26; 95% CI, 0.09-0.74; $P = 0.01$). However, neither the clopidogrel group (HR, 0.63; 95% CI, 0.15-2.65; $P = 0.53$) nor the dual antiplatelets group (HR, 0.67; 95% CI, 0.28-1.60; $P = 0.37$) showed a significant association with risk of HCC development (Table 2). The cumulative incidence rates of HCC at 5 years were 1.6%, 1.3%, and 1.8% in the aspirin, clopidogrel, and dual antiplatelets groups, respectively (Fig. 2B).

The primary diagnosis of HCC in the other patients (237 patients, 14.2%) lost to follow-up was identified using data obtained from the Korean National Health Insurance Service database. Among patients in the antiplatelet group, 41 (7.3%) were lost to follow-up;

TABLE 2. Time-Varying Cox Proportional Hazards Regression Analysis for HCC Development in the Entire Cohort

	Univariable		Multivariable	
	HR (95% CI)	*P**	HR (95% CI)	*P**
Age (years)	1.04 (1.02, 1.07)	0.001	1.04 (1.01, 1.06)	0.002
Male, n (%)	1.54 (0.88, 2.69)	0.13		
DM, n (%)	1.08 (0.59, 1.99)	0.81		
Cirrhosis, n (%)	2.48 (1.40, 4.40)	0.002	0.97 (0.49, 1.92)	0.93
CTP class				
B versus A (reference)	1.75 (0.57, 4.08)	0.26		
C versus A (reference)	9.84 (0.08, 68.52)	0.11		
HBeAg positivity, n (%)	0.98 (0.54, 1.78)	0.95		
HBV DNA (log_{10} IU/mL)	0.73 (0.48, 1.12)	0.15		
ALT (IU/L)				
80-200 versus < 80	0.87 (0.24, 2.21)	0.80		
>200 versus < 80	0.61 (0.01, 4.22)	0.73		
Albumin (g/dL)	0.60 (0.39, 0.93)	0.02	0.68 (0.40, 1.15)	0.15
Total bilirubin (mg/dL)	0.97 (0.71, 1.33)	0.84		
Creatinine (mg/dL)	0.90 (0.66, 1.23)	0.51		
PT INR	2.97 (1.22, 7.19)	0.02	1.81 (0.56, 5.78)	0.32
Platelet ($\times 10^3$ /μL)	0.990 (0.986 0.995)	<0.001	0.991 (0.985, 0.997)	0.002
Viral breakthrough	2.10 (1.19, 3.73)	0.01	2.46 (1.36, 4.46)	0.003
Antiplatelet therapy, n (%)	0.58 (0.31, 0.86)	0.04	0.44 (0.23, 0.85)	0.01
Aspirin	0.33 (0.12, 0.91)	0.03	0.26 (0.09, 0.74)	0.01
Clopidogrel	0.97 (0.24, 3.99)	0.97	0.63 (0.15, 2.65)	0.53
Dual antiplatelets†	0.96 (0.41, 2.23)	0.91	0.67 (0.28, 1.60)	0.37

*P value estimated by Cox proportional hazard regression with Firth's penalized likelihood.
†Combination treatment of aspirin and clopidogrel.
Abbreviations: ALT, alanine aminotransferase; CTP, Child-Turcotte-Pugh; DM, diabetes mellitus; HBeAg, hepatitis B virus e antigen; PT INR, international normalized ratio for prothrombin time.

