Daily aspirin associated with a reduced risk of hepatocellular carcinoma in patients with non-alcoholic fatty liver disease: a population-based cohort study

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eClinicalMedicine
2023;61: 102065
Published Online xxx
https://doi.org/10.1016/j.eclinm.2023.102065

Summary

Background Emerging laboratory and animal studies suggest that aspirin may prevent non-alcoholic fatty liver disease (NAFLD)-related hepatocellular carcinoma (HCC), however clinical evidence remains lacking.

Methods Using Taiwan's National Health Insurance Research Database, we screened 145,212 NAFLD patients from 1997 through 2011. After excluding any confounding conditions, 33,484 patients who continuously received a daily dose of aspirin for 90 days or more (treated group), along with 55,543 patients who had not received antiplatelet therapy (untreated group), were respectively recruited. Inverse probability of treatment weighting using the propensity score was applied to balance the baseline characteristics. Cumulative incidence of, and hazard ratio (HR) for HCC occurrence were analyzed after adjusting competing events. The high-risk patients, who were defined as age ≥ 55 years & elevated serum alanine aminotransferase, were further analyzed.

Findings The 10-year cumulative incidence of HCC in the treated group was significantly lower than that in the untreated group (0.25% [95% CI, 0.19–0.32%] *vs.* 0.67% [95% CI, 0.54–0.81%]; P < 0.001). Aspirin therapy was significantly associated with a reduced HCC risk (adjusted HR [aHR] 0.48 [95% CI, 0.37–0.63]; P < 0.001). In the high-risk patients, the 10-year cumulative incidence of HCC in the treated group was significantly lower than that in the untreated group (3.59% [95% CI, 2.99–4.19%] *vs.* 6.54% [95% CI, 5.65–7.42%]; P < 0.001). Aspirin therapy remained associated with a reduced HCC risk (aHR 0.63 [95% CI, 0.53–0.76]; P < 0.001). Subgroup sensitivity analyses verified this significant association in nearly all subgroups. In the time-varying model amongst aspirin users, HCC risk was significantly lower through the use of aspirin for ≥ 3 years (aHR 0.64 [95% CI, 0.44–0.91]; P = 0.013), when compared with short-term use (< 1 year).

Interpretation Daily aspirin therapy is significantly associated with a reduced HCC risk in NAFLD patients.

Funding Ministry of Science and Technology, Ministry of Health and Welfare, and Taichung Veterans General Hospital, Taiwan.

Keywords: Antiplatelet; Prevention; Chemoprevention; Liver cancer

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Research in context

Evidence before this study

The prevalence of non-alcoholic fatty liver disease (NAFLD) is continuing to increase globally, and NAFLD may eventually become the main cause of hepatocellular carcinoma (HCC). However, no NAFLD-specific medications have been approved by the Food and Drug Administration, and an effective therapy in the prevention of NAFLD-related HCC is highly expected, particularly for patients at a high risk of HCC development. Emerging laboratory data suggest that aspirin can reduce the risk of NAFLD-induced HCC; however, clinical evidence of aspirin therapy in preventing NAFLD-related HCC remains limited, with data coming only from certain subgroup analyses.

Added value of this study

In this large-scale, population-based, long-term retrospective cohort study, we first reported that daily low-dose aspirin therapy is significantly associated with a reduced risk of HCC in NAFLD patients. Aspirin therapy not only was significantly associated with a reduced HCC risk in the whole patient cohort, but also with that in the high-risk patient cohort, which was defined as older patients with impaired liver function. In addition, HCC risk was significantly lower through the use of aspirin for 3 or more years, when compared with short-term use.

Implications of all the available evidence

With a high prevalence of NAFLD in the general population, although the incidence of HCC is not high, NAFLD-related HCC remains a substantial threat to public health, particularly to high-risk patients. As observed findings taken from the present study, a 37% risk of HCC was reduced through aspirin therapy for high-risk NAFLD patients, making aspirin therapy a worthy consideration in view of public health. A proof-of-concept trial should be encouraged in the future.

Introduction

Non-alcoholic fatty liver disease (NAFLD) has been one of the most common liver diseases globally, with its prevalence continuing to increase in both Western or Eastern countries.[1,2] NAFLD can eventually result in chronic hepatitis, advanced fibrosis, cirrhosis and the development of hepatocellular carcinoma (HCC).[3] In the United States, the number of patients diagnosed with NAFLD-related HCC has reportedly been increasing by 9% per year, with the estimation that NAFLD may eventually become the main cause of liver cancer.[4] NAFLD is certainly going to become one of the most concerning topics in worldwide public health discussions. However, although several new drugs put forth in clinical trials have been shown to offer certain benefits towards improving NAFLD, an effective medicine has not yet been approved by the Food and Drug Administration.[5] Therefore, developing a therapy which can help prevent NAFLD-related HCC remains of urgent concern.

With its anti-inflammatory properties, aspirin has been widely investigated for its chemoprevention effects in cancers which involve chronic inflammation. For example, aspirin use has been recommended for the primary prevention of colorectal cancer.[6] As one of the well-known processes in HCC development, hepatocarcinogenesis is basically related to chronic liver inflammation, and growing data has suggested that aspirin can be considered for HCC chemoprevention.[7,8] For example, in our previous studies, we reported that daily aspirin therapy was related to a reduced risk of HCC development in patients with chronic hepatitis B virus (HBV) infection[9] or chronic hepatitis C virus (HCV) infection.[10] However, a clinical study focusing on the chemoprevention effect of aspirin therapy in patients with NAFLD remains lacking.

