

Aspirin and the risk of hepatocellular carcinoma development in patients with alcoholic cirrhosis

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Abstract

Aspirin therapy has shown protective effects against hepatocellular carcinoma (HCC) in preclinical studies. However, it is unclear whether aspirin therapy lowers the risk of HCC in patients with alcoholic cirrhosis.

A retrospective analysis of data from 949 consecutive patients with alcoholic cirrhosis who abstained from alcoholic drinking was performed. The primary and secondary outcomes were development of HCC and gastrointestinal bleeding events, respectively. Risk was compared between patients with aspirin treatment and patients who were not treated (non-aspirin group) using a time-varying Cox proportional hazards model for total population and propensity score-matching analysis.

The aspirin group included 224 patients and the non-aspirin group had 725 patients. During the study period of median duration of 3.1 years, 133 patients (13.6%) developed HCC. In time-varying Cox proportional analyses, the aspirin group showed a significantly lower risk of HCC (adjusted hazard ratio [aHR]: 0.13; 95% confidence interval [CI]: 0.08–0.21; $P < .001$). In propensity score-matched pairs, aspirin therapy significantly reduced the risk of HCC (aHR: 0.14; 95% CI: 0.09–0.22; $P < .001$). In bleeding risk, treatment with aspirin alone was not significantly associated with a higher bleeding risk (aHR: 0.81; 95% CI: 0.45–1.44; $P = .46$).

Aspirin therapy was associated with the lower risk of HCC in patients with alcoholic cirrhosis.

Abbreviations: ALC = alcoholic liver cirrhosis, CI = confidence interval, CP = Child-Pugh, HCC = hepatocellular carcinoma, HR = hazard ratio, MELD = model for end-stage liver disease.

Keywords: alcoholic liver cirrhosis, aspirin, hepatocellular carcinoma

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SS and SH contributed equally to this work.

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1. Introduction

Liver cirrhosis is an advanced stage of liver fibrosis which presents with regenerative nodules surrounded by fibrous bands that develop in response to chronic liver injury.^[1] Although alcohol is one of the common causes of liver cirrhosis worldwide and alcoholic liver cirrhosis (ALC) is responsible for development of hepatocellular carcinoma (HCC) and a high rate of mortality,^[2,3] limited resources except alcohol abstinence have been invested into research on ALC, particularly chemoprevention for HCC development.

In hepatitis B models, recent preclinical studies have suggested potential therapeutic applications of aspirin therapy. Platelets are key facilitators of this immune-mediated injury, as they promote accumulation of CD8⁺ T cells.^[4] 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.^[5] In clinical studies, a large population-based study in the National Institutes of Health 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 non-use.^[6] In addition, nationwide cohort study^[7] and retrospective cohort study using in-hospital database^[8] have also observed that aspirin use was associated with a 37% to 56% lower risk of HCC compared to non-use.

Although previous studies showed that aspirin use was significantly associated with reduced risk of HCC in chronic hepatitis B patients, there was lack of studies reporting whether aspirin use can be significantly associated with reduced risk of HCC developed from other etiologies such as ALC. Previous studies showed that patients

with ALC have also increased platelet activation which is a target of aspirin,^[9] and aspirin therapy was associated with lower liver fibrosis risk with validated noninvasive fibrosis markers among US adults with suspected alcoholic liver disease.^[10] However, there are no data regarding association between HCC development and aspirin therapy in ALC patients.

In this study, we investigated whether aspirin therapy is associated with a reduction in HCC incidence in patients with ALC and the risk of gastro-intestinal bleeding.

2. Materials and methods

2.1. Study design

The study population was obtained from inpatient and outpatient database files between August 1, 2003 and May 31, 2016 at Kangwon National University Hospital (Chuncheon, Korea) and consisted of a cohort of 949 consecutive adult ALC patients. Patients were excluded if they met any of the following criteria: active alcoholism; younger than age 18 or older than age 85; diagnosis with HCC before study enrollment; infection with hepatotropic viruses (i.e., hepatitis B, hepatitis C or D virus) or human immunodeficiency virus; duration of aspirin therapy shorter than 6 months; diabetes mellitus; liver transplantation.