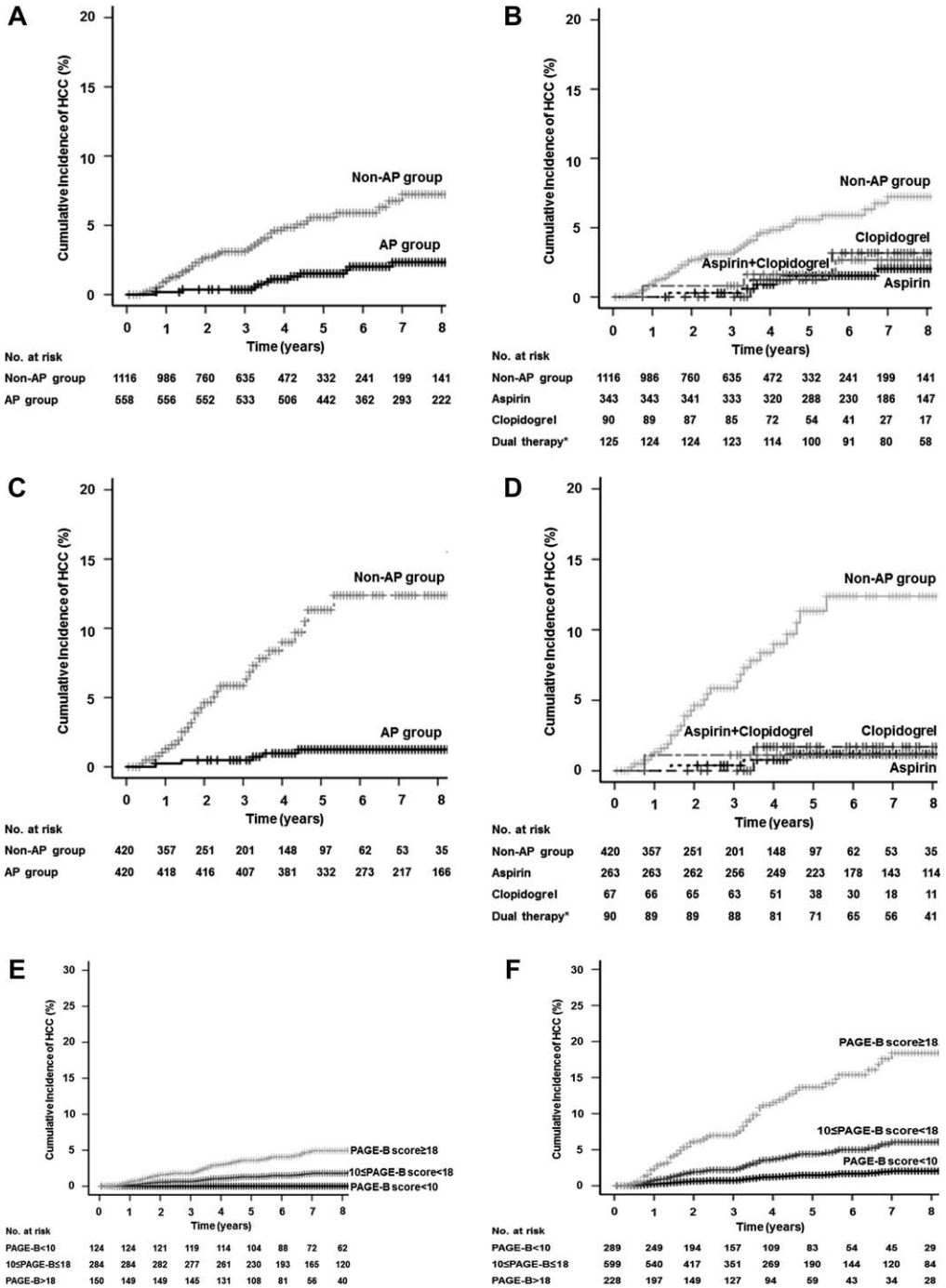

FIG. 2. Kaplan-Meier estimates of cumulative incidence of HCC in the entire cohort and propensity score–matched cohorts. Propensity score matching of the entire cohort created 420 matched pairs of patients. (A) Cumulative incidence of HCC in non-antiplatelet and antiplatelet groups in the entire cohort: non-antiplatelet group versus antiplatelet group ($P = 0.04$ by log-rank test). (B) Cumulative incidence of HCC according to antiplatelet drugs in the entire cohort: non-antiplatelet group versus aspirin ($P = 0.02$ by log-rank test), clopidogrel ($P = 0.98$ by log-rank test), and dual antiplatelets groups ($P = 0.64$ by log-rank test). (C) Cumulative incidence of HCC in the non-antiplatelet and antiplatelet groups in the propensity score–matched cohorts: non-antiplatelet group versus antiplatelet group ($P = 0.007$ by log-rank test). (D) Cumulative incidence of HCC according to antiplatelet drugs in propensity score–matched cohorts: non-antiplatelet group versus aspirin ($P < 0.001$ by log-rank test), clopidogrel ($P = 0.02$ by log-rank test), and dual antiplatelets groups ($P = 0.001$ by log-rank test). (E) Cumulative incidence of HCC in the antiplatelet group according to PAGE-B score ($P = 0.007$ by log-rank test). (F) Cumulative incidence of HCC in the non-antiplatelet group according to PAGE-B score ($P < 0.001$ by log-rank test). Risk for HCC occurrence was stratified by PAGE-B score: low-risk, PAGE-B score < 10: intermediate-risk, $10 \leq$ PAGE-B score < 18; and high-risk, PAGE-B score ≥ 18. *Dual antiplatelet therapy means a combination therapy of aspirin and clopidogrel. Abbreviation: AP, antiplatelet.