Emerging laboratory and animal studies suggest that antiplatelet therapy, e.g., aspirin, can reduce the risk of NAFLD-induced HCC. In animal model studies, T cell-oriented immune responses played a key role in NAFLD progression and hepatocarcinogenesis,[11,12] and platelets demonstrated closed connections to the immune responses.[13] Antiplatelet therapy can effectively reduce NAFLD severity, immune cell infiltration and HCC development.[13,14] In a prospective cohort study, daily aspirin therapy was found to be related to a reduced risk for fibrosis progression in patients with biopsy-confirmed NAFLD.[15] Nevertheless, clinical evidence of aspirin therapy in preventing NAFLD-related HCC remains limited, with data coming only from certain subgroup analyses.[16–18] Although the incidence of HCC amongst NAFLD patients is low in general, older patients experiencing serum alanine aminotransferase (ALT) elevation have been classified and placed into the high-risk stratification.[17,19] We therefore aimed to conduct a population-based cohort study to investigate the association of daily aspirin therapy with HCC risk in NAFLD patients, including high-risk patients.

Methods

Study design

In this retrospective population-based cohort study, data was retrieved from the National Health Insurance Research Database (NHIRD) of Taiwan for the period

January 01, 1997 to December 31, 2011. The claim data of the NHIRD is comprised of more than 99% of the 23.38 million residents in Taiwan.[20] As outlined in our previously published studies,[9,10,17,21,22] comprehensive information, including patient demographic data, dates of clinic visits or hospitalization, disease diagnosis, medical examinations or procedures, and details of prescriptions (including drug names, dosages, frequency, administration routes, dates and durations) can be exactly obtained from the database. The quality of data taken from the NHIRD, in terms of both disease diagnosis and accuracy of medical administrations, has been fully validated in previous research.[23,24] The diagnosis of diseases has been defined by using the *International Classification of Diseases*, 9th *Revision (ICD-9)* coding system, and the ICD codes employed in this study have been marshaled in Supplemental Table S1. For identifying patients with established disease diagnosis, the diseases must have been coded at least 3 times during outpatient visits, or once during hospitalization.

Hospital-based validation study for NAFLD in the NHIRD

Because ICD codes have been used to identify NAFLD patients in this NHIRD study, we conducted a hospital-based validation study to confirm the process in NAFLD patient selection. We screened all patients with a diagnosis of ICD 571.8 in Taichung Veterans General Hospital from 2009 through 2011, and patients with any malignancy, HBV infection, HCV infection, other infectious hepatitis, HIV infection, excess alcohol use/alcohol-related disorder, toxic hepatitis, biliary cirrhosis or autoimmune hepatitis were excluded. Among finally identified 594 NAFLD patients, 578 (97.8%) patients received a liver ultrasound examination, and fatty liver was diagnosed by ultrasound in 569 (95.8%) patients. In addition, detailed laboratory data such as serum ALT could not be directly obtained from the NHIRD. Because hepatoprotectants (e.g., silymarin, liver hydrolysate and choline bitartrate) were reimbursed only for patients who needed to fulfill the criteria of serum ALT elevation, we used a prescription of hepatoprotectants as a surrogate marker of serum ALT elevation.[17] As shown in Supplemental Table S2, in this hospital-based validation study, the median level of serum ALT in hepatoprotectant users was significantly higher than that in non-users (101.5 [IQR: 66.2–144.0] *vs.* 37.0 [IQR: 23.0–62.0] U/L). Both the diagnosis of NAFLD and serum ALT elevation in the NHIRD study can be validated.

Ethics statement

This study has been approved by the Institutional Review Board of Taichung Veterans General Hospital (CE21003B & CE18314A), and the need for written informed consent was waived.

Study population & high-risk patient cohort

The patient selection process is shown in Fig. 1. We screened 145,212 patients with NAFLD from the nationwide database, and no missing values were found. According to the reimbursement criteria of Taiwan's NHI, long-term daily aspirin use is indicated for antiplatelet therapy, and this indication has not been changed until now. Because regular drug users can receive a refillable prescription over a duration of 3 months in Taiwan, we used an inclusion criterion of 90 consecutive days for enrolling regular aspirin users in this study. Patients who continuously received daily aspirin therapy for 90 days or more were identified as the aspirin-treated cohort, while patients who never received antiplatelet therapy, defined as being a cumulative dose $\leq$ 90 days during the study period, were allocated to the untreated cohort. After matching the index dates to the start of follow-up, patients with any malignancy (including HCC and other types of cancer), chronic HBV infection, chronic HCV infection, other infectious hepatitis, HIV infection, excess alcohol use/alcohol-related disorder, toxic hepatitis, biliary cirrhosis or autoimmune hepatitis were excluded. In order to avoid any observational bias, we also excluded patients who did not call for a medical visit at least one time in a year during the follow-up period. Ultimately, 33,484 patients who continuously received a daily dose of aspirin for 90 days or more, and 55,543 patients who had not received antiplatelet therapy were recruited into the treated and the untreated groups, respectively.

The selection process for high-risk patients is shown in Supplemental Fig. S2. We defined high-risk patients according to the findings in our prior NAFLD study,[17] in which cumulative HCC incidence was highest in older (age > 55 years) patients with serum ALT elevation. As shown in the patient selection process, 7048 patients who continuously received a daily dose of aspirin for 90 days or more, and 7140 patients who had not received antiplatelet therapy were recruited into the treated and the untreated groups, respectively.

