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ORIGINAL ARTICLE

Distinct effects of hepatic steatosis and metabolic dysfunction on the risk of hepatocellular carcinoma in chronic hepatitis B

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Abstract

Objective Chronic hepatitis B (CHB) and metabolic dysfunction-associated fatty liver disease (MAFLD) are the leading causes of hepatocellular carcinoma (HCC). We aim to explore the impact of concurrent MAFLD on the risk of HCC in CHB.

Methods Patients with CHB were consecutively recruited from 2006 to 2021. MAFLD was defined by steatosis and either obesity, diabetes mellitus, or other metabolic abnormalities. The cumulative incidence of HCC and associated factors were compared between the MAFLD and non-MAFLD groups.

Results 10,546 treatment-naïve CHB patients were included with a median follow-up of 5.1 years. CHB patients with MAFLD ($n = 2212$) had fewer hepatitis B e antigen (HBeAg)-positivity, lower HBV DNA levels, and Fibrosis-4 index compared with the non-MAFLD group ($n = 8334$). MAFLD was independently associated with a 58% reduced risk of HCC (adjusted hazard ratio [aHR] 0.42, 95% confidence interval [CI] 0.25–0.68, $p < 0.001$). Furthermore, steatosis and metabolic dysfunction had distinct effects on HCC. Steatosis was protective against HCC (aHR 0.45, 95% CI 0.30–0.67, $p < 0.001$), while a greater burden of metabolic dysfunction increased the risk (aHR 1.40 per dysfunction increase, 95% CI 1.19–1.66, $p < 0.001$). The protective effect of MAFLD was further confirmed in analysis with inverse probability of treatment weighting (IPTW), patients who had undergone antiviral therapy, those with probable MAFLD, and after multiple imputation for missing data.

Conclusions Concurrent hepatic steatosis is independently associated with a lower risk of HCC, whereas the increasing burden of metabolic dysfunction aggravates the risk of HCC in untreated CHB patients.

Keywords Hepatitis B virus (HBV) · Metabolic dysfunction-associated fatty liver disease (MAFLD) · Non-alcoholic fatty liver disease (NAFLD) · Liver cancer · Metabolic syndrome

Abbreviations

AASLD	The American Association for the Study of Liver Diseases
AFP	Alpha-fetoprotein
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
aHR	Adjusted hazard ratio
BMI	Body Mass Index
CHB	Chronic hepatitis B
CI	Confidence interval
CAP	Controlled Attenuation Parameter
DM	Diabetes mellitus
FIB-4	Fibrosis-4
HBcAg	Hepatitis B core antigen
HBsAg	Hepatitis B surface antigen
HBeAg	Hepatitis B e antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HCC	Hepatocellular carcinoma
HIV	Human immunodeficiency virus
HR	Hazard ratio
IFN	Interferon
IPTW	Inverse probability of treatment weighting
IQR	Interquartile range

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MAFLD	Metabolic dysfunction-associated fatty liver disease
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
NA	Nucleos(t)ide analogue

Introduction

Non-alcoholic fatty liver disease (NAFLD) is an emerging chronic liver disease with growing clinical significance. The prevalence of NAFLD is estimated to affect nearly a third of people in Asia [1, 2] and worldwide [3]. NAFLD contributes to the development of hepatocellular carcinoma (HCC), especially in those with cirrhosis [4]. Because metabolic dysfunction is the fundamental pathogenesis of NAFLD, metabolic dysfunction-associated fatty liver disease (MAFLD) has been proposed and widely accepted to simplify the diagnostic criteria and avoid excluding patients with other concomitant liver diseases, such as chronic hepatitis B (CHB) [5]. In clinical practice, MAFLD can select those with greater liver disease severity than the criteria of NAFLD [6–8].