TABLE 3. Characteristics of Propensity Score–Matched Cohorts

	Non-Antiplatelet Group (n = 420)	Antiplatelet Group (n = 420)	P*
Age (years)	54.7 ± 10.5	54.5 ± 11.1	0.69
Male, n (%)	258 (61.4%)	259 (61.7%)	0.94
DM, n (%)	82 (19.5%)	85 (20.2%)	0.71
Cirrhosis, n (%)	34 (8.1%)	43 (10.2%)	0.25
CTP score	5.2 ± 0.6	5.2 ± 0.5	0.85
MELD score	8.6 ± 4.0	8.6 ± 3.0	0.94
HBeAg positivity (%)	68 (16.2%)	65 (15.5%)	0.75
ALT (IU/L)	28 (18, 48)	27 (19, 39)	0.71
Albumin (g/dL)	4.2 (4.0, 4.4)	4.2 (3.9, 4.5)	0.93
Total bilirubin (mg/dL)	0.8 (0.6, 1.1)	0.9 (0.6, 1.1)	0.68
Creatinine (mg/dL)	0.9 (0.7, 1.0)	1.0 (0.8, 1.1)	0.92
PT INR	1.0 (1.0, 1.1)	1.0 (1.0, 1.1)	0.73
Platelet ($\times 10^3/\mu L$)	189 (165, 220)	196 (156, 239)	0.56
Viral breakthrough	44 (10.5%)	41 (9.8%)	0.74

*P value estimated by paired t-test for continuous variables and McNemar test for categorical variables.
Abbreviations: ALT, alanine aminotransferase; CTP, Child-Turcotte-Pugh; DM, diabetes mellitus; HBeAg, hepatitis B virus e antigen; MELD, model for end-stage liver disease; PT INR, international normalized ratio for prothrombin time.

196 patients (17.6%) in the non-antiplatelet group were lost to follow-up. When those patients were included in both groups, the risk of HCC development in the antiplatelet group was significantly lower than that in the non-antiplatelet group (HR, 0.45; 95% CI, 0.24-0.86; $P = 0.008$) (not shown as a table). Regarding respective antiplatelet agents, the aspirin group (HR, 0.28; 95% CI, 0.11-0.75; $P = 0.009$) was significantly associated with a lower risk of HCC. Additionally, both the clopidogrel group (HR, 0.65; 95% CI, 0.16-2.64; $P = 0.55$) and the dual antiplatelets group (HR, 0.68; 95% CI, 0.29-1.58; $P = 0.38$) displayed a lower risk for HCC development, but this failed to reach statistical significance.

Viral breakthrough was independently associated with a significantly higher risk of HCC development (HR, 2.46; 95% CI, 1.36-4.46; $P = 0.003$) (Table 2). In the subgroup of patients who did not experience viral breakthrough during the study period, antiplatelet therapy (n = 508) was significantly associated with lower development of HCC compared to the non-antiplatelet group (n = 990) (HR, 0.48; 95% CI, 0.23-0.97; $P = 0.04$) (Supporting Table S5). Regarding respective antiplatelet agents, the aspirin group (HR, 0.27; 95% CI, 0.08-0.85; $P = 0.03$) was significantly associated with a lower risk of HCC. Both the clopidogrel group (HR, 1.02; 95% CI, 0.25-4.18; $P = 0.98$) and the dual antiplatelets group (HR, 0.71; 95% CI, 0.26-1.96; $P = 0.50$) failed to reach statistical significance. However, among patients who experienced viral breakthrough, antiplatelet therapy (n = 50) was not significantly associated with lower development of HCC compared to the non-antiplatelet group (n = 126) (HR, 0.87; 95% CI, 0.25-3.04; $P = 0.83$) (Supporting Table S5). Respective antiplatelet agents were not significantly associated with lower development of HCC compared to the non-antiplatelet group: the aspirin group (HR, 0.72; 95% CI, 0.08-2.92; $P = 0.70$), the clopidogrel group (HR, 2.29; 95% CI, 0.02-15.97; $P = 0.61$), and the dual antiplatelets group (HR, 2.15; 95% CI, 0.41-7.06; $P = 0.32$).