The entire cohort was divided according to aspirin therapy: the non-aspirin group consisted of patients not treated with aspirin and the aspirin group was patients who were treated with aspirin (aspirin 100mg/day). All aspirin agents were prescribed by the physicians at the Kangwon National University Hospital for the patients visiting the outpatient clinic on a regular basis. We excluded patients in the non-aspirin group who had taken aspirin, including over-the-counter (OTC) 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-aspirin group who had taken aspirin or OTC 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., aspirin and anticoagulants) before endoscopic surveillance every 1 to 2 years for esophageal varix or gastric cancer.

This study was approved by the Institutional Review Board of Kangwon National University Hospital, and the requirement for informed consent from patients was waived.

2.2. Outcomes and follow-up evaluation

The primary outcome of interest in this study was HCC development. Secondary outcomes evaluated included gastrointestinal bleeding events. The index date was defined as the first date that the patient was diagnosed with ascites, hepatic encephalopathy or esophageal/gastric varices. The censored date was defined as the date of patient's death, last date of follow-up, or cutoff date (i.e., May 31, 2016).

Patients regularly underwent clinical examinations, liver function tests, and measurement of serum variables every 6 months. Abstinence from alcohol drinking was confirmed by both meticulous reviews of medical records written by physicians who prescribed the agents and serial follow up of blood tests such as liver function test and gamma-glutamyl transferase test every 6 months.^[3] When results of surveillance tests were equivocal, 3-month interval screenings were permitted. Compliance with

aspirin agents in the aspirin group was assessed when the patients visited the outpatient clinic where physicians had first prescribed aspirin agents such as the departments of cardiology, endocrinology, neurology, family medicine, and neurosurgery.

2.3. Definitions

Clinical diagnosis of alcoholic liver cirrhosis was determined as follows: excess alcohol consumption >60 g/d and (i) platelet count of <150,000/mL and ultrasonography findings suggestive of cirrhosis, including a blunted, nodular liver edge accompanied by splenomegaly (>12 cm) or (ii) clinical signs of portal hypertension, such as ascites, esophageal or gastric varices, and hepatic encephalopathy.^[3,11,12] Diagnosis of HCC was established based on the guidelines of the American Association for the Study of Liver Diseases.^[13,14] The diagnosis of HCC was based on the radiological findings of dynamic CT and/or MRI, which included intense arterial uptake followed by a "washout" of contrast during the venous-delayed phases. For patients who had equivocal findings on CT, MRI with liver-specific contrast was performed for the diagnosis of HCC. If characteristic findings of HCC on MRI were not found, a liver biopsy was performed. Diagnosis of gastrointestinal bleeding events in this study were defined when symptoms such as hematemesis, melena, or hematochezia were occurred.

2.4. Statistical analyses

Data are presented as the mean and standard deviation (SD) or median and interquartile range (IQR) for continuous variables, and frequencies and proportions are shown for categorical variables. Comparisons of baseline demographic and clinical characteristics were performed using *t* test, analysis of variance, Mann-Whitney *U* test, or Kruskal-Wallis test for continuous variables, and χ^2 test, or Fisher exact test for categorical variables.

In this retrospective observational study, immortal time bias for HCC incidences in the aspirin group can arise because there were two different dates: time of diagnosis with ALC in the non-aspirin group and time of taking first aspirin agents after diagnosis with alcoholic liver cirrhosis in the aspirin group. Because the initiation date of aspirin therapy varied between patients, hazard ratios and 95% confidence intervals were computed by a time-varying Cox proportional hazards regression model. In this model, exposure to aspirin agent was treated as a time-dependent variable.^[15,16] In brief, among aspirin users, a patient was coded as a non-user before initiation of aspirin agent, then recoded to user on the date when the aspirin agent was initiated. Among patients who never used an aspirin agent, coding as non-user was applied throughout.

