CLINICAL and MOLECULAR HEPATOLOGY

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Spears and shields for liver transplantation

Sorafenib versus Lenvatinib Post-ATE/BEV in HCC
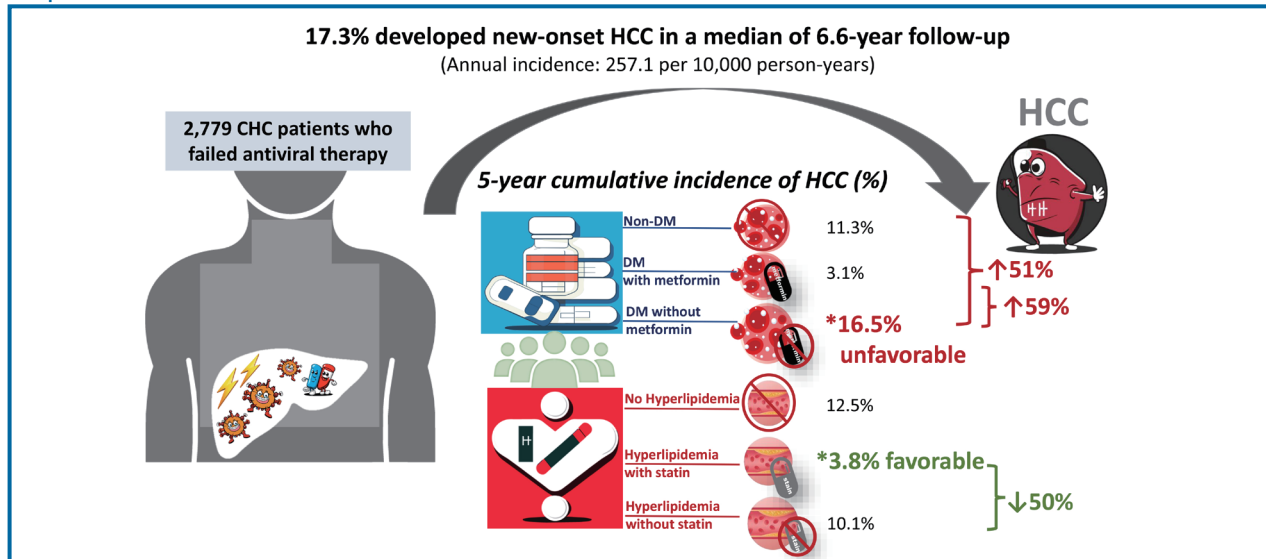Bariatric intervention improves MASH
Non-liver cancer risk of ETV vs TDF
Dynamic changes in MASLD and HCC risk

KASL

Original Article

Metformin and statins reduce hepatocellular carcinoma risk in chronic hepatitis C patients with failed antiviral therapy

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Graphical Abstract

17.3% developed new-onset HCC in a median of 6.6-year follow-up
(Annual incidence: 257.1 per 10,000 person-years)

2,779 CHC patients who failed antiviral therapy

5-year cumulative incidence of HCC (%)

HCC

Non-DM — 11.3%
DM with metformin — 3.1%
DM without metformin — *16.5% unfavorable

↑51%
↑59%

No Hyperlipidemia — 12.5%
Hyperlipidemia with statin — *3.8% favorable
Hyperlipidemia without statin — 10.1%

↓50%

Study Highlights

What is known

- Successful antiviral therapy reduces but doesn't eliminate HCC risk.
- 25% of CHC patients failing therapy remain at high risk of HCC.
- Strategies to lower HCC risk post-therapy failure need exploration.

What is new here

- CHC patients with DM not on metformin use have a 1.51-fold higher risk of HCC than those without DM in antiviral therapy failures.
- CHC patients with HLP on statins use have a 50% reduced HCC risk than those without HLP in antiviral therapy failures.
- Metformin and statins use may prevent HCC in antiviral therapy.

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Abbreviations:

CHC, chronic hepatitis C; HCV, hepatitis C virus; HCC, hepatocellular carcinoma; DM, diabetes mellitus; HTN, hypertension; HLP, hyperlipidemia; LC, liver cirrhosis; LT, liver transplant; DAA, Direct-acting antiviral agent; BMI, body mass index; SVR, sustained virological response; ALT, alanine aminotransferase; AST, aspartate aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis index based on the four factors; GT, genotype; SHR, sub-distribution hazard ratio; aSHR, adjusted sub-distribution hazard ratio; CI, confidence interval; ICD-9-CM, International Classification of Diseases, ninth revision; ICD-10, International Classification of Disease, tenth revision

Background/Aims: Chronic hepatitis C (CHC) patients who failed antiviral therapy are at increased risk for hepatocellular carcinoma (HCC). This study assessed the potential role of metformin and statins, medications for diabetes mellitus (DM) and hyperlipidemia (HLP), in reducing HCC risk among these patients.

Methods: We included CHC patients from the T-COACH study who failed antiviral therapy. We tracked the onset of HCC 1.5 years post-therapy by linking to Taiwan's cancer registry data from 2003 to 2019. We accounted for death and liver transplantation as competing risks and employed Gray's cumulative incidence and Cox sub-distribution hazards models to analyze HCC development.

Results: Out of 2,779 patients, 480 (17.3%) developed HCC post-therapy. DM patients not using metformin had a 51% increased risk of HCC compared to non-DM patients, while HLP patients on statins had a 50% reduced risk compared to those without HLP. The 5-year HCC incidence was significantly higher for metformin non-users (16.5%) versus non-DM patients (11.3%; adjusted sub-distribution hazard ratio [aSHR]=1.51; P=0.007) and metformin users (3.1%; aSHR=1.59; P=0.022). Statin use in HLP patients correlated with a lower HCC risk (3.8%) compared to non-HLP patients (12.5%; aSHR=0.50; P<0.001). Notably, the increased HCC risk associated with non-use of metformin was primarily seen in non-cirrhotic patients, whereas statins decreased HCC risk in both cirrhotic and non-cirrhotic patients.

Conclusions: Metformin and statins may have a chemopreventive effect against HCC in CHC patients who failed antiviral therapy. These results support the need for personalized preventive strategies in managing HCC risk. **(Clin Mol Hepatol 2024;30:468-486)**

Keywords: Hepatitis C; Hepacivirus; Hepatocellular carcinoma; Metformin; Statins

INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health concern, because it frequently leads to liver cirrhosis (LC) and hepatocellular carcinoma (HCC), which impose significant burdens on many countries.[1] HCV treatment has changed from interferon-based therapies to direct-acting antivirals (DAAs), with significant improvements in sustained virological response (SVR) rates.[2] Achieving SVR through anti-HCV therapy greatly reduces the risk of HCC, liver failure,[3] and liver-related mortality.[4]

The risk of HCC can be reduced but cannot be eliminated completely, even after successful antiviral therapy,[5] particularly among patients with persistent advanced fibrosis, insulin resistance, and diabetes mellitus (DM), as well as among the elderly.[6-8] Moreover, patients in whom antiviral therapy fails are still at a high risk of developing HCC, especially those with advanced fibrosis and DM.[9]

Chronic hepatitis C (CHC) has been associated with an increased risk of DM,[10] and the comorbidity of DM increases the risk of developing HCC in patients with CHC.[11] Metformin use has been associated with a reduced risk of HCC compared with other oral hypoglycemic agents (OHAs) or insulin among patients with DM.[12] We previously showed that metformin use in DM significantly reduces the risk of HCC in patients with CHC after successful antiviral therapy.[13]

Statin use has been associated with reduced development of cirrhosis and incidence of HCC, which is mainly observed in patients receiving lipophilic statins.[14] Although the chemopreventive effects of metformin in patients with DM and statins in patients with hyperlipidemia (HLP) reduce the risk of HCC in patients with CHC, which has been proven after achieving SVR,[13] the impact on HCC risk reduction remains unclear in patients with CHC who have failed antiviral therapy.