Main outcome

The major measured outcome was the occurrence of HCC. Patients in the aspirin-treated group were followed up for HCC occurrence from the 90th day (the index date) of initiating aspirin therapy. For purposes of avoiding any immortal time bias, the index dates of patients in the untreated group were matched with those in the treated group. Patients who had developed HCC prior to the index dates were excluded. Study subjects were followed up until the date of HCC occurrence, mortality or the end of the study period. Patients who withdrew from the nationwide NHI program had typically deceased and were identified as mortality. The diagnosis of HCC was validated by a concurrent registration in the Registry for Catastrophic Illness Patient Database (RCIPD). The RCIPD is based

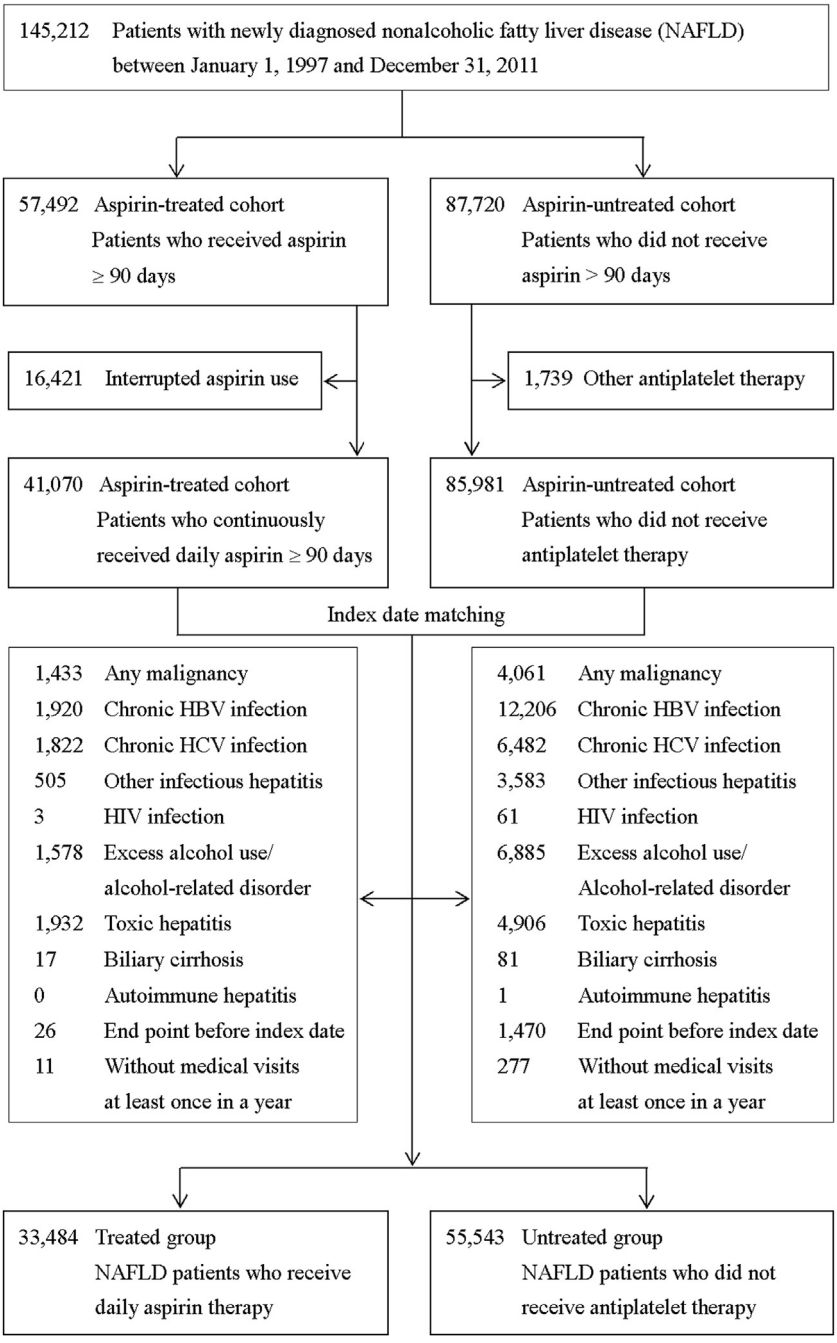

***Fig. 1:* Selection of study participants in the whole patient cohort**. HBV = hepatitis B virus, HCV = hepatitis C virus, HIV = human immunodeficiency virus, NAFLD = nonalcoholic fatty liver disease.

upon an official identification system which can provide a certification to patients with catastrophic diseases for purposes of copayment deduction, as the diagnosis of HCC requires either histopathological confirmation or typical image presentations confirming the diagnostic criteria of HCC.[9,10,17,21,22]

Risk factor assessment

Baseline patient characteristics and major underlying comorbidities, which may be associated with the measured outcome, were evaluated. Study subjects who regularly received specific medicines for diabetes, hyperlipidemia or hypertension were also identified

for diagnosis of any underlying comorbidities. In addition, the use of metformin or statins, which were considered as possibly having potentially chemopreventive effects, were particularly analyzed. Drug users were defined as those patients taking the drugs for more than one day per week prior to the index dates of outcome follow-up.