In an endemic area of chronic hepatitis B (CHB), the co-existence of HBV infection and hepatic steatosis is frequently observed [9]. Nevertheless, There are still controversies about the impact of hepatic steatosis on chronic HBV infection [10, 11]. A population-based study reported negative associations between hepatic steatosis and HBV infection [12]. Another study enrolling 1915 patients of CHB showed hepatic steatosis was associated with a lower degree of inflammation and fibrosis in liver histology compared with those without steatosis [13]. In addition, a higher degree of steatosis was associated with a lower risk of HCC occurrence [14, 15]. Recently, a meta-analysis disclosed that about 30% of patients with CHB had hepatic steatosis, which was inversely associated with viral activity but not hepatic fibrosis [16]. In contrast, other studies demonstrated higher risks of advanced fibrosis, HCC, and other adverse liver outcomes in patients of CHB with the coexistence of fatty liver disease [17–19].

Considering the increasing prevalence of MAFLD in patients of CHB, we thus investigated the impact of MAFLD on CHB patients for the development of HCC.

Patients and methods

Study population

From January 2006 to April 2021, patients aged 20 or older with chronic liver diseases were retrospectively screened from the Integrated Medical Database of the National Taiwan University Hospital (NTUH), a tertiary center in Taiwan. The database included all diagnoses, laboratory data, imaging studies, and prescription records in NTUH, as well as the nationwide death registry; its quality has been validated by systemic data mining and statistical analysis with clinical interpretation [20].

CHB patients should have at least twice CHB diagnoses on different dates of the emergency department or outpatient clinic visits or once from the discharge diagnosis of hospitalization. All these patients also had a positive hepatitis B surface antigen (HBsAg) or detectable serum HBV DNA level for confirmation.

This study was approved by the Institutional Review Board of NTUH (202104086RIND) and conformed to the ethical principles for medical research involving human subjects of the Declaration of Helsinki updated in 2013. The informed consent was waived because this is a retrospective study conducted by a review of medical records only.

Definition of MAFLD in CHB patients

MAFLD was defined according to the proposed criteria [5]. First, patients should have hepatic steatosis, which was investigated by abdominal ultrasonography with the presence of fatty liver, defined as increased echogenicity compared with renal cortex or spleen along with beam attenuation [21]. The accuracy of ultrasonography for the detection of steatosis in NTUH reached ninety percent among CHB patients receiving liver biopsy in our biopsy cohort [7, 9]. Second, they should have one of the three following clinical criteria, namely (a) overweight/obesity (BMI $\geq$ 23 kg/m^2); (b) type 2 diabetes mellitus (DM) or receiving specific hypoglycemic drug treatment; (c) $\geq$ 2 metabolic abnormalities: hypertension or receiving specific anti-hypertensive drug treatment, plasma triglycerides $\geq$ 150 mg/dl, HDL-cholesterol < 40 mg/dl for male and < 50 mg/dl for female, prediabetes (fasting glucose levels 100 to 125 mg/dl or HbA1c 5.7% to 6.4%), plasma high-sensitivity C-reactive protein level > 2 mg/l, and Homeostatic Model Assessment-Insulin Resistance $\geq$ 2.5 [5]. Patients were screened for MAFLD and classified into two groups (MAFLD and non-MAFLD groups).

Patients fulfilling MAFLD criteria with intermittent fatty liver by serial abdominal ultrasonography were designated as the probable MAFLD group for sensitivity analysis. Those receiving anti-HBV therapy (including nucleos(t)ide analogue [NA] or interferon) before HCC development would be included for sensitivity analysis as well. We excluded patients with co-existing HCV, HIV infection, with other malignancies or metastatic tumors at baseline. Patients with prior HCC and HCC developed within 1 year were

excluded to avoid a small undiagnosed HCC at baseline. HCC surveillance was performed by abdominal ultrasonography and/or serum alpha-fetoprotein (AFP) measurement every 3–12 months in CHB patients according to the current guidelines [22–24].