When the causes of antiplatelet therapy were considered in the development of HCC, coronary heart disease, arrhythmia, and brain infarcts were found not to be independent predictors in this study (Supporting Information). A significant association between antiplatelet therapy and lower HCC development was maintained after adjusting for the three factors.

Analyses After Balancing the Two Groups

Propensity score matching of the entire study population yielded 420 matched pairs of patients. The non-antiplatelet and antiplatelet groups within this matched cohort did not significantly differ in their baseline characteristics (Table 3). In the propensity score–matched cohort, the risk of HCC development was significantly lower in the antiplatelet group than in the non-antiplatelet group (HR, 0.34; 95% CI, 0.15-0.77; $P = 0.01$; Supporting Table S6). The 5-year cumulative incidence rates of HCC were 1.4% in the antiplatelet group and 9.9% in the non-antiplatelet group (Fig. 2C). Regarding respective antiplatelet

agents, the aspirin (HR, 0.07; 95% CI, 0.02-0.24; $P < 0.001$), clopidogrel (HR, 0.11; 95% CI, 0.01-0.80; $P = 0.03$), and dual antiplatelets (HR, 0.11; 95% CI, 0.03-0.46; $P = 0.002$) groups were associated with a significantly lower risk of HCC development than the non-antiplatelet group. In patients treated with aspirin, clopidogrel, and dual antiplatelets, the cumulative incidence rates of HCC at 5 years were 1.3%, 1.8%, and 1.4%, respectively (Fig. 2D).

With regard to patients who did not experience viral breakthrough in propensity score-matching analysis, antiplatelet therapy was significantly associated with a lower risk of HCC compared to the non-antiplatelet group (HR, 0.20; 95% CI, 0.07-0.56; $P = 0.002$; Supporting Table S6). Regarding respective antiplatelet agents, the aspirin group (HR, 0.16; 95% CI, 0.04-0.68; $P = 0.01$) was significantly associated with a lower risk of HCC. Both the clopidogrel (HR, 0.44; 95% CI, 0.06-3.24; $P = 0.42$) and the dual antiplatelets (HR, 0.17; 95% CI, 0.02-1.27; $P = 0.09$) groups exhibited a lower risk of HCC, but this failed to reach statistical significance. However, of the patients who exhibited viral breakthrough, antiplatelet therapy was not significantly associated with a lower development of HCC compared to the non-antiplatelet group (HR, 0.62; 95% CI, 0.18-2.16; $P = 0.45$). Regarding respective antiplatelet agents, a significant difference in the risk of HCC development in each treatment group compared to the non-antiplatelet group was not observed: aspirin (HR, 0.49; 95% CI, 0.05-1.99; $P = 0.36$), clopidogrel (HR, 2.58; 95% CI, 0.02-18.57; $P = 0.57$), and dual antiplatelets (HR, 1.49; 95% CI, 0.29-4.91; $P = 0.59$) groups.

Competing Risk Analyses

The three competing outcomes in this study were death, transplantation, and HCC. It is possible that the more frequent occurrences of death or transplantation in the antiplatelet group may have reduced the number of patients at risk for HCC. Thus, risks were adjusted using competing risk analysis (competing events for death and transplantation) in both the entire cohort and the propensity score–matched cohort. In the entire cohort, HCC risk was significantly lower in the antiplatelet group than in the non-antiplatelet group (HR, 0.27; 95% CI, 0.14-0.52; $P < 0.001$). Regarding respective antiplatelet agents, the aspirin group showed a significantly lower risk of HCC than the non-antiplatelet group (HR, 0.21; 95% CI, 0.09-0.48; $P < 0.001$). The clopidogrel (HR, 0.32; 95% CI, 0.08-1.33; $P = 0.12$) and dual antiplatelets (HR, 0.42; 95% CI, 0.16-1.05; $P = 0.06$) groups had lower risk, but this failed to reach statistical significance (Supporting Fig. S1). In the propensity score–matched cohort, HCC risk was significantly lower in the antiplatelet group than in the non-antiplatelet group (HR, 0.08; 95% CI, 0.03-0.21; $P < 0.001$). All of the respective antiplatelet agents showed a significantly lower risk of HCC than the non-antiplatelet group: aspirin (HR, 0.06; 95% CI, 0.02-0.21; $P < 0.001$), clopidogrel (HR, 0.11; 95% CI, 0.01-0.81; $P = 0.03$), and dual antiplatelets (HR, 0.11; 95% CI, 0.03-0.46; $P = 0.002$), respectively.