Statistical analysis

Inverse probability of treatment weighting (IPTW) using the propensity score was applied to balance the baseline characteristics between the two study groups.[25] Propensity scores were consisted of age, gender, cirrhosis, liver decompensation, serum ALT elevation and cardiovascular-related comorbidities, i.e., diabetes, hyperlipidemia, hypertension, coronary arterial disease, cerebral vascular disease, cardiac dysrhythmias and peripheral vascular disease, along with use of other potentially chemopreventive medicines (i.e., metformin and statins). Liver cirrhosis complicated with hepatic encephalopathy, ascites or hepatorenal syndrome was defined as liver decompensation. Propensity score-based adjustment weighed the study subjects by the inverse of the probability of aspirin treatment, with aspirin treatment assignment being independent of measured baseline covariates.[25] With an optimal balance in the measured confounders between the two study groups being achieved, the average effect of aspirin therapy could be estimated. By respectively using a modified Gray method and the Kaplan–Meier method, cumulative incidences of HCC were calculated and compared.[26] Patient mortality or liver transplantation prior to HCC development was treated as a competing risk event. The differences in the full time-to-event distributions between the two study groups were compared using a modified log-rank test.[26] In further analyses, we treated aspirin use as a time varying exposure, and aspirin use or not was categorized from each single day onward until the outcome follow-up period.[27] Using Cox proportional hazard regression models, hazard ratios (HRs) were calculated after IPTW and adjusted based on subdistribution of the competing risk.[28] Furthermore, interaction analyses were performed by adding interaction terms between aspirin therapy and the subgroup factors in the multivariable analyses. We utilized the "cmprsk" package for R to construct the Cox proportional hazard models,[29] and the models were verified by the log–log survival curves (Supplemental Fig. S1).[30] The data of this study were managed through SAS, version 9.3 software (SAS Institute Inc).

Role of funding source

The funding sources had no role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

Results

Study participants

Of the NAFLD patients ultimately included for outcome analysis, 33,484 and 55,543 patients were respectively placed in the aspirin-treated and untreated groups (Fig. 1). As shown in Table 1, the median age in the treated group was 63.5 years, with the percentage of males being mildly lower to females (47.4% *vs.* 52.6%). The overwhelming majority of the patients (98.4%) were prescribed aspirin at a dosage of 100 mg or less, a level that was clinically used for the purpose of cardiovascular event prevention. The median duration of aspirin therapy was quite long during the study period: 8.4 (IQR: 5.0–11.5) years. A small proportion of patients with compensated or decompensated liver cirrhosis were diagnosed when initiating the regular aspirin therapy. As the data presented in the real-world, the patient percentage of serum ALT elevation was small (8.7%). The proportions of patients with major cardiovascular-related diseases were significantly higher in the aspirin-treated group when compared to those in the untreated group. However, after being adjusted by IPTW (Supplemental Table S3), the baseline covariates were well-balanced between the two study groups, i.e., both at a standardized mean difference < 0.1.

Furthermore, as shown in Supplemental Fig. S2, patients at a high HCC risk, i.e., age ≥ 55 years & elevated serum ALT, were identified: 7048 and 7140 patients were respectively recruited in the treated and the untreated groups. The baseline patient characteristics are presented in Table 2. The baseline covariates were also well-balanced between the two study groups after being adjusted by IPTW (Supplemental Table S4).

Hepatocellular carcinoma

Fig. 2A demonstrates the 10-year cumulative incidence of HCC in the whole patient cohort after adjustment through IPTW (Supplemental Fig. S3A presents the data before IPTW). With a median 7.5 (IQR: 4.5–10.5) years of follow-up, the 10-year cumulative incidence of HCC in the aspirin-treated group was significantly lower than that in the untreated group (0.25% [95% CI, 0.19–0.32%] *vs.* 0.67% [95% CI, 0.54–0.81%]; P < 0.001). In multivariable regression analysis, aspirin therapy was independently associated with a reduced HCC risk (adjusted HR 0.48 [95% CI, 0.37–0.63]; P < 0.001).

Fig. 2B demonstrates the 10-year cumulative incidence of HCC in the high-risk patient cohort after adjustment through IPTW (Supplemental Fig. S3B presents the data before IPTW). With a median 7.9 (IQR: 5.0–10.8) years of follow-up, the 10-year cumulative incidence of HCC in the treated group was significantly lower than that in the untreated group (3.59% [95% CI, 2.99–4.19%] *vs.* 6.54% [95% CI, 5.65–7.42%]; P < 0.001). In multivariable regression analysis, aspirin therapy was significantly associated with a reduced HCC risk (adjusted HR 0.63 [95% CI, 0.53–0.76]; P < 0.001).

Characteristics	Aspirin-treated	Aspirin-untreated	SMD before IPTW	SMD after IPTW
	n = 33,484	n = 55,543		
Age, years			0.912	0.075
Mean ± SD	62.6 ± 11.2	51.4 ± 13.2		
Median (IQR)	63.5 (54.7–70.9)	51.0 (42.2–60.3)		
Gender, n (%)			0.145	0.021
Male	15,866 (47.4%)	30,339 (54.6%)		
Female	17,618 (52.6%)	25,204 (45.4%)		
Aspirin dosage		NA		
≤100 mg/day	32,959 (98.4%)			
>100 mg/day	525 (1.6%)			
Aspirin duration, year		NA		
Mean ± SD	8.1 ± 4.0			
Median (IQR)	8.4 (5.0–11.5)			
Cirrhosis				
Compensated	503 (1.5%)	687 (1.2%)	0.023	0.011
Decompensated	159 (0.5%)	321 (0.6%)	0.014	0.001
Serum ALT elevation	2922 (8.7%)	3794 (6.8%)	0.071	0.022
CV-related diseases				
Diabetes	21,716 (64.9%)	17,454 (31.4%)	0.710	0.058
Hyperlipidemia	12,805 (38.2%)	9045 (16.3%)	0.509	0.045
Hypertension	22,308 (66.6%)	11,664 (21.0%)	1.036	0.034
CAD	15,556 (46.5%)	4643 (8.4%)	0.945	0.067
CVA	10,114 (30.2%)	2530 (4.6%)	0.719	0.083
Cardiac arrhythmia	4928 (14.7%)	2388 (4.3%)	0.361	0.018
PVD	802 (2.4%)	513 (0.9%)	0.115	0.001
Drug use				
Metformin	15,240 (45.5%)	11,679 (21.0%)	0.538	0.051
Statin	7778 (23.2%)	4467 (8.0%)	0.428	0.025
PPI	889 (2.7%)	1220 (2.2%)	0.030	0.013
H2RA	1977 (5.9%)	1766 (3.2%)	0.131	0.009