Data collection

The baseline demographics, laboratory data, and abdominal ultrasonography [25] were collected. To evaluate the influence of metabolic dysfunction, the fulfilled criteria of metabolic dysfunction, namely (a) BMI ≥ 23 kg/m^2, (b) DM, and (c) ≥ 2 metabolic risk abnormalities, were calculated cumulatively as the burden of metabolic dysfunction.

Definition of clinical outcome

HCC diagnosis was based on either pathology or two typical dynamic imaging studies with AFP ≥ 200 ng/ml before 2010, and one typical dynamic imaging study after 2010 according to the American Association for the Study of Liver Diseases (AASLD) guidelines [26]. The study endpoint was the occurrence of HCC. Patients were censored at the time of HCC or their last clinical visits.

Statistical analysis

The continuous variables were expressed by median (interquarter range [IQR]) and compared by the Mann–Whitney *U* test. The categorical data were expressed in numbers (percentage) and compared by Chi-square test. The cumulative incidence of HCC was calculated by the Kaplan–Meier method. Factors associated with HCC occurrence were included in the univariable Cox regression model, and those with $p < 0.05$ were further included in the multivariable Cox regression model. Patients with missing data were omitted in the original analysis and were additionally managed by multiple imputation via multivariate imputation by chained equation (MICE) package in R as the sensitivity analysis [27]. In addition, stabilized inverse probability of treatment weighting (IPTW) method was performed to balance the baseline characteristics between the two groups [28]; a logistic regression analysis with MAFLD as the dependent variable and associated factors (including age, sex, HBeAg, HBV DNA level, cirrhosis, and FIB-4 index) as independent variables was performed to calculate the predicted probability of MAFLD, and the inverse of predicted probability was used as the weight with balanced baseline characteristics between the two groups. The statistical comparison was performed by R (Version 4.1.0, R Foundation for Statistical Computing, Vienna, Austria), and a two-tailed *p* value < 0.05 were defined as statistically significant.

Results

128,417 patients with chronic liver disease were screened, and 35,100 patients with confirmed CHB were identified. After exclusion, 10,546 patients were included in the final analysis. Of these patients, 2212 (21%) had concurrent MAFLD, while the other 8334 were in the non-MAFLD group (Supplementary Fig. 1).

Baseline demographics and laboratory data

Compared with the non-MAFLD group, CHB patients with MAFLD were significantly older, male-predominant, and had higher BMI, more DM, more hypertension, and less cirrhosis. After a median follow-up of 5.1 years, 23 (1.0%) and 161 (1.9%) patients developed HCC in the MAFLD and non-MAFLD groups, respectively (Table 1). The number of deaths before HCC development was 54 (2.4%) and 453 (5.4%) in the MAFLD and non-MAFLD groups. Regarding the viral factors, CHB patients with MAFLD had a lower proportion of hepatitis B e antigen (HBeAg) positivity and lower median HBV DNA levels than non-MAFLD patients. The details of other laboratory data are shown in Table 1.

Factors associated with HCC occurrence

The 3-, 5-, and 10-year cumulative incidence of HCC was 0.8%, 1.0%, and 1.5% in the MAFLD group, and 1.1%, 1.9%, and 3.3% in the non-MAFLD group (Fig. 1). In the univariable analysis, age ≥ 50 years, male sex, BMI ≥ 23 kg/m^2, DM, hypertension, alcoholic liver disease, HBeAg positivity, higher aspartate aminotransferase (AST), AFP ≥ 20 ng/ml, and FIB-4 index ≥ 1.45 were associated with HCC occurrence, whereas the presence of MAFLD was inversely associated with HCC occurrence (Table 2).