The current study aimed to assess the impact of metformin for DM and/or statins for HLP on the risk of HCC among CHC patients in who failed antiviral therapy. The findings of this study will improve our understanding of the potential benefits of chemoprevention in reducing the risk of HCC in patients with difficult-to-cure CHC.

The study was approved by the institutional review boards of Kaohsiung Medical University Hospital (KMUHIRB-E(I)-20210378) and was conducted in accordance with the Declaration of Helsinki and the ethical

guidelines. All participants provided written informed consent.

MATERIALS AND METHODS

Study population

The study included patients aged ≥20 years who were diagnosed with CHC either through liver histology or by testing positive for anti-HCV or HCV RNA for > six months. These patients were drawn from a large-scale, multicenter cohort in Taiwan (Taiwanese Chronic Hepatitis C Cohort, T-COACH) and had undergone antiviral interferon-based therapy for at least four weeks since 2003.

We excluded patients who lacked virological outcome data, achieved SVR, had coinfection with human immunodeficiency virus or hepatitis B virus (HBV), died within six months of end-of-treatment (EOT), and developed HCC within 1.5 years after EOT. Finally, the analysis focused on 2,779 patients with CHC who experienced antiviral therapy failure (Fig. 1).

Independent variables

Data collected on the independent variables included: 1) demographic characteristics: age, sex, and body mass index (BMI); 2) medical history: DM, HLP, and hypertension (HTN); 3) laboratory data: AST, ALT, platelet count, creatinine, liver fibrosis (FIB-4 score; fibrosis index based on four factors), renal function (estimated glomerular filtration rate, eGFR); and 4) clinical features: renal function impairment (eGFR ≤60), advanced fibrosis (FIB-4 ≥3.25), LC, and sustained virological response (SVR; HCV RNA seronegativity 24 weeks after interferon-based therapy). Related assessments of the liver and renal function were performed accordingly. The FIB-4 score was calculated using age, AST, ALT, and platelet count of the patient: [age (years)×AST(IU/L)]/[platelet (x1,000/µL)×ALT (IU/L)^0.5]. The eGFR was calculated using the patient's creatinine levels, age, and sex: $186 \times$ creatinine $(mg/dL)^{-1.154} \times$ age $(year)^{-0.203} \times 0.742$ (if female). LC was based on any of the following: liver histology,[15] transient elastography (FibroScan®; Echosens, Paris, France) >12 kPa,[16] acoustic radiation force impulse >1.98 m/s[17], FIB-4 >6.5,[18] or the presence of clinical, radiologi-

cal, endoscopic, or laboratory evidence of cirrhosis and portal hypertension.

Study endpoints and linked databases

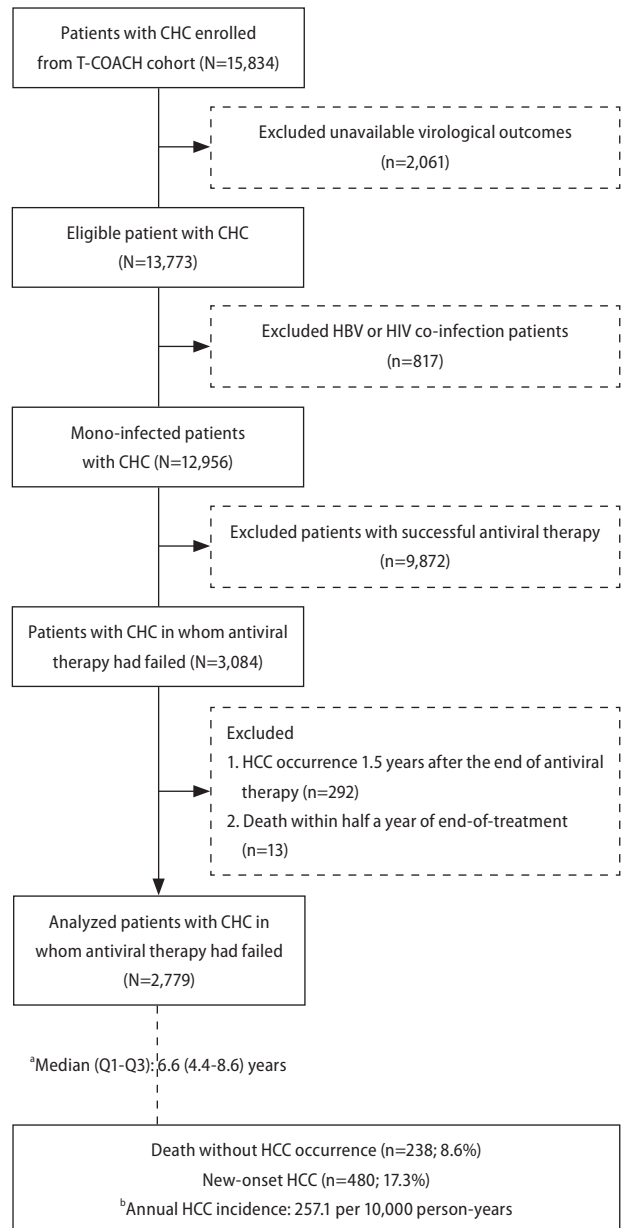The data for the study were obtained from the National Health Insurance Research Database, which covers ap-

Figure 1. Patient enrollment of the cohort. [a]Median (quartile 1–quartile 3) was shown. [b]The annual incidence of HCC was calculated as new-onset HCC divided by the sum of person-years. CHC, chronic hepatitis C; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; HBV, hepatitis B virus.

proximately 26 million Taiwanese people since 1995.

Participants were considered to have DM if they met any of the following criteria: a history of DM on treatment with OHAs with or without insulin, fasting glucose ≥126 mg/dL, or 2-hour plasma glucose ≥200 mg/dL. Patients with a DM diagnosis who had taken metformin for ≥84 days were considered metformin users, whereas those who had taken metformin for <84 days were considered metformin non-users after six months of EOT. Participants were considered to have HLP if they had a history of HLP and used medication. Diagnosed HLP statin users were defined as patients with an HLP diagnosis who had taken statins for ≥84 days, whereas those who had taken statins for <84 days were considered HLP statin non-users after six months of EOT.

All diseases, including HCC and liver transplant (LT), were identified using specific codes from the International Classification of Diseases, 9th or 10th revision (ICD-9-CM and ICD-10). In this study, the occurrence of HCC was determined based on data from the Cancer Registry, whereas the LT cases were identified from the registry of catastrophic illnesses. Information on patient deaths was obtained from the death registry. The relevant medication codes for the diseases were linked to the corresponding entries in the detailed health insurance inpatient/outpatient records (Supplementary Table 1).

New-onset HCC was defined as HCC occurring in patients 1.5 years after antiviral EOT. The follow-up period began 1.5 years after antiviral EOT and continued until the censored events (HCC and death/LT) or December 31, 2019.

Statistical analysis

Continuous variables are presented as mean ± standard deviation, while categorical variables are expressed as numbers (percentages). Chi-square was used to compare subgroups with categorical parameters, and Student's t-test was used for continuous parameters, as appropriate.