During a median period of 4.8 years, HCC-related mortality rates in the antiplatelet group were not significantly different from those in the non-antiplatelet group (HR, 0.31; 95% CI, 0.07-1.32; $P = 0.11$) after competing risk analysis (competing events for HCC-unrelated death and liver transplantation). The 5-year mortality rates were 1.6% in the antiplatelet group and 1.5% in the non-antiplatelet group (Supporting Fig. S2).

Subgroup Analyses According to HCC Risk Models

When we compared the predictive performance of Chinese University-HCC, modified Guide With Age and Gender-HCC, REACH-B, and PAGE-B scores for HCC development, all four HCC risk scores showed an acceptable predictive function (all concordance indices ≥ 0.73; Supporting Table S7). According to HCC risk stratification by PAGE-B score, intermediate-risk or high-risk patients in the antiplatelet group (Fig. 2E) had a significantly lower risk for HCC development compared to intermediate-risk or high-risk patients in the non-antiplatelet group (Fig. 2F): intermediate-risk patients in the antiplatelet group versus intermediate-risk patients in the non-antiplatelet group (HR, 0.34, 95% CI, 0.13-0.79, $P = 0.02$); high-risk patients in the antiplatelet group versus high-risk patients in the non-antiplatelet group (HR, 0.32; 95% CI, 0.13-0.69; $P = 0.007$) (Supporting Table S8). In low-risk patients, there was no significant difference of HCC risk between the antiplatelet and non-antiplatelet groups (HR, 0.13; 95% CI, 0.01-1.43; $P = 0.20$).

In subgroup analyses according to the other three HCC risk models (Chinese University-HCC, modified Guide With Age and Gender-HCC, and REACH-B), antiplatelet therapy was significantly or

marginally associated with a lower risk of HCC development compared to the non-antiplatelet group in most subgroups (Supporting Table S8). However, the majority of subgroup analyses in HCC risk comparisons between the non-antiplatelet and antiplatelet groups showed a significantly lower risk of HCC development in the antiplatelet group. Although some comparison groups did not show significant differences, probably due to small patient numbers in the subgroups, most adjusted HRs were below 1.00.

Subgroup Analyses According to Persistent Viremia

Of 1,498 patients in whom viral breakthrough did not occur, 16 had persistent detectable serum HBV DNA in the absence of viral breakthrough during antiviral therapy. The persistently detectable low-level viremia (<2,000 IU/mL) was not associated with a risk of HCC development. The risk of HCC development in the 16 patients was comparable to that of those with persistently undetectable HBV DNA (<12 IU/mL) (n = 911; HR, 4.24; 95% CI, 0.55-32.63; $P = 0.17$) and to that of those with intermittently detectable low-level viremia (<2,000 IU/mL) (n = 571; HR, 1.97; 95% CI, 0.25-15.35; $P = 0.52$).

ASSOCIATION BETWEEN BLEEDING EVENTS AND ANTIPLATELET THERAPY

During the study period, 1.8% (20 of 1,116) of patients in the non-antiplatelet group and 9.5% (53 of 558) of those in the antiplatelet group experienced bleeding events. The major event in both groups was upper gastrointestinal tract bleeding (Supporting Table S9). The results of bleeding sources and major bleeding events were described in the Supporting Information.

Overall, bleeding risk in the antiplatelet group was significantly higher than that in the non-antiplatelet group (HR, 3.28; 95% CI, 1.98-5.42; $P < 0.001$). In a time-varying Cox regression analysis of the entire cohort, bleeding risk in patients treated with aspirin was not significantly different from risk in the non-antiplatelet group (HR, 1.11; 95% CI, 0.48-2.54; $P = 0.81$). However, bleeding risk in patients treated with clopidogrel or dual antiplatelets was significantly higher than in the non-antiplatelet group: the clopidogrel group (HR, 4.71; 95% CI, 1.81-12.22; $P = 0.001$) and the dual antiplatelet group (HR, 7.37; 95% CI, 4.20-12.92; $P < 0.001$). The cumulative incidence rates of bleeding events at 5 years were 2.7% in the non-antiplatelet group and 2.6%, 12.6%, and 19.7% in the aspirin, clopidogrel, and dual antiplatelets group, respectively (Fig. 3A).