In the entire cohort, cumulative incidence rates of HCC and bleeding events were delineated using a Kaplan–Meier estimator, and log-rank test was to compare aspirin drugs. A time-varying Cox proportional hazard regression analysis was applied to assess the effects of aspirin therapy on HCC development and gastrointestinal bleeding events after adjustment of covariates.

Propensity score matching was performed to reduce selection bias and confounders between the aspirin and non-aspirin groups at baseline. To derive propensity scores, the following variables were included in a multiple logistic regression: age, gender, Child-Pugh (CP) score, model for end-stage liver disease (MELD) score, aspartate aminotransferase alanine aminotransferase, albumin, total bilirubin, creatinine, prothrombin time INR, and platelet count. One-to-one propensity score matching was performed by

Table 1
Baseline Characteristics.

	Non-aspirin group (n=725)	Aspirin group (n=224)	P value*
Age, years	57.9 ± 12.2	64.6 ± 10.8	<.001
Male, N (%)	524 (72.3%)	150 (67.0%)	<.001
Child- Pugh score	5.3 ± 0.7	5.6 ± 1.0	<.001
MELD score	8.5 ± 3.3	9.9 ± 5.3	<.001
AST, U/L	34.4 ± 21.6	49.7 ± 40.0	<.001
ALT, U/L	20.5 ± 12.3	35.0 ± 29.6	<.001
Albumin, g/dL	3.9 ± 0.5	3.7 ± 0.6	.003
Total bilirubin, mg/dL	1.0 ± 1.0	1.2 ± 1.3	.006
Creatinine, mg/dL	0.9 ± 0.6	1.2 ± 0.5	<.001
INR	1.08 ± 0.20	1.07 ± 0.26	.09
Platelet, ×10 ³ /μL	136 ± 77	131 ± 79	.28

CI=confidence interval, HR=hazards ratios, INR=international normalized ratio for prothrombin time, MELD=model for end-stage liver disease.

* P value estimated by paired t-test for continuous variables and McNemar test for categorical variables.

nearest neighbor matching with a caliper width of 0.1 multiplied by the SD of the linearly transformed propensity scores. For the propensity score-matched cohort, differences in demographic and clinical characteristics were examined with a paired t-test for continuous variables and McNemar test for categorical variables.

All statistical analyses were conducted with R version 3.2.1 (<http://www.r-project.org>). P values less than .05 were considered statistically significant.

3. Results

3.1. Baseline characteristics

The total study population was comprised of 949 ALC patients: 725 patients were without aspirins (non-aspirin group) and 224 patients were taking aspirin therapy (aspirin group). During the study period of median duration of 3.1 years (interquartile [IQR], 1.3–5.9 years), a total of 133 (13.6%) patients developed HCC. The two groups differed significantly in terms of baseline characteristics (Table 1). Patients in the non-aspirin group were significantly younger (mean, 58 years vs 65 years) compared to those in the aspirin group. The MELD scores in the aspirin group were 9.9 significantly higher than 8.5 in the non-aspirin group.

Serum total bilirubin and creatinine levels in the aspirin group were significantly higher than those in the non-aspirin group; serum albumin levels in the aspirin group were significantly lower than those in the non-aspirin group. The indications for the aspirin therapy in the aspirin group in this study were as below: 61.6% (n=138) of patients for prevention of cardiovascular disease, 22.3% (n=50) of patients for coronary heart disease, 10.3% (n=23) of patients for arrhythmia prevention, 5.8% (n=13) patients for others such as brain infarct, peripheral vascular disease or others.

3.2. Association between aspirin therapy and HCC development

In the aspirin group, the median duration of exposure to aspirin therapy was 18.5 months (IQR, 6.2–48.4 months). 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=949; Table 2). Aspirin therapy was independently associated with a significantly lower risk of HCC development (adjusted hazard ratio [HR]: 0.13; 95% confidence interval [CI]: 0.08–0.21; P<.001; Fig. 1A).

To minimize potential bias, we tried to balance variables between the non-aspirin group and the aspirin group by the propensity score matching at the baseline index date. Propensity score matching of the entire study population yielded 179 matched pairs of patients. Non-aspirin and aspirin groups within this matched cohort did not significantly differ in their baseline characteristics (Table 3). After setting up the propensity-score matched cohort, we performed time-varying Cox proportional hazards analysis for HCC development.