Person-years were calculated as the number of years that each participant contributed to the study from 1.5 years after EOT to the date of the first diagnosis of HCC, death/LT, or December 31, 2019, whichever occurred first. The annual incidence of HCC was calculated as the number of new-onset HCC cases divided by the sum of person-

years and the groups were compared by Poisson method.

The study considered death or LT as competing events, meaning that patients who died or underwent LT before developing HCC were no longer at risk of developing HCC. To account for this, the study modified the Kaplan-Meier method using Gray's cumulative incidence method.[19] Cox proportional hazard regression was used to calculate subdistribution hazard ratios (SHRs)[20] for HCC development before and after adjusting for various factors such as age, sex, LC, HCV genotype (GT), HCV RNA and aspirin use. In addition, we compared the cumulative incidence of new-onset HCC between subgroups by stratifying the patients according to their LC status.

Sensitivity analyses were conducted to ensure robustness of the findings. First, advanced fibrosis was substituted with LC to validate the robustness of the results in the multivariate analysis. Second, in this study, participants were defined as metformin users when they used metformin six months after EOT. Patients who used metformin either before or during antiviral therapy were classified as metformin non-users. To strengthen our findings, we reclassified previous metformin users to confirm the effect of metformin on the risk of new-onset HCC in patients with DM. A similar approach was employed for HLP statin users. Third, to avoid potential bias related to specific clinical scenarios including treatment by DAA for IFN-failed patients or patients with diabetes and severe renal impairment (eGFR <30) who are typically not candidates for metformin therapy, we conducted further analysis to validate our findings. All statistical analyses were performed using SAS Enterprise Guide, and *P*-values less than 0.05 were considered statistically significant.

RESULTS

Patient characteristics

The clinical characteristics of the 2,779 CHC patients who failed antiviral therapy are presented in Table 1. The mean age of the patients was 56.1±10.7 years, mean log-transformed HCV RNA levels were 6.1±0.9, 52.7% were female, 34.7% had advanced fibrosis (FIB-4 ≥3.25), 16.2% had liver cirrhosis, 6.7% had an eGFR <60 mL/min/1.73m^2, and 63.1% were infected with HCV GT1. Furthermore,

Table 1. The characteristics of CHC patients in whom antiviral therapy failed

Variables	All (n=2,779)	DM patients			HLP patients		
		Non-metformin (n=288)	Metformin (n=332)	P-value	Non-statin (n=106)	Statin (n=500)	P-value
Age (years)	56.1±10.7	57.9±9.0	56.4±9.5	0.056	57.6±10.1	56.5±9.7	0.275
>65	553 (19.9)	64 (22.2)	53 (16.0)	0.047	23 (21.7)	97 (19.4)	0.590
Female	1,464 (52.7)	128 (44.4)	159 (47.9)	0.391	52 (49.1)	250 (50.0)	0.860
BMI (kg/m^2)	25.0±3.0	25.9±3.8	25.5±2.8	0.088	26.0±3.2	25.3±3.2	0.041
≥27	488 (17.6)	91 (31.6)	68 (20.5)	0.002	33 (31.1)	93 (18.6)	0.004
DM	620 (22.3)	-	-		38 (35.9)	198 (39.6)	0.472
HTN	315 (11.3)	88 (30.6)	46 (13.9)	<0.001	44 (41.5)	71 (14.2)	<0.001
HLP	606 (21.8)	92 (32.0)	144 (43.4)	0.004	-	-	-
Metformin use	332 (12.0)	0 (0.0)	332 (100.0)	-	12 (11.3)	132 (26.4)	<0.001
Statin use	500 (18.0)	66 (22.9)	132 (39.8)	<0.001	0 (0.0)	500 (100.0)	-
Aspirin use	289 (10.4)	35 (12.2)	60 (18.1)	0.041	9 (8.5)	110 (22.0)	0.002
AST (IU/L)	87.6±59.2	90.1±61.9	90.8±52.6	0.883	83.6±48.1	80.0±49.7	0.502
≥2X (80)	1,193 (42.9)	127 (44.1)	156 (47.0)	0.471	45 (42.5)	180 (36.0)	0.212
ALT (IU/L)	121.0±92.5	120.5±88.6	132.9±87.0	0.080	115.9±72.4	117.2±82.6	0.875
≥ 2X (80)	1,722 (62.0)	173 (60.1)	234 (70.5)	0.007	64 (60.4)	298 (59.6)	0.882
Platelet (x1,000/µL)	164.6±55.7	158.6±72.3	160.7±55.1	0.677	171.2±55.5	172.0±59.1	0.895
Creatinine (mg/dL)	1.00±1.23	1.20±1.70	0.92±0.75	0.007	1.05±1.12	1.09±1.61	0.739
FIB-4	3.26±2.77	3.90±3.32	3.40±3.19	0.057	3.11±2.05	2.82±1.93	0.159
≥3.25	964 (34.7)	121 (42.0)	115 (34.6)	0.059	37 (34.9)	126 (25.2)	0.041
Liver cirrhosis	451 (16.2)	74 (25.7)	56 (16.9)	0.007	18 (17.0)	64 (12.8)	0.253
eGFR (mL/min/1.73m^2)	88.6±25.5	87.4±30.9	87.8±22.6	0.853	83.6±24.8	86.3±25.6	0.308
<60	185 (6.7)	42 (14.6)	17 (5.1)	<0.001	10 (9.4)	44 (8.8)	0.835
HCV RNA (log10 IU/mL)	6.1±0.9	6.0±0.9	6.1±0.8	0.278	6.2±0.9	6.2±0.9	0.688
HCV genotype							
GT 1	1,753 (63.1)	189 (65.6)	192 (57.8)	0.048	69 (65.0)	313 (62.6)	0.659
Follow-up (person-years)	18,668	1,663	2,848		634	3,994	

Table 1. Continued

Variables	All (n=2,779)	DM patients			HLP patients		
		Non-metformin (n=288)	Metformin (n=332)	P-value	Non-statin (n=106)	Statin (n=500)	P-value
Follow-up duration (years)							
Mean±SD	6.72±3.18	5.78±2.90	8.58±3.01	<0.001	5.98±2.68	7.99±2.91	<0.001
Median (Q1–Q3)	6.57 (4.41–8.64)	5.76 (3.41–8.05)	8.43 (6.25–10.35)		5.65 (3.92–8.24)	7.82 (5.92–9.55)	
New-onset HCC	480 (17.3)	68 (23.6)	57 (17.2)	0.002	14 (13.2)	47 (9.4)	<0.001
Annual incidence of HCC (per 10,000 person-years)	257.1	408.9	200.1	<0.001	220.8	117.7	0.036
Competing death	238 (8.6)	40 (13.9)	25 (7.5)		13 (12.3)	18 (3.6)	

Values are presented as mean±standard deviation (SD) or number (%).
CHC, chronic hepatitis C; BMI, body mass index; DM, diabetes mellitus; HTN, hypertension; HLP, hyperlipidemia; AST, aspartate aminotransferase; ALT, alanine aminotransferase; FIB-4, fibrosis index based on four factors; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma.

17.6% of the patients were obese (BMI ≥27), 22.3% had DM, 11.3% had HTN, and 21.8% had HLP. Among the patients with DM, 53.5% were metformin users. Among patients with HLP, 82.5% were statin users. In addition, 10.4% of the patients were aspirin users. Overall, 238 (8.6) patients died before the development of HCC and 480 (17.3%) developed new-onset HCC during a total of 18,668 person-years of follow-up (median: 6.6 years). The annual incidence of HCC was 257.1 cases per 10,000 person-years (Table 1).