Among 215 patients who received clopidogrel, 2 were also treated with omeprazole. Because it is well known that omeprazole decreases the effect of clopidogrel,[19] we performed subgroup analyses after excluding those 2 patients taking both medications. In those subgroup analyses, risk of HCC development in patients treated with clopidogrel or dual antiplatelets was also not significantly different from that in the non-antiplatelet group: clopidogrel group (HR, 0.97; 95% CI, 0.24-3.99; $P = 0.97$) and dual antiplatelet group (HR, 0.97; 95% CI, 0.41-2.26; $P = 0.94$).

In propensity score-matching analysis, bleeding risk was not significantly higher in the antiplatelet group than in the non-antiplatelet group (HR, 2.06; 95% CI, 0.93-4.57; $P = 0.07$). Bleeding risk in patients treated with aspirin or clopidogrel did not significantly differ from those not treated with antiplatelet therapy: aspirin (HR, 0.85; 95% CI, 0.31-2.29; $P = 0.75$) and clopidogrel (HR, 2.81; 95% CI, 0.92-8.59; $P = 0.07$). Bleeding risk in patients treated with dual antiplatelets was significantly higher than in the non-antiplatelet group (HR, 5.36; 95% CI, 2.27-12.66; $P < 0.001$). In the propensity score–matched set, the cumulative incidence rates of bleeding events at 5 years were 2.7% in the non-antiplatelet group and 2.1%, 9.6%, and 10.1% in the aspirin, clopidogrel, and dual antiplatelets groups, respectively (Fig. 3B).

Competing risks analysis was performed for bleeding events in the entire cohort and the propensity score–matched cohort; liver transplantation was considered a competing event. After competing risk analysis, bleeding risk was significantly higher in the antiplatelet group than in the non-antiplatelet group (HR, 3.75; 95% CI, 2.30-6.11; $P < 0.001$ in the entire cohort and HR, 2.28; 95% CI, 1.18-4.42; $P = 0.01$ in the propensity score–matched cohort). Bleeding risk in patients treated with aspirin did not significantly differ from risk in the non-antiplatelet group (HR, 1.32; 95% CI, 0.60-2.90; $P = 0.48$ in the entire cohort and HR, 0.54; 95% CI, 0.16-1.87; $P = 0.34$ in the propensity score–matched cohort). However, bleeding risk in patients treated with clopidogrel or dual antiplatelets was significantly higher than in the non-antiplatelet group in the entire cohort: clopidogrel (HR, 6.07; 95% CI, 2.65-13.90; $P < 0.001$) and dual antiplatelets (HR, 8.27; 95% CI, 4.74-14.45; $P < 0.001$). In the propensity score–matched cohort, bleeding risk in patients