In the propensity score-matched cohort, the risk of HCC development in the aspirin group was significantly lower than in the non-aspirin group (adjusted HR: 0.14; 95% CI: 0.09–0.22; P<.001; Fig. 1B). In time-varying Cox proportional analyses in the propensity score-matched cohort, aspirin therapy significantly reduced the risk of HCC (aHR: 0.15; 95% CI: 0.09–0.27; P<.001; Table 4). The risk of HCC development in the non-aspirin group was significantly increased 1.63 times higher than that in the aspirin group as the CP score went 1 point higher in the univariable analysis (95% CI 1.22–2.18, P<.001). However, in the multivariable analysis, the CP score was not significantly an independent risk factor for HCC development (P=.36).

Table 2
Time-varying Cox Proportional Hazards Analysis for HCC Development in the Entire Cohort.

	Univariable		Multivariable	
	HR (95% CI)	P value*	HR (95% CI)	P value*
Age (per years)	1.05 (1.04, 1.07)	<.001	1.05 (1.04, 1.07)	<.001
Male (vs female)	1.32 (0.92, 1.89)	.14		
Albumin, g/dL	0.34 (0.27, 0.44)	<.001	0.40 (0.26, 0.62)	<.001
Total bilirubin, mg/dL	1.13 (1.05, 1.21)	<.001	1.04 (0.94, 1.16)	.46
AST, IU/L	1.001 (0.997, 1.006)	.55		
ALT, IU/L	0.99 (0.98, 1.00)	.05		
Platelet, ×10 ³ /μL	0.999 (0.997, 1.001)	.53		
INR	2.27 (1.37, 3.76)	.002	1.08 (0.49, 2.35)	.85
Creatinine, mg/dL	0.97 (0.86, 1.09)	.58		
MELD score	1.05 (1.02, 1.08)	<.001	1.04 (1.00, 1.09)	.05
Child-Pugh score	1.63 (1.44, 1.85)	<.001	1.27 (0.93, 1.71)	.13
Aspirin use (vs non-use)	0.31 (0.21, 0.48)	<.001	0.13 (0.08, 0.21)	<.001

CI=confidence interval, HR=hazards ratios, INR=international normalized ratio for prothrombin time, MELD=model for end-stage liver disease.

* P value estimated by Cox proportional hazard regression.