In the multivariable Cox regression model, the levels of AST, which were already used in the calculation of FIB-4, were not included. Age ≥ 50 versus < 50 years (adjusted HR [aHR] 1.80, 95% CI 1.16–2.80, $p = 0.009$), male sex (aHR 3.19, 95% CI 2.13–4.79, $p < 0.001$), BMI ≥ 23 versus < 23 kg/m^2 (aHR 1.53, 95% CI 1.07–2.18, $p = 0.019$), HBeAg positivity (aHR 2.71, 95% CI 1.75–4.18 $p < 0.001$), FIB-4 index ≥ 1.45 versus < 1.45 (aHR 3.52, 95% CI 2.33–5.34, $p < 0.001$), and AFP ≥ 20 versus < 20 ng/ml (aHR 2.60, 95% CI 1.51–4.45, $p < 0.001$) were associated with HCC occurrence, while the presence of MAFLD had a reduced risk of HCC (aHR 0.42, 95% CI 0.25–0.68, $p < 0.001$, Table 2).

Because high HBV viral load is associated with increased HCC risk, it was adjusted with different cut-off values in another multivariable Cox regression model. Increasing HBV DNA level was an independent risk predictor for

Table 1 Baseline characteristics and laboratory data of patients with chronic hepatitis B (N = 10,546)

Characteristics	Sample number	CHB		*p* value
		without MAFLD (*n* = 8334)	with MAFLD (*n* = 2212)	
Age, years	10,546	47 (37–57)	52 (44–60)	< 0.001
Male sex	10,546	3880 (47)	1505 (68)	< 0.001
DM	10,546	407 (4.9)	451 (20.4)	< 0.001
Hypertension	10,546	1244 (14.9)	802 (36.3)	< 0.001
Alcoholic liver disease	10,546	44 (0.5)	11 (0.5)	0.859
Cirrhosis	10,546	283 (3.4)	18 (0.8)	< 0.001
BMI, kg/m2	7893	21.9 (20.1–24.0)	26.4 (24.4–28.8)	< 0.001
HBeAg positivity	8093	882 (13.7)	111 (6.7)	< 0.001
HBV DNA, IU/ml	4929	2290 (115–100,000)	565 (28–12,100)	< 0.001
ALT, U/L	10,508	22 (16–35)	32 (22–50)	< 0.001
AST, U/L	10,430	24 (19–31)	26 (21–34)	< 0.001
AFP, ng/ml	10,054	2.67 (2.00–3.88)	2.71 (2.06–3.77)	0.120
Platelet, k/uL	8854	213 (175–254)	228 (194–268)	< 0.001
FIB-4 index	8765	1.13 (0.76–1.75)	1.06 (0.77–1.49)	< 0.001
Glucose, mg/dl	6630	89 (84–96)	98 (90–111)	< 0.001
TG, mg/dl	6534	80 (61–108)	136 (100–182)	< 0.001
HDL, mg/dl	4340	55 (45–66)	42 (37–49)	< 0.001
Follow-up duration, years	10,546	5.2 (2.2–8.9)	4.7 (2.0–8.1)	< 0.001
HCC occurrence	10,546	161 (1.9)	23 (1.0)	0.004

The data are expressed as median (IQR) or number (%) accordingly

CHB chronic hepatitis B, *MAFLD* metabolic dysfunction-associated fatty liver disease, *BMI* Body Mass Index, *HCC* hepatocellular carcinoma, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *AFP* Alpha-Fetoprotein, *TG* triglyceride, *HDL* High-density lipoprotein, *FIB-4* Fibrosis-4