ALT = alanine aminotransferase, CAD = coronary arterial disease, CV = cardiovascular, CVD = cerebral vascular disease, H2RA = histamine 2 receptor antagonist, PPI = proton pump inhibitor, PVD = peripheral vascular disease, IPTW = inverse probability of treatment weighting, IQR = interquartile range, NA = not applicable, SD = standard deviation, SDM = standardized mean difference. [a]Treated patients were those who continuously received daily aspirin therapy for 90 days or more; untreated patients were those who had not received antiplatelet therapy, defined as cumulative doses ≤90 days.

Table 1: **Demographic characteristics of the study participants in the whole patient cohort.**[a]

Sensitivity analyses for high-risk patients

With a higher incidence of HCC in high-risk patients, aspirin therapy may be more suggestive for HCC chemoprevention. Therefore, we performed several sensitivity analyses in the high-risk patient cohort. First, the multivariable stratified analysis for each subgroup of patients. Because some medical conditions might be changed over time, we also performed a time sensitivity analysis: The year of patient inclusion was classified into three periods, i.e., 1997–2000, 2001–2004, and 2005–2011, in which the case numbers in different periods were balanced. As shown in Fig. 3, the association between aspirin therapy and reduced HCC risk was verified in all patient subgroups (all HRs < 0.1). Moreover, a statistical significance was reached in nearly all patient subgroups, including age ≤ 65 years, female or male gender, no compensated cirrhosis, no liver decompensation, no diabetes, hyperlipidemia or not, hypertension or not, metformin non-use, and statin use or non-use. On the interaction analysis, we did not find any statistically significant interactions between aspirin therapy and the subgroup factors (Supplemental Table S5). Second, using a time varying model for patients in the treated group, aspirin therapy remained independently associated with a reduced HCC risk (adjusted HR 0.37 [95% CI, 0.28–0.50]; P < 0.001), when compared to no aspirin use (aspirin discontinuation). Third, for estimating the duration-dependent effect between aspirin use and HCC risk, the HCC risk amongst aspirin users in different therapy durations was evaluated in a multivariable regression model, which consisted of age, gender, compensated cirrhosis, decompensated cirrhosis, diabetes, hyperlipidemia, hypertension, coronary arterial disease, cerebral vascular disease, cardiac dysrhythmias, peripheral vascular disease, metformin use and statin use. Compared to that of

Characteristics	Aspirin-treated	Aspirin-untreated	SMD before IPTW	SMD after IPTW
	n = 7048	n = 7140		
Age, years			0.382	0.048
Mean ± SD	66.5 ± 7.2	63.9 ± 6.6		
Median (IQR)	66.0 (60.7–71.5)	62.7 (58.3–68.1)		
Gender, n (%)			0.018	0.019
Male	3154 (44.8%)	3133 (43.9%)		
Female	3894 (55.2%)	4007 (56.1%)		
Aspirin dosage		NA		
≤100 mg/day	6922 (98.2%)			
>100 mg/day	126 (1.8%)			
Aspirin duration, year		NA		
Mean ± SD	8.4 ± 4.0			
Median (IQR)	8.9 (5.5–11.8)			
Cirrhosis				
Compensated	197 (2.8%)	228 (3.2%)	0.023	0.012
Decompensated	44 (0.6%)	65 (0.9%)	0.033	0.003
CV-related diseases				
Diabetes	4025 (57.1%)	2329 (32.6%)	0.508	0.019
Hyperlipidemia	2400 (34.1%)	1195 (16.7%)	0.406	0.021
Hypertension	4745 (67.3%)	2124 (29.7%)	0.811	0.027
CAD	3502 (49.7%)	938 (13.1%)	0.857	0.07
CVA	2163 (30.7%)	523 (7.3%)	0.624	0.093
Cardiac arrhythmia	1184 (16.8%)	452 (6.3%)	0.332	0.040
PVD	214 (3.0%)	89 (1.2%)	0.124	0.036
Drug use				
Metformin	2781 (39.5%)	1574 (22.0%)	0.384	0.021
Statin	1494 (21.2%)	607 (8.5%)	0.363	0.017
PPI	208 (3.0%)	206 (2.9%)	0.004	0.001
H2RA	519 (7.4%)	394 (5.5%)	0.075	0.015

ALT = alanine aminotransferase, CAD = coronary arterial disease, CV = cardiovascular, CVD = cerebral vascular disease, H2RA = histamine 2 receptor antagonist, PPI = proton pump inhibitor, PVD = peripheral vascular disease, IPTW = inverse probability of treatment weighting, IQR = interquartile range, NA = not applicable, SD = standard deviation, SDM = standardized mean difference. [a]The high-risk patients were defined as age ≥ 55 years & elevated serum ALT.