HCC risk between patients with/without DM and on/not on metformin

Of the 2,779 CHC patients in whom antiviral therapy failed, 620 (22.3%) had DM. After a median follow-up of 7.27 years, 65 died before HCC developed, and 125 developed HCC (annual incidence: 277.1 per 10,000 person-years). For the other 2,159 (77.7%) patients without DM, 173 died before HCC developed, and 355 developed HCC (annual incidence: 250.8 per 10,000 person-years) after a median follow-up of 6.56 years. The 5-year cumulative incidence rate of HCC was not significantly different between patients with and without DM (9.3% vs. 11.3%, Gray's P=0.419) (Fig. 2A). Notably, patients with DM who were not on metformin showed a significant increase in the risk of HCC compared to metformin users (annual incidence: 408.9 per 10,000 person-years in metformin non-users vs. 200.1 per 10,000 person-years in metformin users, P<0.001, Table 1). The 5- and 10-year cumulative incidence rates of HCC were 11.3% and 21.6%, respectively, in non-DM patients; 3.1% and 19.6%, respectively, in DM metformin users; and 16.5% and 28.6%, respectively, in DM metformin non-users. HCC risk was significantly higher in metformin non-users than in metformin users (adjusted sub-distribution hazard ratio (aSHR=1.59, 95% CI=1.07–2.36, P=0.022) and in patients without DM (aSHR=1.51, 95% CI=1.12–2.04, P=0.007). However, there was no difference in HCC risk between metformin users and patients without DM (aSHR=1.05, 95% CI=0.76–1.44, P=0.763) (Fig. 2).
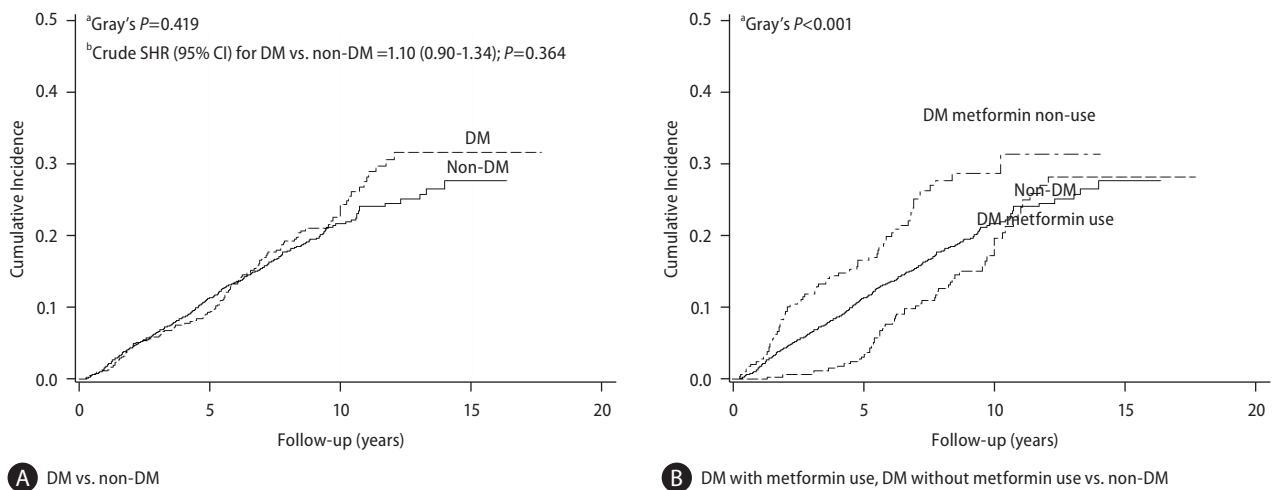
HCC risk between patients with/without HLP and on/not on statins

Of 606 patients with HLP, 31 died before HCC developed, and 61 developed HCC after a median follow-up of 7.64 years (annual incidence: 131.8 per 10,000 person-years). Of the 2,173 patients without HLP, 207 died before HCC developed, and 419 developed HCC (annual incidence: 298.4 per 10,000 person-years) after a median follow-up of 6.46 years. The 5-year cumulative incidence rate of HCC was significantly lower in patients with HLP than in those without HLP (4.8% vs. 12.5%, Gray's $P<0.001$). After further stratification by statin use in HLP patients, the annual incidences were 220.8 per 10,000 person-years in statin non-users vs. 117.7 per 10,000 person-years in statin users

($P=0.036$, Table 1). The 5-year cumulative incidence rates of HCC were 12.5%, 3.8%, and 10.1% in patients without HLP, HLP statin users, and HLP statin non-users, respectively. The HCC risk was significantly lower in HLP statin users than in patients without HLP (3.8% vs. 12.5%; aSHR=0.50, 95% CI=0.36–0.68, $P<0.001$), but there was no difference between patients with HLP who were statin non-users and those who did not (Fig. 3).

Factors associated with HCC risk

After accounting for death as a competing risk, univariate Cox regression analysis showed that being elderly (≥65 years), female, having DM without metformin use, HCV GT1, high AST, high ALT, advanced fibrosis and LC were

A. DM vs. non-DM

B. DM with metformin use, DM without metformin use vs. non-DM

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value	[b]*Adjusted SHR (95% CI) P-value
DM	No. at risk	620	609	560	473	266	114	1.10 (0.90-1.34)	-
	Cumulative Incidence (%)		1.1	5.8	9.3	19.2	24.3	$P=0.364$	
metformin non-use	No. at risk	288	277	234	175	74	21	1.56 (1.20-2.03)	1.51 (1.12-2.04)	1.59 (1.07-2.36)
	Cumulative Incidence (%)		2.4	11.8	16.5	27.7	28.6	$P<0.001$ $P=0.007$	$P=0.022$
metformin use	No. at risk	332	332	326	298	192	93	0.81 (0.62-1.05)	0.95 (0.69-1.31)	1
	Cumulative Incidence (%)		0	0.6	3.1	12.6	19.6	$P=0.116$ $P=0.763$	
Non-DM	No. at risk	2,159	2,098	1,917	1,463	694	265	1	1	1.05 (0.76-1.44)
	Cumulative Incidence (%)		1.5	6.5	11.3	17.9	21.6			$P=0.763$

Figure 2. The risk of HCC in CHC patients who failed antiviral therapy between with/without DM (A) and on/not on metformin (B) while considering death as a competing risk. [a]After considering death as a competing risk, a Kaplan–Meier plot was constructed using Gray's cumulative incidence method. [b]All SHRs (95% CIs) and P-values were calculated using the Cox sub-distribution hazards method. *Adjusted for age, sex, LC, HCV GT1, HCV RNA, aspirin, and HLP/statin. DM, diabetes mellitus; HLP, hyperlipidemia; HCC, hepatocellular carcinoma; SHR, sub-distribution hazard ratio; LC, liver cirrhosis; GT, genotype.

independently associated with a higher risk of HCC, while aspirin use, HLP statin use and low HCV viral load were associated with a significantly lower risk of HCC. In multivariate analysis, the significant factors associated with increased HCC risk were LC (aSHR=2.27, 95% CI=1.81–2.85), elderly (≥65 years; aSHR=1.89, 95% CI=1.52–2.36), HCV GT1 (aSHR=1.30, 95% CI=1.04–1.63) and DM without metformin use (vs. no DM; aSHR=1.51, 95% CI=1.12–2.04). Conversely, patients with HLP who were on statins had a significantly lower risk of HCC than those without HLP (aSHR=0.50, 95% CI=0.36–0.68) (Table 2). Aspirin use also exhibited a significantly lower HCC risk than non-aspirin use (aSHR=0.71, 95% CI=0.51–1.00, P=0.049) (Supplementary Fig. 1A).