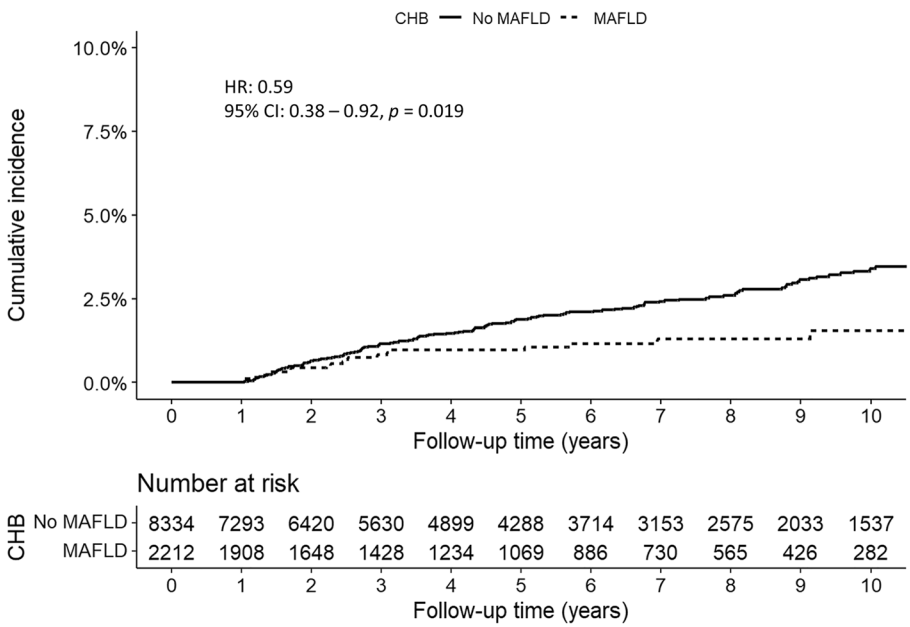

Fig. 1 Cumulative incidence of HCC in untreated CHB patients with versus without MAFLD. Those with MAFLD had lower risks of HCC occurrence (HR 0.59, 95% CI 0.38–0.92, $p = 0.019$). *HCC* hepatocellular carcinoma, *CHB* chronic hepatitis B, *MAFLD* metabolic dysfunction-associated fatty liver disease, *HR* hazard ratio, *CI* confidence interval

HCC in these CHB patients (HBV DNA ≥ 200000 versus < 200000 IU/ml: aHR 1.47, 95% CI 1.01–2.14, $p = 0.046$, Supplementary Table 1). The presence of MAFLD was associated with a reduced risk of HCC occurrence (aHR 0.44, 95% CI 0.27–0.72, $p = 0.001$, Supplementary Table 1). Additionally, the FIB-4 index was replaced by

Table 2 Factors associated with HCC occurrence ($N = 10,546$)

HCC occurrence	Univariable analysis			Multivariable analysis		
Variables	Hazard Ratio	95% CI	*p* value	Hazard Ratio	95% CI	*p* value
Age (≥ 50 versus < 50 years)	3.05	2.22–4.19	< 0.001	1.80	1.16–2.80	0.009
Sex (male versus female)	3.99	2.77–5.75	< 0.001	3.19	2.13–4.79	< 0.001
BMI (≥ 23 versus < 23 kg/m^2)	1.41	1.05–1.90	0.022	1.53	1.07–2.18	0.019
Diabetes mellitus	2.68	1.83–3.92	< 0.001	1.22	0.75–1.99	0.414
Hypertension	1.88	1.37–2.58	< 0.001	1.35	0.91–2.00	0.134
Alcoholic liver disease	6.22	2.31–16.77	< 0.001	0.50	0.07–3.65	0.497
MAFLD	0.59	0.38–0.92	0.019	0.42	0.25–0.68	< 0.001
HBeAg positivity	2.11	1.45–3.06	< 0.001	2.71	1.75–4.18	< 0.001
HBV DNA (≥ 2000 versus < 2000 IU/ml)	1.30	0.95–1.78	0.101	1.01	0.73–1.42	0.932
ALT (per U/L increase)	1.001	1.000–1.002	0.136			
AST (per U/L increase)	1.002	1.001–1.003	0.003			
AFP (≥ 20 versus < 20 ng/ml)	6.10	3.79–9.81	< 0.001	2.60	1.51–4.45	< 0.001
FIB-4 (≥ 1.45 versus < 1.45)	6.54	4.70–9.09	< 0.001	3.52	2.33–5.34	< 0.001

HCC hepatocellular carcinoma, *CI* confidence interval, *BMI* Body Mass Index, *MAFLD* metabolic dysfunction-associated fatty liver disease, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *ALT* alanine aminotransferase, *AST* aspartate aminotransferase, *AFP* Alpha-Fetoprotein, *FIB-4* Fibrosis-4

the sonographic cirrhosis in another model, and MAFLD remained associated with a lower risk of HCC (aHR 0.45, 95% CI 0.28–0.74, $p = 0.002$, Supplementary Table 2).