Table 2: **Demographic characteristics of the study participants in the high-risk patient cohort [a].**

aspirin non-users, the HCC risk could have been lowered in short-term (3 months to < 1 year) aspirin users (adjusted HR 0.72 [95% CI, 0.54–0.98]; P = 0.013). Moreover, compared to that of short-term aspirin users, the HCC risk was lowered with use of aspirin over a period of 1 to < 3 years (adjusted HR 0.71 [95% CI, 0.50–1.01]; P = 0.058) and ≥ 3 years (adjusted HR 0.64 [95% CI, 0.44–0.91]; P = 0.013).

Major bleeding risk in high-risk patients

According to the international criteria surrounding the evaluation of major bleeding risk during antiplatelet therapy,[31,32] both intracranial bleeding and peptic ulcer bleeding were the measured safety outcomes in this study. As shown in Supplemental Fig. S4, the cumulative incidences of intracranial bleeding in the aspirin-treated group were similar to those in the untreated group over a span of 10 years (2.23% [95% CI, 1.78–2.67%] *vs.* 1.98% [95% CI, 1.45–2.51%]; P = 0.259). However, as shown in Supplemental Fig. S5, the 10-year cumulative incidence of peptic ulcer bleeding (PUB) in the aspirin-treated group was modest although significantly higher than that in the untreated group over a period of 10 years (8.63% [95% CI, 7.77–9.48%] *vs.* 6.44% [95% CI, 5.64–7.23%]; P < 0.001), with PUB being defined as a major diagnosis of peptic ulcer disease that eventually led to both hospitalization and blood transfusions.

Discussion

As the threat of NAFLD to public health remains increasing, it is unfortunate that effective therapies remain limited. Currently, no NAFLD-specific medications have yet been approved by the Food and Drug Administration.[5] Lifestyle modification, particularly weight loss, is the mainstay of treatment, however one's set goals are often unreachable. Importantly, while HCC is one of the most concerning adverse outcomes in NAFLD patients, an effective therapy in the prevention

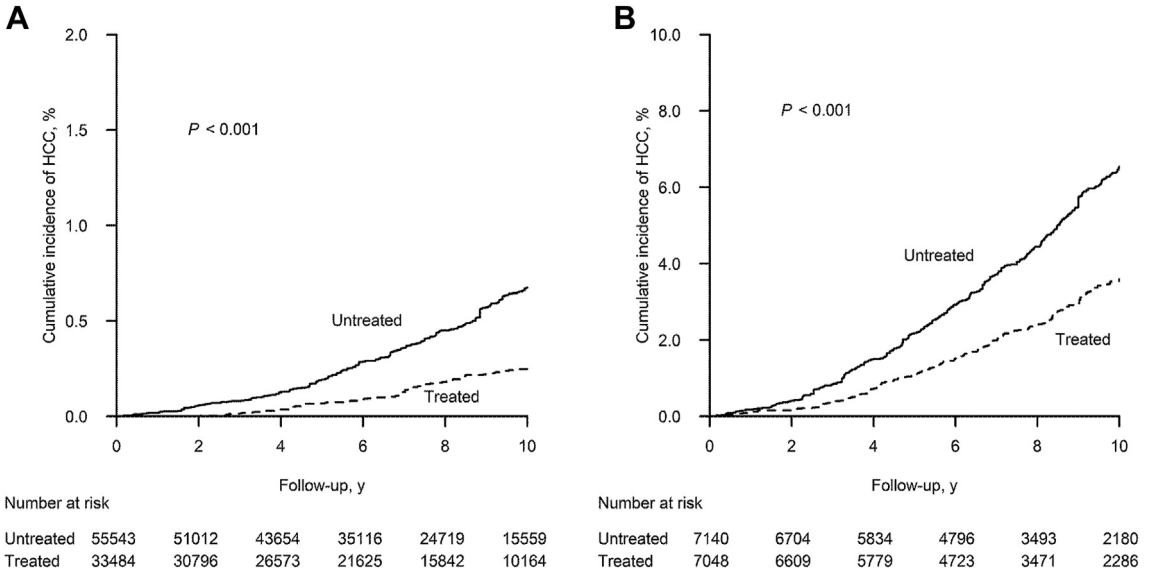

Fig. 2: **Cumulative incidence of hepatocellular carcinoma development after inverse probability of treatment weighting, accounting for patient mortality or liver transplantation as a competing risk**. (A) The whole patient cohort; (B) The high-risk patient cohort. Follow-up from the 90th day after daily aspirin therapy in the treated group and the matched index date in the untreated group. Treated patients were those who continuously received daily aspirin therapy for 90 days or more; untreated patients were those who had not received antiplatelet therapy cumulatively ≥90 days.

of NAFLD-related HCC is highly expected in the future, particularly for NAFLD patients who are at a high risk of HCC development. In the present study, we first reported that daily low-dose aspirin therapy is significantly associated with a reduced risk of HCC in NAFLD patients. With the therapy offering a potential effect towards HCC prevention in NAFLD patients, a proof-of-concept trial should be encouraged in the future.