Subgroup analysis

LC is the most significant risk factor for HCC. Therefore, we stratified the patients according to their cirrhosis status to evaluate the impact of metformin or statin use among different subgroups.

Among patients without LC, the 5-year cumulative incidence rates of HCC were 9.2%, 3.0%, and 13.5% among those without DM, metformin users, and metformin non-users, respectively. The HCC risk was significantly higher in DM metformin non-users than in patients without DM, with an aSHR of 1.73 (95% CI=1.24–2.40, P=0.001) and in patients with DM on metformin, with an aSHR of 1.71 (95% CI=1.11–2.63, P=0.014) (Fig. 4A). No significant differences were observed between metformin users and patients
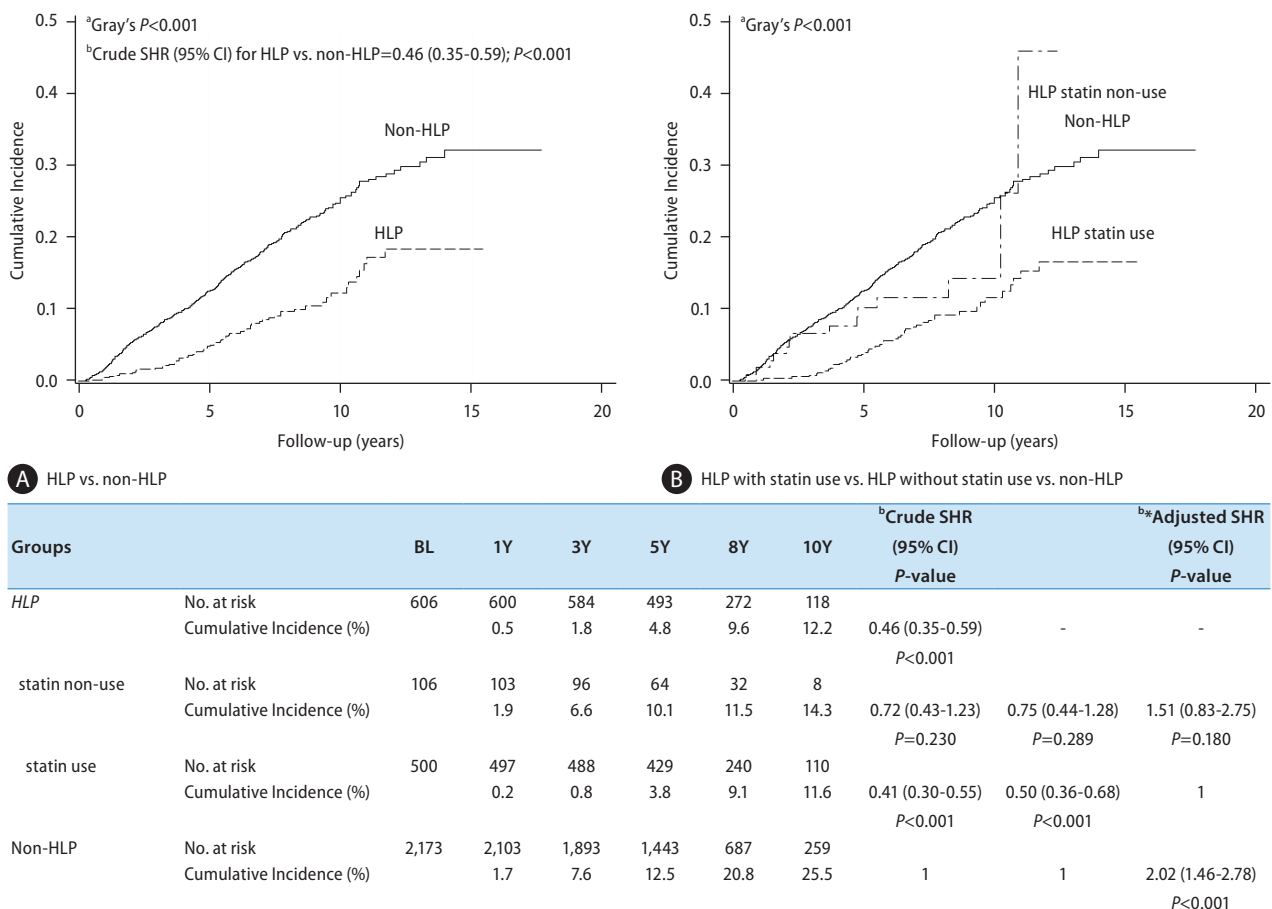
A HLP vs. non-HLP

B HLP with statin use vs. HLP without statin use vs. non-HLP

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value	[b]*Adjusted SHR (95% CI) P-value
HLP	No. at risk	606	600	584	493	272	118	0.46 (0.35-0.59) P<0.001	- -
	Cumulative Incidence (%)		0.5	1.8	4.8	9.6	12.2		
statin non-use	No. at risk	106	103	96	64	32	8	0.72 (0.43-1.23) P=0.230	0.75 (0.44-1.28) P=0.289	1.51 (0.83-2.75) P=0.180
	Cumulative Incidence (%)		1.9	6.6	10.1	11.5	14.3		
statin use	No. at risk	500	497	488	429	240	110	0.41 (0.30-0.55) P<0.001	0.50 (0.36-0.68) P<0.001	1
	Cumulative Incidence (%)		0.2	0.8	3.8	9.1	11.6		
Non-HLP	No. at risk	2,173	2,103	1,893	1,443	687	259	1	1	2.02 (1.46-2.78) P<0.001
	Cumulative Incidence (%)		1.7	7.6	12.5	20.8	25.5		

Figure 3. The risk of HCC in CHC patients who failed antiviral therapy between with/without HLP (A) and on/not on statin (B) while considering death as a competing risk. [a]After considering death as a competing risk, a Kaplan–Meier plot was constructed using Gray's cumulative incidence method. [b]All SHRs (95% CIs) and P-values were calculated using the Cox sub-distribution hazards method. *Adjusted for age, sex, LC, HCV GT1, HCV RNA, aspirin, and DM/metformin. DM, diabetes mellitus; HLP, hyperlipidemia; HCC, hepatocellular carcinoma; SHR, sub-distribution hazard ratio; LC, liver cirrhosis; GT, genotype.

Table 2. Cox sub-distribution hazards model for risk factors of new-onset HCC among CHC patients in whom antiviral therapy failed while considering death as a competing risk