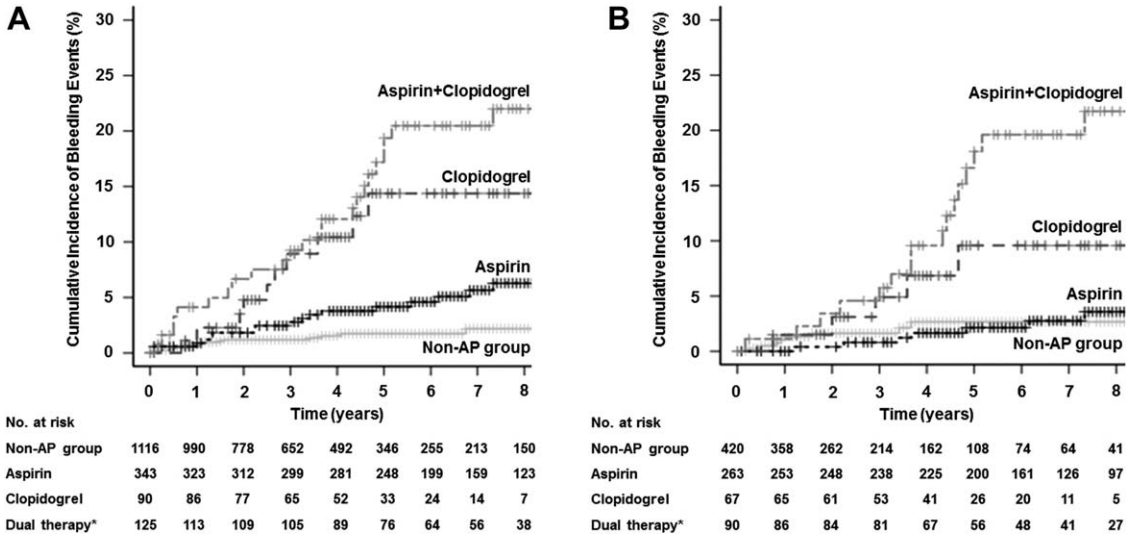

FIG. 3. Kaplan-Meier estimates of cumulative incidence of bleeding events in the entire cohort and propensity score–matched cohorts. (A) Cumulative incidence of bleeding events according to antiplatelet drugs in the entire cohort: non-antiplatelet group versus aspirin ($P = 0.80$ by log-rank test), clopidogrel ($P = 0.001$ by log-rank test), and dual antiplatelets group ($P < 0.001$ by log-rank test). (B) Cumulative incidence of bleeding events according to antiplatelet drugs in the propensity score–matched cohorts: non-antiplatelet group versus aspirin ($P = 0.76$ by log-rank test), clopidogrel ($P = 0.05$ by log-rank test), and dual antiplatelets group ($P < 0.001$ by log-rank test). *Dual antiplatelet therapy means a combination therapy of aspirin and clopidogrel. Abbreviation: AP, antiplatelet.

treated with clopidogrel was not significantly higher than in the non-antiplatelet group (HR, 3.24; 95% CI, 0.94-11.17; $P = 0.06$). In patients treated with dual antiplatelets, bleeding risk was significantly higher than in the non-antiplatelet group (HR, 5.97; 95% CI, 2.90-12.31; $P < 0.001$). Difference in bleeding-related deaths was not applicable between the antiplatelet and the non-antiplatelet groups (no death in the non-antiplatelet group and 2 deaths in the antiplatelet group) (Supporting Information).

When the causes of antiplatelet therapy were considered for bleeding events, coronary heart disease, arrhythmia, and brain infarcts were found not to be independent predictors in this study (Supporting Information). When adjusting for the three factors, bleeding risk in patients treated with dual antiplatelets, not with aspirin or clopidogrel, was significantly higher than in the non-antiplatelet group.

ASSOCIATION BETWEEN OTHER COMORBIDITIES AND ANTIPLATELET THERAPY

Fifty-six patients (3.4%) developed malignancies other than HCC, and there was no significant difference during the study period: 2.6% (29 of 1,116) in the non-antiplatelet group and 4.8% (27 of 558) in the antiplatelet group (Supporting Table S10). The risk of malignancy other than HCC, end-stage kidney disease, and cardiovascular events including myocardial infarction was described in the Supporting Information.

Discussion

This observational study showed that antiplatelet therapy is associated with a significantly lower risk of HCC development in CHB patients with controlled HBV DNA levels using antiviral treatment. Immortal time bias is often unrecognized in epidemiologic studies of drug effect on cancer development. To minimize immortal time bias for HCC development in this study, we used a time-varying Cox regression analysis. A time-varying Cox proportional hazards regression analysis is more accurate for defining antiplatelet exposure status than the conventional Cox proportional hazards analysis because the analysis takes into account variations in the timing of antiplatelet initiation in patients and considers the period of nonexposure to antiplatelet agents.[20] After immortal time bias was controlled for HCC development by this method,

HCC incidence rates in the antiplatelet group were significantly lower than those in the non-antiplatelet group. These results were observed consistently in several analyses, including a time-varying Cox proportional hazards model for the entire cohort, competing risks, and propensity score–matched analyses.

Antiplatelet therapy had additive effects on antiviral treatment for risk reduction of HCC development in this study. This result implies that underlying chronic inflammation in the liver is important in facilitating HCC development, as well as uncontrolled HBV DNA. In CHB patients, virus-induced $CD8^+$ lymphocytes produce inflammatory cytokines to cope with the infection, but they also damage the remaining liver tissue if the viral clearance is unsuccessful. This repeated damage to hepatocytes eventually results in the development of cirrhosis and HCC.[21,22] During this microinflammation, platelets play a key role to accumulate $CD8^+$ T cells in the liver.[23] Platelets also produce platelet-derived growth factor-β to activate hepatic stellate cells and promote fibrosis in animal models. Antiplatelet therapy will decrease platelet activation, decrease $CD8^+$ cells, and thus prevent HCC development[21] and selectively inhibit platelet-derived growth factor-β to reduce biliary fibrosis in patients with liver disease.[24]

Given that aspirin alone did not significantly increase the bleeding risk in all of the time-varying Cox regression, propensity score–matched, and competing risk analyses compared to the non-antiplatelet group, aspirin use is a feasible option for preventing HCC development in CHB patients with controlled HBV DNA levels. Although the dual antiplatelets group showed the strongest preventive effects against HCC occurrence in preclinical studies, the clinical benefit of dual antiplatelet therapy in an actual clinical setting was not satisfactory as bleeding risk in patients treated with dual antiplatelets was much higher than in those not treated with antiplatelets.