Emerging data supports using aspirin in the chemoprevention of NAFLD-related HCC. In animal model studies regarding NAFLD, an increased number of intrahepatic platelets, platelet aggregation, and platelet activation were disclosed, while early platelet recruitment would be a contributing factor towards further liver damage.[13] Platelets play an important role in the pathogenesis of HCC, including their attraction to circulating CD8+ T cells,[33] interaction with Kupffer cells,[13] and the release of platelet-derived growth factors, cytokines or chemokines.[34] In contrast, evidence suggests that antiplatelet therapy can prevent HCC development. In animal model studies, aspirin has been shown to be significantly beneficial in reducing steatosis, atherosclerosis, and serum cholesterol,[35] while also attenuating NAFLD severity, abrogating intrahepatic immune cell infiltration, and inhibiting HCC development.[13] However, these laboratory findings have not been fully verified through clinical studies. Although a prospective cohort study reported that daily aspirin therapy was associated with a reduced risk for fibrosis progression occurring in NAFLD,[15] any clinical research designed for investigating the prevention of NAFLD-related HCC remains unreported. The clinical effect of aspirin therapy on HCC prevention has mainly been investigated in regards to viral hepatitis,[9,10,36] with the present study able to fill in the study gap for NAFLD. However, even though the association of aspirin use with better outcomes has been recently reported in patients receiving sorafenib for advanced HCC,[37] the role of aspirin use during HCC treatment needs further investigations.

The high prevalence of NAFLD remains growing in Western and Eastern countries, causing NAFLD to actually become a huge challenge to public health.[1,2] Although only a small proportion of patients with NAFLD will have their condition evolve into liver cirrhosis or HCC, NAFLD has the potential to become the fastest growing cause of HCC in liver transplant candidates.[38] Therefore, a practical way to identify high-risk patients is highly needed for HCC prevention. Although liver pathology remains the gold standard in the evaluation of NAFLD severity, particularly the degree of steatohepatitis, a liver biopsy is rarely performed due to its invasive shortcomings.[5] Fortunately, certain non-invasive risk factors, e.g., serum ALT elevation,[17,19] can be used to identify patients who are at a high risk of HCC development. Although the 10-year cumulative incidence of NAFLD-related HCC was not high in general, it could be significantly increased amongst older patients diagnosed with abnormal liver function.[17] Even though ALT may not be a perfect biomarker to

Characteristics	Event, No./Total No.		
Strata	Treated	Untreated	HR (95% CI)
Age group, y			
≤ 65	98/3133	244/4365	0.47 (0.36-0.63)
> 65	168/3915	196/2775	0.81 (0.64-1.03)
Gender			
Female	121/3894	199/4007	0.63 (0.48-0.83)
Male	145/3154	241/3133	0.63 (0.51-0.80)
Year of patient inclusion			
1997-2000	149/2394	237/2269	0.60 (0.48-0.77)
2001-2004	85/2476	148/2542	0.76 (0.55-1.04)
2005-2011	32/2178	55/2329	0.55 (0.32-0.92)
Compensated cirrhosis			
No	252/6851	406/6912	0.63 (0.52-0.76)
Yes	14/197	34/228	0.67 (0.35-1.27)
Decompensated cirrhosis			
No	264/7004	432/7075	0.63 (0.53-0.76)
Yes	2/44	8/65	0.51 (0.11-2.41)
Diabetes			
No	143/3023	354/4811	0.55 (0.44-0.69)
Yes	123/4025	86/2329	0.86 (0.62-1.19)
Hyperlipidemia			
No	227/4648	410/5945	0.67 (0.56-0.81)
Yes	39/2400	30/1195	0.46 (0.24-0.86)
Hypertension			
No	115/2303	350/5016	0.59 (0.47-0.74)
Yes	151/4745	90/2124	0.71 (0.52-0.98)
Metformin			
No	183/4267	385/5566	0.59 (0.48-0.72)
Yes	83/2781	55/1574	0.84 (0.56-1.27)
Statin			
No	248/5554	430/6533	0.67 (0.56-0.80)
Yes	18/1494	10/607	0.30 (0.11-0.82)
Overall	266/7048	440/7140	0.63 (0.53-0.76)

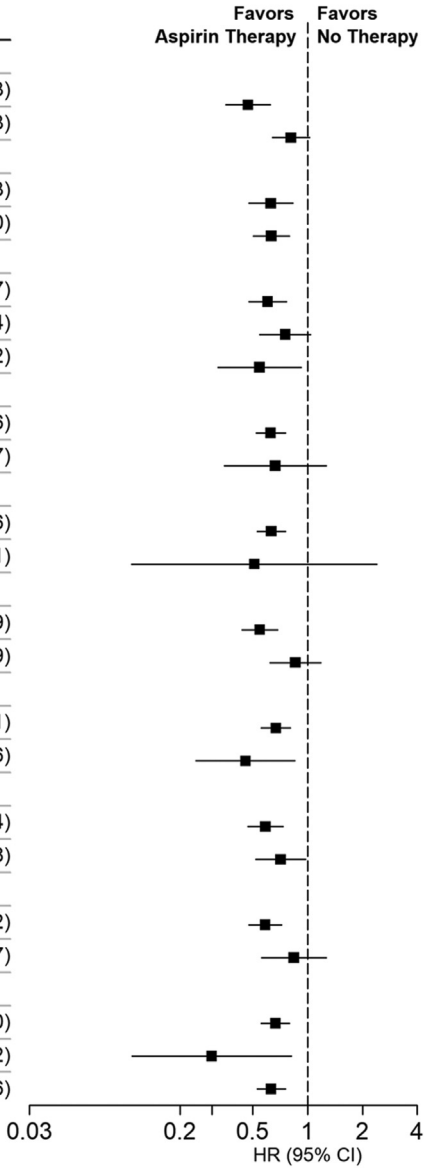

Fig. 3: **Subgroup sensitivity analyses of the association between aspirin therapy and hepatocellular carcinoma development in the high-risk patient cohort**. HR = hazard ratio.

identify all high-risk NAFLD patients, e.g., cirrhotic patients, it can be widely used in clinical practice. Fortunately, as seen findings taken from the present study, a 37% risk of HCC was reduced through aspirin therapy for high-risk NAFLD patients, making aspirin therapy a worthy consideration in view of public health.