Variables	Levels	No.	New-Onset HCC, n (%)	Competing death, n (%)	Crude SHR (95% CI)	Crude P-value	Adjusted SHR (95% CI)	Adjusted P-value	Adjusted SHR (95% CI)	Adjusted P-value
Age (years)	<65	2,226	335 (15.1)	156 (7.0)	1		1		1	
	≥65	553	145 (26.2)	82 (14.8)	1.93 (1.59–2.35)	<0.001*	1.89 (1.52–2.36)	<0.001*	1.89 (1.52–2.36)	<0.001*
Gender	Male	1,315	206 (15.7)	139 (10.6)	1		1		1	
	Female	1,464	274 (18.7)	99 (6.8)	1.23 (1.03–1.47)	0.025*	1.14 (0.92–1.40)	0.225	1.14 (0.92–1.40)	0.225
BMI (kg/m²)	<27	2,291	390 (17.0)	186 (8.1)	1		-	-	-	-
	≥27	488	90 (18.4)	52 (10.7)	1.11 (0.88–1.39)	0.378	-	-	-	-
Aspirin	No	2,490	435 (17.5)	213 (8.6)	1		1		1	
	Yes	289	45 (15.6)	25 (8.7)	0.68 (0.50–0.91)	0.009*	0.71 (0.51–1.00)	0.049*	0.71 (0.51–1.00)	0.049*
DM/Metformin	Non-DM	2,159	355 (16.4)	173 (8.0)	1		1		1.05 (0.76–1.44)	0.763
	DM/metformin (+)	332	57 (17.2)	25 (7.5)	0.81 (0.62–1.05)	0.116	0.95 (0.69–1.31)	0.763	1	
	DM/metformin (-)	288	68 (23.6)	40 (13.9)	1.56 (1.20–2.03)	<0.001*	1.51 (1.12–2.04)	0.007*	1.59 (1.07–2.36)	0.022*
HLP/Statin	Non-HLP	2,173	419 (19.3)	207 (9.5)	1		1		2.02 (1.46–2.78)	<0.001*
	HLP/statin (+)	500	47 (9.4)	18 (3.6)	0.41 (0.30–0.55)	<0.001*	0.50 (0.36–0.68)	<0.001*	1	
	HLP/statin (-)	106	14 (13.2)	13 (12.3)	0.72 (0.43–1.03)	0.230	0.75 (0.44–1.28)	0.289	1.51 (0.83–2.75)	0.180
AST (IU/L)	<80	1,586	186 (11.7)	103 (6.5)	1		-§		-§	
	≥80	1,193	294 (24.6)	135 (11.3)	2.03 (1.69–2.44)	<0.001*	-§		-§	
ALT (IU/L)	<80	1,057	119 (11.3)	74 (7.0)	1		-§		-§	
	≥80	1,722	361 (21.0)	164 (9.5)	1.67 (1.36–2.05)	<0.001*	-§		-§	

Table 2. Continued

Variables	Levels	No.	New-Onset HCC, n (%)	Competing death, n (%)	Crude SHR (95% CI)	P-value	Adjusted SHR (95% CI)	P-value	Adjusted SHR (95% CI)	P-value
FIB-4	<3.25	1,815	185 (10.2)	120 (6.6)	1		-§		-§	
	≥3.25	964	295 (30.6)	118 (12.2)	3.39 (2.82–4.07)	<0.001*	-§		-§	
Liver cirrhosis	No	2,328	339 (14.6)	170 (7.3)	1		1		1	
	Yes	451	141 (31.3)	68 (15.1)	2.43 (1.99–2.95)	<0.001*	2.27 (1.81–2.85)	<0.001*	2.27 (1.81–2.85)	<0.001*
eGFR (mL/min/1.73m^2)	≥60	2,594	446 (17.2)	201 (7.8)	1		-		-	
	<60	185	34 (18.4)	37 (20.0)	1.03 (0.73–1.45)	0.871	-		-	
HCV RNA (IU/mL)	≤8,000,000	2,078	370 (17.8)	182 (8.8)	1		1		1	
	>8,000,000	332	38 (11.5)	16 (4.8)	0.66 (0.48–0.93)	0.016*	0.73 (0.51–1.04)	0.079	0.73 (0.51–1.04)	0.079
HCV genotype	Non-1	855	126 (14.7)	82 (9.6)	1		1		1	
	1	1,753	320 (18.3)	133 (7.6)	1.24 (1.01–1.52)	0.039*	1.30 (1.04–1.63)	0.022*	1.30 (1.04–1.63)	0.022*

§Due to AST, ALT and FIB-4 were associated with composition in the diagnosis of LC, we did not put these variables in the multivariate analysis.
BMI, body mass index; DM, diabetes mellitus; AST, aspartate aminotransferase; ALT, alanine aminotransferase; eGFR, estimated glomerular filtration rate; FIB-4, fibrosis index based on the 4 factors; eGFR, estimated glomerular filtration rate; HCC, hepatocellular carcinoma; SHR, sub-distribution hazard ratio.
*P<0.05.

without DM. Among patients with LC, the 5-year cumulative incidence of HCC was 23.3%, 3.6%, and 25.1% in patients without DM, metformin users, and metformin non-users, respectively. However, the difference in HCC risk between metformin non-users and the other two groups was not significant (Fig. 4B).

Among patients without LC, the 5-year cumulative incidence rates of HCC were 10.4%, 3.1%, and 6.1% in patients without HLP, in HLP statin users, and in HLP statin non-users, respectively. The HCC risk was significantly lower in HLP statin users than in patients without HLP, with an aSHR of 0.43 (95% CI=0.30–0.61, P<0.001), while there was no difference in HCC risk between HLP statin users and HLP statin non-users (Fig. 5A). Among patients with LC, the 5-year cumulative incidence of HCC was 22.8%, 8.4%, and 30.5% in patients without HLP, HLP statin users, and HLP statin non-users, respectively. HCC incidence

was significantly lower in HLP statin users than in patients without HLP (aSHR=0.47, 95% CI=0.26–0.85, P=0.012) and HLP statin non-users (aSHR=0.35, 95% CI=0.14–0.91, P=0.032), whereas there was no difference in HCC risk between patients without HLP and HLP statin non-users (Fig. 5B).

The aspirin use was not associated with a lower risk of HCC in the subgroup of non-LC (aSHR=0.76, 95% CI=0.53–1.08, P=0.122, Supplementary Fig. 1B) and LC (aSHR=0.68, 95% CI=0.39–1.18, P=0.167, Supplementary Fig. 1C).

Sensitivity analysis

Four sensitivity analyses were conducted to validate the robustness of the main findings and enhance the results.
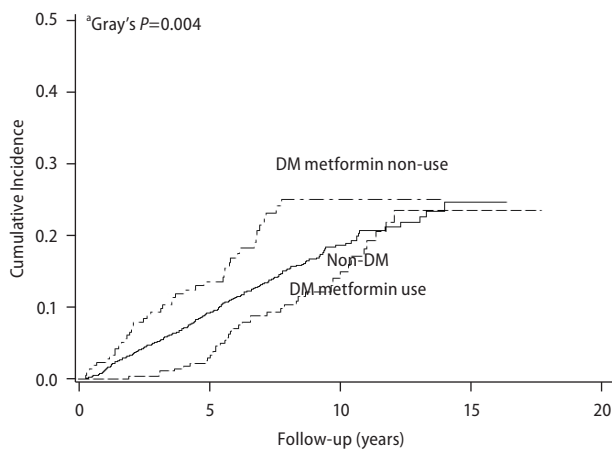
A Non-LC

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value		[b]*Adjusted SHR (95% CI) P-value
DM metformin non-use	No. at risk	214	207	182	138	60	18			
	Cumulative Incidence (%)		2.3	9.4	13.5	25.0	25.0	1.55 (1.12-2.15) P=0.009	1.73 (1.24-2.40) P=0.001	1.71 (1.11-2.63) P=0.014
DM metformin use	No. at risk	276	276	273	248	159	81			
	Cumulative Incidence (%)		0	0.4	3.0	10.3	14.9	0.77 (0.56-1.07) P=0.121	1.01 (0.72-1.41) P=0.955	1
Non-DM	No. at risk	1,838	1,797	1,667	1,290	620	243			
	Cumulative Incidence (%)		1.2	5.2	9.2	15.2	18.6	1	1	0.99 (0.71-1.39) P=0.955

Figure 4. The risk of HCC in CHC patients who failed antiviral therapy between with/without DM and on/not on metformin stratified by baseline liver cirrhosis status: non-LC (A) and LC (B). [a]After considering death as a competing risk, a Kaplan–Meier plot was constructed using Gray's cumulative incidence method. [b]All SHRs (95% CIs) and P-values were calculated using the Cox sub-distribution hazards method. *Adjusted for age, sex, HCV GT1, HCV RNA, aspirin, and HLP/statin. DM, diabetes mellitus; HLP, hyperlipidemia; HCC, hepatocellular carcinoma; SHR, sub-distribution hazard ratio; LC, liver cirrhosis; GT, genotype.