Subgroup analyses of the association between MAFLD and HCC occurrence

After adjustment for age, sex, BMI, DM, hypertension, HBeAg, and FIB-4 index, MAFLD was consistently associated with a lower risk of HCC occurrence in patient subgroups regardless of age, sex, presence of DM, levels of alanine aminotransferase (ALT) and HBV viral load. In addition, concurrent MAFLD decreased risks of HCC in patients with BMI ≥ 23 kg/m^2, HBeAg negativity, absence of cirrhosis, FIB-4 < 1.45, as well as FIB-4 between 1.45 and 3.25 (Table 3).

The association of steatosis and metabolic dysfunction on HCC occurrence

Steatosis and metabolic dysfunction were two critical components of MAFLD. There were 1225, 736, and 251 patients with 1, 2, and 3 categories of metabolic dysfunction in the MAFLD group, and 5730, 2028, 425, and 151 patients with 0, 1, 2, and 3 categories in the non-MAFLD group. Steatosis was independently protective against HCC occurrence (aHR 0.45, 95% CI 0.30–0.67, $p < 0.001$, Table 4), whereas metabolic dysfunction increased the risk (aHR 1.40 per 1 dysfunction increase, 95% CI 1.19–1.66, $p < 0.001$, Table 4). Of the metabolic risk factors considered (DM, overweight, and hypertension), DM exhibited the most significant impact on the HCC risk (aHR 1.57, 1.02–2.39, $p = 0.039$, Supplementary Table 3).

After stratification by MAFLD, the dose-dependent association of metabolic dysfunction and risk of HCC remained significant for those without MAFLD (aHR 1.33 per 1 dysfunction increase, 95% CI 1.11–1.60, $p = 0.002$); while it was marginally significant for those with MAFLD (aHR 1.71 per 1 dysfunction increase, 95% CI 0.99–2.97, $p = 0.055$). The cumulative incidence of HCC in patients with concurrent MAFLD and variable burden of metabolic dysfunction is shown in Fig. 2.

Balancing baseline characteristics by IPTW method

To balance the baseline viral factors and hepatic fibrosis between the MAFLD and non-MAFLD groups, stabilized IPTW method was used. The FIB-4 index, HBV DNA levels, the proportion of cirrhosis (4.9% versus 4.7%), and HBeAg positivity (13.9% versus 14.6%) were well-balanced between the two groups after IPTW (all $p > 0.05$) as shown in Supplementary Table 4. In the multivariable analysis, concurrent MAFLD remained an independent factor associated with fewer HCC occurrence (aHR 0.50, 95% CI 0.26–0.96, $p = 0.037$) after adjustment for age, sex, BMI, DM, hypertension, alcoholic liver disease, HBeAg, HBV DNA, AFP and FIB-4 levels (Supplementary Table 5).

Table 3 Subgroup analysis of the association of MAFLD with HCC occurrence in patients with chronic hepatitis B using multivariable Cox regression models