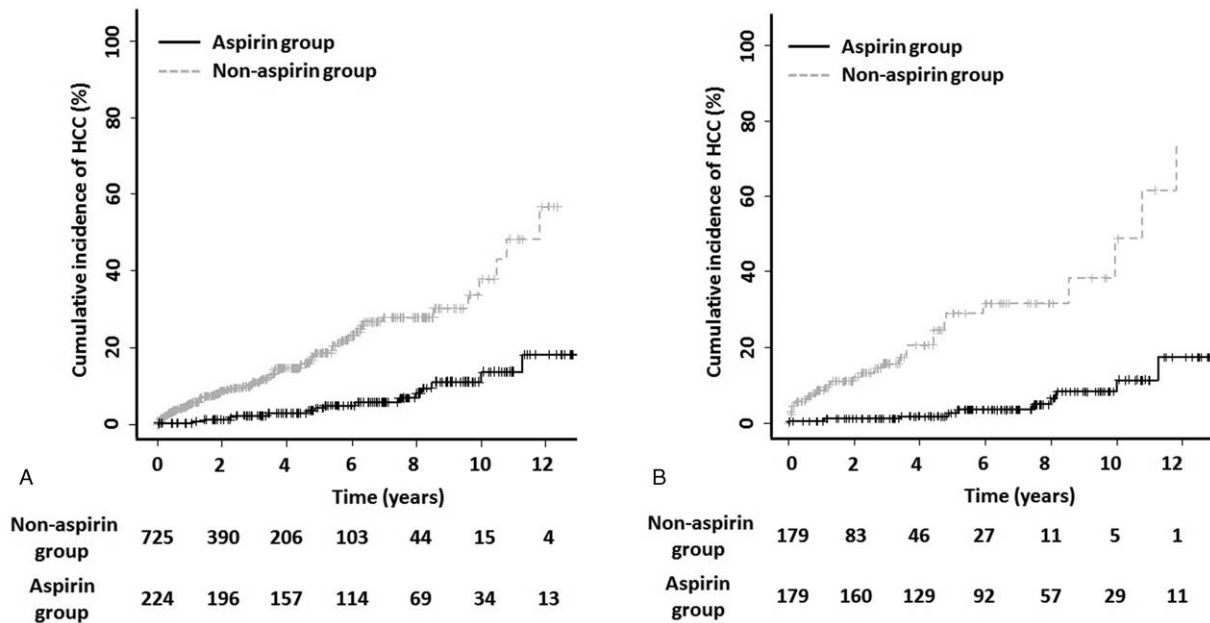


Figure 1. Kaplan–Meier estimates of cumulative incidence of HCC in the entire cohort and propensity score-matched cohorts. Propensity score matching of the entire cohort created 179 matched pairs of patients. (A) Cumulative incidence of HCC according to aspirin use in the entire cohort ($P < .001$ by log-rank test). (B) Cumulative incidence of HCC in the non-aspirin and aspirin groups for patients included in the propensity score-matched cohort ($P < .001$ by log-rank test).

3.3. Association between gastrointestinal bleeding events and aspirin therapy

During median study duration of 3.1 years (IQR, 1.3–5.9 years), 13.5% (98 of 725) patients in the non-aspirin group and 15.6% (35 of 224) in the aspirin group experienced gastrointestinal bleeding events. Main cause of gastrointestinal bleeding was esophageal variceal bleeding in both groups: 56.1% in the non-aspirin group and 28.6% in the aspirin group (Supplementary Table 1, <http://links.lww.com/MD/D879>). There was a significantly difference of bleeding causes between the two groups ($P < .001$): bleeding events related to liver cirrhosis (9.5% of total non-aspirin users) such as esophageal or gastric variceal bleeding and portal hypertensive gastropathy in the non-aspirin group were significantly higher than those (5.4% of total aspirin users)

in the aspirin group ($P < .001$). All of patients with gastrointestinal bleeding events received gastroscopy and/or colonoscopy examinations: gastroscopy exams were done when patients showed hematemesis, melena, or hematochezia, and colonoscopy exams were done when patients showed melena or hematochezia. Regarding ulcer risk in patients who received gastroscopy ($n = 133$), 25 patients (18.8%) among them had ulcer in the stomach or duodenum. In the aspirin users who received endoscopy exams ($n = 35$), 8 patients (22.9%) had ulcer: gastric ulcer ($n = 5$) or duodenal ulcer ($n = 3$). In the non-aspirin users who received endoscopy exams ($n = 98$), 17 patients (2.3%) had ulcer: gastric ulcer ($n = 12$) or duodenal ulcer ($n = 5$). During median study duration of 3.1 years (IQR, 1.3–5.9 years), there was not a significant difference of ulcer risk between the two group ($P = .63$ by the log-rank test).

	Non-aspirin group (n=179)	Aspirin group (n=179)	P value*
Age	65.2 ± 12.1	64.3 ± 10.5	.39
Male, N (%)	121 (67.6%)	114 (63.7%)	.50
Child- Pugh score	5.5 ± 0.7	5.5 ± 0.9	.92
MELD score	8.7 ± 3.8	9.0 ± 3.8	.06
AST, U/L	41 ± 37	42 ± 28	.13
ALT, U/L	26 ± 20	27 ± 19	.10
Albumin, g/dL	3.8 ± 0.5	3.8 ± 0.6	.27
Total bilirubin, mg/dL	1.0 ± 0.7	1.1 ± 1.3	.12
Creatinine, mg/dL	1.0 ± 0.8	1.1 ± 1.3	.66
INR	1.06 ± 0.21	1.07 ± 0.27	.95
Platelet, × 10 ³ /μL	133 ± 78	131 ± 79	.85

CI=confidence interval, HR=hazards ratios, INR=international normalized ratio for prothrombin time, MELD=model for end-stage liver disease.