Assessment of outcomes with metformin or statin use before or after the end-of-antiviral therapy

To avoid underestimating the duration of metformin or statin use, we redefined metformin or statin use, either before or after EOT. The 5-year cumulative incidence rate of HCC was higher in patients taking metformin before or after EOT, with rates of 11.1%, 8.1%, and 29.7% in patients without DM, metformin users, and metformin non-users, respectively. HCC risk remained significantly higher in DM metformin non-users than in DM metformin users (aSHR=2.28, 95% CI=1.48–3.46, P<0.001) or patients without DM (aSHR=2.45, 95% CI=1.95–4.24, P<0.001) (Supplementary Fig. 2A). The 5-year cumulative incidence of HCC was higher in patients with HLP on statins before or after EOT, with rates of 12.7%, 5.1%, and 12.7% in patients without HLP, HLP statin users, and HLP statin non-users, respectively. HCC risk remained significantly lower in HLP statin users than in patients without HLP (aSHR=0.43, 95%

CI=0.34–0.63, P<0.001) (Supplementary Fig. 2B). The results were consistent between groups using different definitions of metformin or statin use.

Outcomes using advanced fibrosis as the critical variable

We used another level of liver fibrosis, advanced fibrosis (FIB-4 score >3.25), to validate our findings. In multivariate analysis, HCC risk was significantly higher in patients with advanced fibrosis than in those without advanced fibrosis (aSHR=3.01, 95% CI=2.45–3.64, P<0.001, see Supplementary Table 2). The predicted model showed that DM metformin non-users continued to have a significantly higher risk of HCC compared to patients without DM (aSHR=1.54, 95% CI=1.16–2.03, P=0.005) and DM metformin users (aSHR=1.60, 95% CI=1.13–2.85, P=0.013). The HCC risk was significantly lower in patients with HLP on statins than in patients without HLP (aSHR=0.47, 95% CI=0.34–0.67, P<0.001). The results of LC and advanced
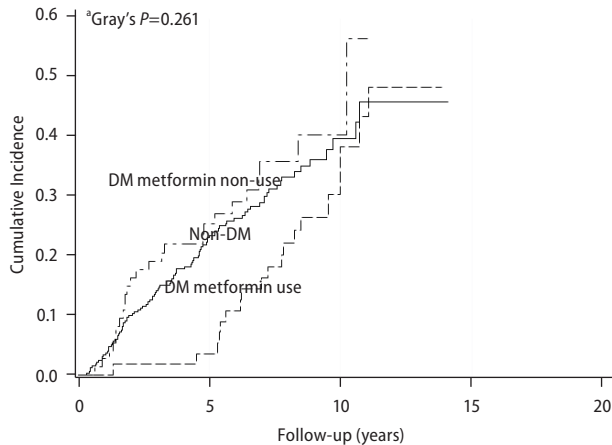
B LC

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value		[b]*Adjusted SHR (95% CI) P-value
DM metformin non-use	No. at risk	74	70	52	37	14	3	1.17 (0.75-1.83) P=0.482	1.22 (0.77-1.94) P=0.394	1.45 (0.81-2.61) P=0.210
	Cumulative Incidence (%)		2.7	18.9	25.1	35.5	40.1			
DM metformin use	No. at risk	56	56	53	50	33	12	0.77 (0.49-1.19) P=0.237	0.84 (0.54-1.32) P=0.449	1
	Cumulative Incidence (%)		0	1.8	3.6	22.1	38.1			
Non-DM	No. at risk	321	301	250	173	74	22	1	1	1.19 (0.76-1.86) P=0.449
	Cumulative Incidence (%)		3.4	14.1	23.3	33	39.3			

Figure 4. Continued.

Figure 5. The risk of HCC in CHC patients who failed antiviral therapy between with/without HLP and on/not on statin stratified by baseline liver cirrhosis status: non-LC (A) and LC (B). [a]After considering death as a competing risk, a Kaplan–Meier plot was constructed using Gray's cumulative incidence method. [b]All SHRs (95% CIs) and P-values were calculated using the Cox sub-distribution hazards method. *Adjusted for age, sex, HCV GT1, HCV RNA, aspirin, and DM/metformin. DM, diabetes mellitus; HLP, hyperlipidemia; HCC, hepatocellular carcinoma; SHR, sub-distribution hazard ratio; LC, liver cirrhosis; GT, genotype.

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value	[b]*Adjusted SHR (95% CI) P-value	
HLP statin non-use	No. at risk	88	85	82	56	27	6			
	Cumulative Incidence (%)		1.1	3.4	6.1	7.8	7.8	0.51 (0.24-1.07) P=0.076	0.47 (0.23-0.95) P=0.036	1.08 (0.50-2.37) P=0.841
HLP statin use	No. at risk	436	433	427	378	214	102			
	Cumulative Incidence (%)		0.2	0.7	3.1	8.0	10.0	0.42 (0.30-0.60) P<0.001	0.43 (0.30-0.61) P<0.001	1
Non-HLP	No. at risk	1,804	1,758	1,613	1,242	598	232			
	Cumulative Incidence (%)		1.4	6.1	10.4	17.7	21.6	1	1	2.33 (1.63-3.33) P<0.001

fibrosis models were consistent.

Minimize potential bias related to specific clinical conditions

To avoid potential bias related to specific clinical scenarios including treatment by DAAs for IFN-failed patients or patients with diabetes and severe renal impairment (eGFR <30) who are typically not candidates for metformin therapy, we conducted further analysis to validate our findings.

After excluding 78 IFN-failed patients who experienced retreatment with DAAs, we found that diabetic patients not treated with metformin had a 1.50-fold increased risk of developing HCC compared to non-diabetic individuals (aSHR=1.50, 95% CI=1.13–1.98, P=0.005) (Supplementary Fig. 3A). Conversely, hyperlipidemia patients who were prescribed statins showed a 55% decrease in HCC risk
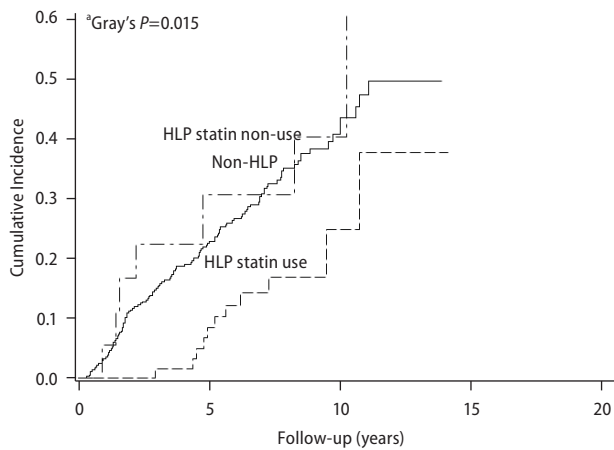
compared to those without hyperlipidemia (aSHR=0.45, 95% CI=0.33–0.60, P<0.001) (Supplementary Fig. 3B).