Subgroups	Number	Adjusted HR (with vs. without MAFLD)	95% CI	*p* value
Age (years)				
< 50	5665	0.35	0.13–0.94	0.038
≥ 50	4881	0.43	0.25–0.75	0.003
Sex				
Female	5161	0.08	0.01–0.66	0.019
Male	5385	0.48	0.29–0.80	0.005
BMI (kg/m^2)				
< 23	4043	0.62	0.15–2.60	0.517
≥ 23	3850	0.38	0.23–0.64	< 0.001
DM				
Yes	858	0.37	0.15–0.88	0.025
No	9688	0.41	0.23–0.73	0.003
Cirrhosis				
Yes	301	0.38	0.08–1.86	0.234
No	10245	0.50	0.30–0.84	0.009
HBeAg				
Positive	993	0.37	0.10–1.33	0.127
Negative	7100	0.44	0.26–0.73	0.002
HBV DNA (IU/ml)				
< 20000	3335	0.51	0.28–0.92	0.024
≥ 20000	1594	0.22	0.08–0.63	0.005
ALT (U/L)				
≤ 41	8211	0.50	0.26–0.96	0.036
> 41	2297	0.28	0.14–0.59	< 0.001
FIB-4				
< 1.45	5874	0.29	0.12–0.70	0.006
1.45–3.25	2259	0.39	0.18–0.85	0.018
> 3.25	632	1.01	0.43–2.38	0.986

The analysis was adjusted for age, sex, BMI, DM, hypertension, HBeAg, and FIB-4 in each subgroup

MAFLD metabolic dysfunction-associated fatty liver disease, *HCC* hepatocellular carcinoma, *HR* hazard ratio, *CI* confidence interval, *BMI* Body Mass Index, *HBeAg* hepatitis B e antigen, *HBV* hepatitis B virus, *FIB-4* Fibrosis-4

Sensitivity analysis of the association between MAFLD and HCC occurrence

The 6661 patients with probable MAFLD (intermittent presence of fatty liver) were used for the sensitivity analysis; after the exclusion by the remaining exclusion criteria, 3583 were finally included, and an intermediate risk of HCC occurrence was found as compared to MAFLD and non-MAFLD groups (aHR 0.38 as compared with the non-MAFLD group, Supplementary Table 6). In addition, the 5065 patients receiving anti-HBV therapy in the exclusion criteria were enrolled, and finally, 4029 were analyzed for HCC risk (Supplementary Fig. 2). Those with MAFLD had a significantly lower risk of HCC occurrence than those without MAFLD (aHR 0.34, Supplementary Table 7).

Furthermore, multiple imputation was performed for missing data in the original cohort (N = 10,546), and MAFLD remained an independent factor associated with a lower risk of HCC occurrence (aHR 0.34, 95% CI 0.21–0.55, $p < 0.001$, Supplementary Table 8).

Table 4 The effect of metabolic dysfunction and steatosis on the risk of HCC occurrence

HCC occurrence	Multivariable analysis		
Variables	Hazard ratio	95% CI	*p* value
Age (≥ 50 versus < 50 years)	1.60	1.07–2.38	0.021
Sex (male versus female)	3.96	2.70–5.82	< 0.001
Burden of metabolic dysfunction (per 1-point increase)	1.40	1.19–1.66	< 0.001
HBeAg positivity	3.14	2.12–4.63	< 0.001
Steatosis	0.45	0.30–0.67	< 0.001
AFP (≥ 20 versus < 20 ng/ml)	2.90	1.75–4.81	< 0.001
FIB-4 (≥ 1.45 versus < 1.45)	4.42	2.98–6.56	< 0.001

HCC hepatocellular carcinoma, *CI* confidence interval, *HBeAg* hepatitis B e antigen, *AFP* Alpha-Fetoprotein, *FIB-4* Fibrosis-4

Discussion

Both CHB and fatty liver disease have been recognized as the major etiologies of chronic liver disease, leading to a significant number of adverse liver outcomes, including the development of HCC. In this study, we found that MAFLD, especially steatosis with only mild metabolic dysfunction, was a favorable prognostic factor against HCC occurrence, whereas a higher burden of metabolic dysfunction aggravated the risk in CHB patients.