* P value estimated by paired t-test for continuous variables and McNemar test for categorical variables.

	Univariable		Multivariable	
	HR (95% CI)	P value	HR (95% CI)	P value
Age (per years)	1.04 (1.01, 1.07)	.008	1.04 (1.02, 1.05)	<.001
Male (vs female)	1.88 (0.90, 3.93)	.09		
Albumin, g/dL	0.40 (0.23, 0.67)	<.001	0.52 (0.31, 0.87)	.012
Total bilirubin, mg/dL	1.04 (0.87, 1.24)	.70		
AST, U/L	1.005 (0.998, 1.013)	.13		
ALT, U/L	0.99 (0.96, 1.01)	.32		
Platelet, × 10 ³ /μL	0.989 (0.984, 0.995)	<.001	0.994 (0.991, 0.997)	<.001
INR	1.68 (0.63, 4.46)	.30		
Creatinine, mg/dL	1.03 (0.81, 1.30)	.82		
MELD score	1.04 (0.97, 1.11)	.27		
Child-Pugh score	1.63 (1.22, 2.18)	<.001	1.16 (0.85, 1.58)	.36
Aspirin use (vs non-use)	0.13 (0.06, 0.26)	<.001	0.15 (0.09, 0.27)	<.001

CI=confidence interval, HR=hazards ratios, INR=international normalized ratio for prothrombin time, MELD=model for end-stage liver disease.

* P value estimated by time-varying Cox proportional hazard regression.

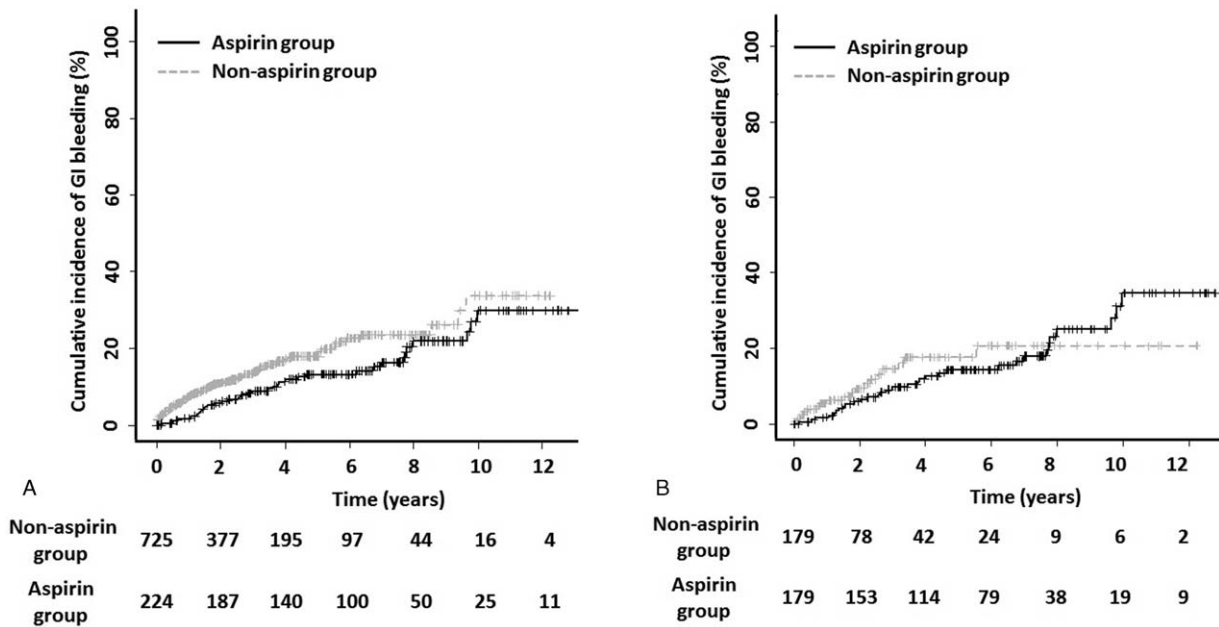


Figure 2. Kaplan–Meier estimates of cumulative incidence of gastrointestinal bleeding in the entire cohort and propensity score-matched cohorts. Propensity score matching of the entire cohort created 179 matched pairs of patients. (A) Cumulative incidence of gastrointestinal bleeding according to aspirin use in the entire cohort ($P = .05$ by log-rank test). (B) Cumulative incidence of gastrointestinal bleeding according to aspirin use in the propensity score-matched cohort ($P = .46$ by log-rank test).