Similarly, after removing 49 patients with eGFR<30, the risk of HCC in diabetic patients not treated with metformin was found to be 1.56-fold higher than that in non-diabetics (aSHR=1.56, 95% CI=1.18–2.07, P=0.002, Supplementary Fig. 4A). Hyperlipidemia patients on statin therapy had a 55% decreased risk of HCC compared to those without hyperlipidemia (aSHR=0.45, 95% CI=0.33–0.60, P<0.001, Supplementary Fig. 4B).

Taken together, our findings from the current study were consistent across these sensitivity analyses.

Interaction of statin and metformin

In this study, statin non-users had a 2.42-fold higher risk of HCC compared to statin users among metformin non-

B LC

Groups		BL	1Y	3Y	5Y	8Y	10Y	[b]Crude SHR (95% CI) P-value	[b]*Adjusted SHR (95% CI) P-value	
HLP statin non-use	No. at risk	18	18	14	8	5	2			
	Cumulative Incidence (%)		5.6	22.2	30.5	30.5	40.2	1.29 (0.62-2.71) P=0.497	1.34 (0.60-3.01) P=0.482	2.84 (1.10-7.33) P=0.032
HLP statin use	No. at risk	64	64	61	51	26	8			
	Cumulative Incidence (%)		0	1.6	8.4	16.8	24.8	0.44 (0.25-0.80) P=0.007	0.47 (0.26-0.85) P=0.012	1
Non-HLP	No. at risk	369	345	280	201	89	27			
	Cumulative Incidence (%)		3.3	14.9	22.8	35.0	43.4	1	1	2.12 (1.18-3.82) P=0.012

Figure 5. Continued.

users, and a 2.23-fold higher risk among metformin users. Whereas, there was no significant difference in HCC risk between metformin non-users and metformin users, both among statin non-users and statin users. Consequently, our analysis did not reveal a significant interaction between statins and metformin in predicting HCC risk (P=0.82, Supplementary Fig. 5).

DISCUSSION

Of the 2,779 CHC patients, 480 (17.3%) developed new-onset HCC and 238 (8.6%) died after antiviral therapy. Patients with DM but no metformin use had a 1.51-fold higher risk of HCC than patients without DM, whereas HCC risk was comparable between patients without DM and those with DM on metformin. The 5-year cumulative HCC incidence (16.5%) was significantly higher in metformin non-users than in those without DM (11.3%, P=0.007) and metformin users (3.1%, P=0.022). Conversely, patients with

HLP who used statins had a 50% lower risk of HCC than those without HLP (5-year cumulative HCC incidence: 3.8% vs. 12.5%, P<0.001). Notably, the unfavorable effect of metformin non-use on increased HCC risk was mainly observed among patients without LC but not among patients with LC. In contrast, a favorable effect of statins on reducing the risk of HCC was observed in patients with and without LC.

HCV infection has been linked to lower lipid profiles, and when a patient with CHC achieves SVR, their lipid profiles may worsen, potentially leading to cardiocerebral events.[21-23] For patients with CHC, DM or HLP, treatment with the antidiabetic agent metformin and cholesterol-lowering statin is commonly used. Several studies have investigated the association between statin use and HCC risk in patients with CHC. Statins are well-known for their preventive role in many cancers, including liver cancer.[24,25] A meta-analysis of 27 studies found strong evidence of statin-related potential in reducing the risk of HCC.[24] Among patients with CHC, statin use has been associated with im-

proved virological response rates to antiviral therapy,[26] decreased liver fibrosis progression, and reduced HCC risk in a dose-dependent manner in both Veteran and Taiwanese insurance cohorts.[14,27] These studies suggested that statin use may have a protective effect against liver cancer in patients with hepatitis C infection. In the present study, we found that statin use reduced the risk of HCC in patients with HLP and CHC after the failure of antiviral therapy.

Several studies have explored the association between metformin use and HCC risk in patients with CHC and DM. A systematic review of 12 studies indicated that metformin might have a protective effect against HCC in patients with DM.[12] A large nationwide Taiwanese study showed that metformin use was linked to a lower HCC risk in a dose-dependent manner in patients with type 2 DM and chronic liver disease.[28] These studies suggested that metformin may exert a protective effect against HCC in patients with CHC and diabetes. Furthermore, our previous study documented that after achieving SVR, LC, and metformin non-use in patients with DM and CHC may result in a higher risk of HCC.[13] Similarly, we also observed a chemopreventive effect of metformin on the risk of HCC development in patients who failed antiviral therapy. Another interesting viewpoint is that among these patients, the HCC risk in the DM metformin use group initially appears lower than in the non-DM group but later aligns with it. This trend implies that the timing and effectiveness of metformin usage may vary. Maybe metformin had a real chemopreventive effect at an earlier period, but it was discontinued later due to progression or uncontrolled diabetes, and the effect faded, resulting in a comparable HCC risk as non-DM. Further validation is needed for these findings.

Our clinical findings revealed that unfavorable DM metformin non-use and favorable HLP statin use were critical in reducing the risk of HCC among patients with CHC after antiviral therapy failed. Several studies have investigated the effects of combination therapy with simvastatin (a statin) and metformin on the growth and migration of different cancer cells, including HBV-related HCC[29] and prostate cancer.[30,31] One study found that simvastatin and metformin inhibited the growth of HBV-related HCC cells by upregulating autophagy.[29] Combining simvastatin with metformin induced G1-phase cell cycle arrest and Ripk1- and Ripk3-dependent necrosis in C4-2B osseous metastatic castration-resistant prostate cancer cells. The synergistic effects of simvastatin and metformin on osseous metastatic castration-resistant prostate cancer cells suggest that this combination may be a promising treatment option for this type of cancer.[30,31]

After HCV infection, host factors, such as older age and fibrosis progression leading to LC, appear to be significant in the development of HCC.[9,32-34] Coexisting conditions such as DM, obesity, and co-infection with HBV or HIV may accelerate the development of HCC.[35,36] Conversely, successful antiviral therapy has been associated with a reduced risk of HCC.[3,37] In this study, we observed that older age, advanced fibrosis, and LC were the independent risk factors for HCC. Exploring chemopreventive approaches, such as using favorable HLP statins and unfavorable DM metformin nonuse among patients with CHC in whom antiviral therapy has failed, may present another opportunity to decrease the risk of HCC.

Our study had several limitations that merit attention. Firstly, the specific impact of the duration, dosage, continuity, and timing of metformin and statin use on the incidence of HCC remains to be determined. Secondly, our analysis did not differentiate between the effects of lipophilic and hydrophilic statins, nor did it consider the potential influence of other OHAs such as sulfonylureas, thiazolidinediones, and DPP4 inhibitors on HCC incidence. Thirdly, we had no information on interferon preparations, specifically pegylated interferon α-2a versus α-2b, in this study.

Despite these limitations, which make further analysis challenging, our research offers significant insights in the era of DAAs for a small subset of patients who do not achieve SVR with DAAs,[38] are non-compliant with treatment,[39] or have contraindications to DAAs.[40] Additionally, this study on the chemopreventive effects of metformin and statins on HCC risk could serve as a benchmark for future research on the long-term risk of HCC among HCV patients and other patients with active liver diseases.

In summary, both metformin for DM and statins for HLP had chemopreventive effects on HCC risk in patients with CHC in whom antiviral therapy failed. These findings underscore the importance of implementing personalized preventive strategies to manage patients with these clinical profiles.

Authors' contribution

Guarantor of the article: Ming-Lung Yu. Conception and

Conflicts of Interest

The authors declare no conflicts of interest in this present study.

SUPPLEMENTARY MATERIAL

Supplementary material is available at Clinical and Molecular Hepatology website (http://www.e-cmh.org).

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