Any increase in bleeding risk associated with aspirin therapy has been well studied in long-term users,[39] with the current practice guidelines recommending that the therapy benefits and drawbacks be balanced.[6] In the present study, the risk of intracranial bleeding was similar between the aspirin-treated group and the untreated group. However, the risk of PUB in the aspirin-treated group was increased when compared to that in the untreated group over a span of 10 years (8.63% *vs.* 6.44%). Therefore, aspirin use may be considered for NAFLD patients at higher HCC risk but not at increased risk of bleeding. Comprehensively evaluating PUB risk factors and properly preventing PUB for high-risk patients should be an important concern prior to initiating aspirin therapy. In addition, a personalized approach to evaluate the individual patient's platelet phenotype may

reduce the potential for harm and should be further investigated.[39]

Several limitations within this study are acknowledged. First, this is an association study. Due to the observational nature of this retrospective study, a causal relationship between aspirin therapy and HCC risk could not be forthrightly concluded. More evidence, preferably the cumulation of data from interventional research, is mandatory before clinical recommendations can be made. Second, the possibility of using over-the-counter aspirin could not be precluded. However, as the cost of aspirin is fully reimbursed by Taiwan's national insurance program, a long-term aspirin user is unlikely to pay for it out of pocket. Moreover, even though some patients in the untreated group purchased over-the-counter aspirin, the difference in HCC risk between the two study groups could thus be underestimated. The conclusion drawn from the study remains unchanged. Third, impaired patient adherence to aspirin therapy might be a concern. However, this concern might be omitted in long-term regular users. Furthermore, even though some patients in the treated group did not adhere to aspirin therapy, the difference in HCC risk between the two study groups could be underestimated. The conclusion of this study remains unchanged. Fourth, the study cohort was not representative of general NAFLD population. With a high proportion of patients with underlying metabolic or cardiovascular disorders, most aspirin users in this study were either middle-aged or older. Even though aspirin therapy remained associated with a reduced HCC risk in the subgroup analysis of those aged < 65 years, the effect of aspirin therapy on the youth requires more studies for validation. Fifth, the overwhelming majority of NAFLD patients in our database did not undergo a liver biopsy, and therefore their liver fibrosis and steatohepatitis data could not be obtained in detail. However, we comprehensively matched the potential confounders between the two study groups, and the proportions of patients with decompensated or decompensation cirrhosis were well adjusted. Using the diagnosis of cirrhosis in order to differentiate NAFLD severity in this study could be closer to that seen in clinical practice. Importantly, based upon a large-scale, population-based, and well-validated database, this study may overcome possible biases to help better illuminate a clinically important association. Sixth, without detailed laboratory and body mass index data in the NHIRD, patients with metabolic-associated fatty liver disease (MAFLD) could not be further identified. MAFLD has been recently recognized as a distinct clinical entity for patients with type 2 DM, obesity, and/or metabolic dysregulation, and MAFLD is better related to the prognosis for cardiovascular diseases than NAFLD.[40] However, the risk of HCC in MAFLD patients remains unclear. Even though a high proportion of NAFLD patients in this study might be also classified as MAFLD patients, the effect of aspirin therapy in NAFLD patients could not be directly inferred. Further well-designed studies for MAFLD-related HCC should be expected. Seventh, the individual alcohol intake amount could not be evaluated in this study. However, not only patients with excess alcohol use but also those with any alcohol-related disorders, including alcoholic fatty liver, alcoholic liver cirrhosis or alcoholic liver damage, were completely excluded, therefore the effect of alcohol-related liver injury should have been minimalized. A well-designed study with detailed alcohol intake records will be helpful to confirm our findings.

In conclusion, this large-scale, population-based, long-term cohort study suggests that daily aspirin therapy is significantly associated with a reduced HCC risk in NAFLD patients. Further well-designed clinical trials regarding this form of therapy should be encouraged, particularly for NAFLD patients at a high HCC risk.

Contributors

TYL and CYW contributed to the conception and design of this study. TYL, YCH, HJH and CYW directly accessed and verified the underlying data, and performed the statistical analysis. JTL, YJC, and CYW provided administrative, technical, and logistic support. TYL, YCH, and CYW drafted the manuscript and all authors provided input into the editing for publication. All authors approved the final version. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Data sharing statement

Data was publicly applied and retrieved from the National Health Insurance Research Database, which was provided by the Bureau of National Health Insurance, the Ministry of Health and Welfare, and managed by the National Health Research Institutes, Taiwan. Requests for data should be directed to the corresponding author.

Declaration of interests

YCH has received research grants from Taiwan's National Science and Technology Council, Gilead Sciences, E-Da Hospital, Stanford University, and Tomorrow Medical Foundation; speaker fees from Gilead Sciences, Abbvie Bristol-Myers Squibb, and Roche; advisory board fees from Gilead Sciences and Sysmex; and support for attending meetings from Gilead Sciences and Abbvie. The other authors have reported no disclosures of interest.

Acknowledgments

This study was supported by unrestricted grants from Ministry of Science and Technology (MOST-110-2327-B-A49A-001, MOST-110-2327-B-010-003, MOST-110-2314-B-A49A-525-MY3; MOST-111-2327-B-A49-005), Ministry of Health and Welfare (MOHW111-TDU-221-014007), and Taichung Veterans General Hospital (TCVGH-1123301C, VTA112-V1-3-3), all in Taiwan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.eclinm.2023.102065.

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