In the entire cohort, NAFLD was an independent risk factor for HCC development (HR, 2.93; 95% CI, 1.06-8.19; $P = 0.04$). However, the proportion of patients with NAFLD in the antiplatelet group (30.3%, 169 of 558) was significantly higher than in the non-antiplatelet group (19.8%, 221 of 1,116, $P < 0.001$). Notably, the risk of HCC development in the antiplatelet group was significantly lower than in the non-antiplatelet group, although the additional HCC risk attributed to NAFLD in the antiplatelet group may be higher than that in the non-antiplatelet group.

There are some limitations in this study. First, the current study was based on retrospective observational data. Thus, our findings are potentially subject to selection bias and confounding effects. There were several imbalanced factors between study groups. To overcome this limitation, an additional analysis using propensity score matching was performed. In this analysis, HCC incidence rates in the antiplatelet group were significantly lower than those in the non-antiplatelet group. Second, the sample size was not sufficient to perform several subgroup analyses. For example, among patients who experienced viral breakthrough during the follow-up period, we did not find a significant association between antiplatelet therapy and HCC risk reduction. The relatively small number of patients treated with antiplatelets (only 50 cases) did not allow for a comprehensive evaluation, particularly in patients who experienced viral breakthrough. Given the very low viral breakthrough rates of high-potency antiviral drugs in this era, viral breakthrough might not be a major hurdle for chemopreventive effects of antiplatelets. In addition, although a tendency existed for greater HCC development in patients with persistently detectable low-level viremia than in those with persistently undetectable and intermittently detectable low-level viremia, there was no significant difference in HCC development among the three groups in this study. This may be explained also by the small number of patients with persistently detectable low-level viremia ($n = 16$) and the low incidence rates of HCC development due to a relatively low rate of cirrhosis (12.2%) compared to that in the original study (50.6%).[25] Third, several clinical data were not available due to the retrospective nature of this study. A previous study showed that, in CHB patients, serum HBV DNA levels <2,000 IU/mL and hepatitis B surface antigen levels >1,000 IU/mL were associated with a significantly higher risk of HCC development.[26] Furthermore, studies also demonstrated that HBV genotype, basal core promoter mutations, pre-S deletion mutants, a family history of HCC, as well as metabolic syndrome were also important risk factors for HCC in CHB patients.[27] Unfortunately, information about serum hepatitis B surface antigen levels, basal core promoter mutations, pre-S deletion mutations, a family history of HCC, serum fasting glucose and insulin levels, and metabolic syndrome was not available for the majority of our patients due to the retrospective nature of our study. Thus, the possible risk of such factors for HCC development could not be evaluated in our study. In further studies, such pivotal factors should be considered in prospective studies of HCC risk assessments.

It was difficult to find out the treatment duration of antiplatelet therapy that determines the risk of HCC development in this study. Although the results suggested that antiplatelet therapy was significantly associated with a lower risk of HCC development (56% reduction) when the median duration of exposure to antiplatelet therapy was 27.6 months, it is unknown exactly how long patients should receive antiplatelet therapy to prevent HCC development. When HCC occurred during the early period of antiplatelet therapy, the effect of antiplatelet therapy on HCC development can be misinterpreted such that antiplatelet therapy increases the HCC risk.[28,29]

In conclusion, the present study in CHB patients with suppressed HBV DNA levels showed that antiplatelet therapy was associated with a significantly lower risk of HCC development. Additive treatment of aspirin in patients with durable viral suppression might be an effective strategy to lower HCC development. Although antiplatelet therapy increased the bleeding risk in CHB patients, only aspirin use did not significantly increase the bleeding risk, in contrast to increased bleeding risk in patients treated with clopidogrel or dual antiplatelets. Given the high fatality rates after HCC diagnosis, further large-scale studies are needed to confirm the chemopreventive effect of aspirin on HCC development, particularly in populations at high risk of HCC development under control of prognostic factors. In addition, prospective studies using paired biopsies are needed to elucidate the mechanism of antiplatelet agents in the future: whether or not chemopreventive effects of antiplatelet agents on HCC development are caused by fibrosis reversal by antiplatelet agents.

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Author names in bold designate shared co-first authorship.

Supporting Information

Additional Supporting Information may be found at onlinelibrary.wiley.com/doi/10.1002/hep.29318/suppinfo.