When time-varying Cox regression analyses to identify factors predictive of gastrointestinal bleeding were performed for the entire cohort ($n = 949$), aspirin therapy was not significantly independent risk factor for gastrointestinal bleeding (HR: 0.67; 95% CI: 0.45–1.00; $P = .05$; Fig. 2A): male (adjusted HR: 2.12; 95% CI: 1.34–3.33; $P = .001$) and low serum albumin levels (adjusted HR: 0.46; 95% CI: 0.30–0.72; $P < .001$) were significantly independent risk factors for gastrointestinal bleeding (Table 5). In the propensity score-matched cohort, the risk of gastrointestinal bleeding in the aspirin group was also similar to that in the non-aspirin group (adjusted HR: 0.81; 95% CI: 0.45–1.44; $P = .46$; Fig. 2B).

4. Discussion

The chemopreventive effect of aspirin on HCC development had been studied mainly on chronic hepatitis B on the basis of rationale that virus-induced CD8⁺ lymphocytes produce micro-inflammation and platelets play a key role to accumulate CD8⁺ T cells in the liver.^[17–19] Previous clinical data also suggested that aspirin therapy was associated with a significantly lower risk of HCC development in chronic hepatitis B patients. However, one of limitation of studies reporting chemoprevention effect of aspirin on HCC development was that the results can be applied to cirrhotic patients who did not have etiology of chronic hepatitis B.

Table 5
Time-varying Cox Proportional Hazards Analysis for Gastrointestinal Bleeding in the Entire Cohort.

	Univariable		Multivariable	
	HR (95% CI)	P value*	HR (95% CI)	P value*
Age (per years)	0.99 (0.97, 1.00)	.05		
Male (vs female)	2.19 (1.40, 3.44)	<.001	2.12 (1.34, 3.33)	.001
Albumin, g/dL	0.47 (0.35, 0.63)	<.001	0.46 (0.30, 0.72)	.001
Total bilirubin, mg/dL	1.03 (0.91, 1.17)	.61		
AST, IU/L	1.004 (0.999, 1.009)	.12		
ALT, IU/L	0.99 (0.98, 1.00)	.07		
Platelet, $\times 10^3 / \mu\text{L}$	0.999 (0.996, 1.001)	.24		
INR	1.89 (1.03, 3.47)	.04	1.33 (0.58, 3.06)	.50
Creatinine, mg/dL	1.00 (0.91, 1.11)	.98		
MELD score	1.04 (1.01, 1.08)	.006	1.01 (0.97, 1.06)	.50
Child-Pugh score	1.42 (1.21, 1.67)	<.001	1.05 (0.77, 1.42)	.78
Aspirin use (vs non-use)	0.67 (0.45, 1.00)	.05		

CI = confidence interval, HR = hazards ratios, INR = international normalized ratio for prothrombin time, MELD = model for end-stage liver disease.

* P value estimated by paired t-test for continuous variables and McNemar test for categorical variables.

Beyond chronic hepatitis B, previous studies performed in basic laboratories suggested that the chemopreventive effect of aspirin can be applied to prevent fibrosis progression from other etiologies, not just chronic hepatitis B. In animal models with advanced fibrosis, aspirin therapy inhibited platelet activation to suppress platelet-derived growth factor- β , thereby inactivating hepatic stellate cells and reducing hepatic fibrosis.^[20,21] However, there was lack of clinical data showing that aspirin therapy can show a significant association with lower risk of HCC development in patients who had other etiologies such as ALC. This observational study showed that aspirin therapy in ALC patients was associated with a significantly lower risk of HCC development. In addition, aspirin therapy was not significantly associated with the risk of gastrointestinal bleeding in ALC patients.

This result implies that aspirin therapy might be helpful to reduce HCC risk in patients with ALC besides chronic hepatitis B. Inhibition of underlying chronic micro-inflammation in the cirrhotic liver can be important in facilitating HCC development not focused on etiology-specific HCC development. It reflects that micro-inflammation might be one of important causes in ongoing process to HCC development even in patients who had high fibrosis burden.

Given that aspirin therapy did not significantly increase the bleeding risk in both of multivariable analysis and propensity score-matched analysis compared to the non-aspirin group, aspirin use is a feasible option in preventing HCC development in ALC patients. Of note, bleeding events related to liver cirrhosis in the aspirin group were significantly lower than those in the non-aspirin group. This result supports the previous study suggesting that aspirin use is associated with lower indices of liver fibrosis.^[10] It suggests that one of mechanism of chemopreventive effects of aspirin for HCC development might be to delay fibrosis progression.

Although diabetes mellitus is an important risk factor for HCC development, patients with diabetes mellitus were excluded in this study to clarify the anticancer effect of aspirin therapy in patients with pure ALC. This is because whether or not glucose levels in patients with diabetes mellitus at baseline is well-controlled during the follow-up period can have a significant effect on HCC development. Previous studies reported that HCC risk was also different according to anti-diabetic drugs such as insulin, sulfonylurea, or biguanides.^[22,23] To minimize the potential effects of various anti-diabetic drugs on HCC development, we excluded the patients with diabetes mellitus at baseline.

The major limitation of the current study is that it was based on retrospective observational data. Thus, our findings are potentially subject to selection bias and confounding effects. First, there were several imbalanced factors between study groups. To overcome this limitation, a subgroup analysis using propensity score matching was performed. HCC incidence rates in the aspirin group were significantly lower than those in the non-aspirin group. Second, although patients with active alcoholism were excluded in this study and it was difficult to provide objective evidence regarding alcohol abstinence, HCC incidence rates in this study were similar to those in the previous study which included only patients without active alcoholism. The results in the study showed 13.4% of HCC incidence during a mean follow-up of 3.7 years in patients with FIB-4 index >3.25 .^[24] This is similar to 13.6% of HCC incidence during a median follow-up of 3.1 years in this study which enrolled only advanced fibrosis (cirrhotic) patients. We believed that most of

patients maintained alcohol abstinence even though the patients could hide real absolute abstinence status in their medical records. Third, there was lack of paired liver biopsy results of fibrosis regression due to retrospective study design and paired transient elastography results because index date in most of patients in this study was before introduction of transient elastography. In addition, although serum levels of ammonia and the spontaneous spleno-renal shunts were important to predict the risk of hepatic decompensation and HCC development,^[25,26] we could not analyze impacts of serum levels of ammonia and the spontaneous spleno-renal shunts on the HCC risk in patients treated with aspirin because there was a lack of information of serum levels of ammonia and the spontaneous spleno-renal shunts in most of patients. Further prospective cohort studies to investigate fibrosis change by aspirin therapy using paired liver biopsy or transient elastography exam, any changes of serum levels of ammonia and the spontaneous spleno-renal shunts in patients treated with aspirin in the long term are needed. Lastly, the criteria for minimum duration of aspirin exposure for HCC prevention was arbitrary in this study. In this study, inclusion criteria for minimum duration of aspirin exposure in the aspirin group was 6 months. In addition, we excluded the patients who took aspirin more than 1 month in the non-aspirin group. However, we think that the effects of minimum duration of aspirin therapy on HCC incidence can be negligible because previous studies indicated that a long-term period of aspirin exposure was needed to observe significant decrease of HCC incidence.^[8,27]

In conclusion, the present study in ALC patients showed that aspirin therapy was associated with a significantly lower risk of HCC development. Aspirin use did not significantly increase the bleeding risk. Given the high fatality rates in cirrhotic patients 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 well control of prognostic factors.

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