Our data showed that CHB patients with MAFLD had lower HBV viral activity than those without MAFLD, suggesting a possibly inverse correlation between MAFLD and HBV replication. A negative association was also found in terms of hepatic steatosis; those with concurrent steatosis had a lower proportion of HBeAg positivity (9% versus 14%, $p < 0.001$) and a lower median level of HBV DNA (794 versus 2390 IU/ml, $p < 0.001$) than those without steatosis in this study. Similar findings have also been demonstrated in previous reports. The HBV viral load was lower in those with steatosis (evaluated by controlled attenuation parameter [CAP]) than in controls with a dose-dependent effect [29]. The proportions of serum HBeAg positivity, HBV viremia, intrahepatic HBsAg and hepatitis B core antigen (HBcAg) positive staining on liver tissue were reduced in patients with steatosis [30]. Another study found that higher levels

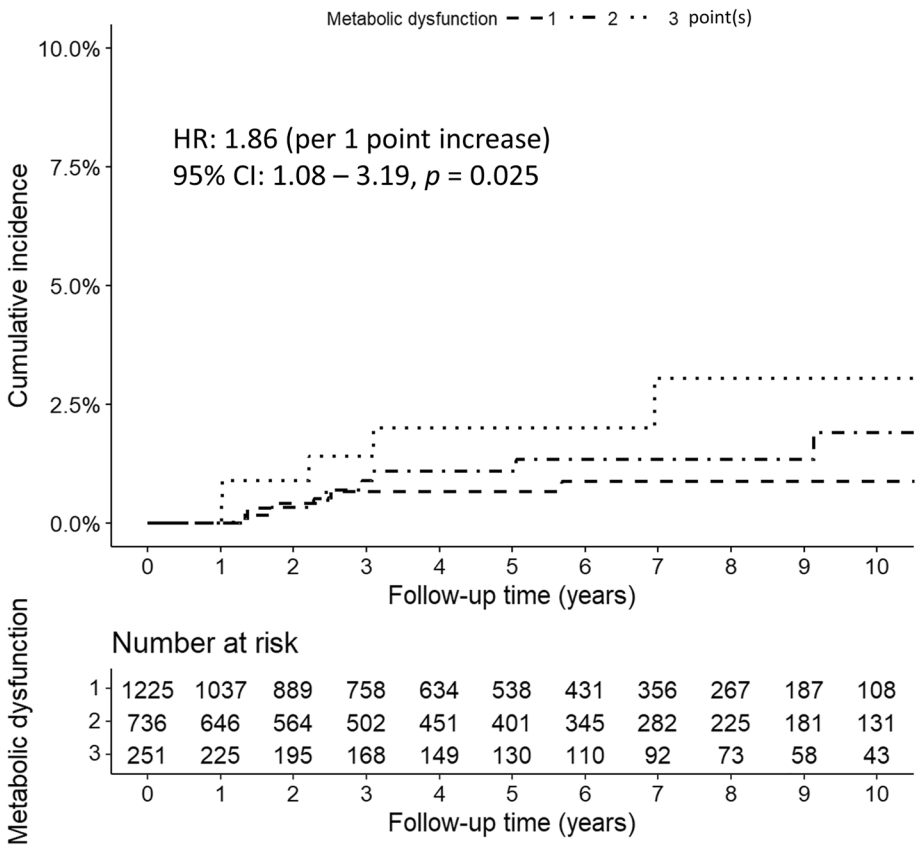

Fig. 2 Cumulative incidence of HCC in untreated CHB patients with concurrent MAFLD. Those with a greater burden of metabolic dysfunction had a higher risk of HCC (HR 1.86 per 1-point increase, 95% CI 1.08–3.19, $p = 0.025$). *HCC* hepatocellular carcinoma, *CHB* chronic hepatitis B, *MAFLD* metabolic dysfunction-associated fatty liver disease, *HR* hazard ratio, *CI* confidence interval

of past viral load trajectories decreased the risk of steatosis, whereas the functional cure of HBV infection was associated with a 1.41-fold risk [31]. Furthermore, a meta-analysis demonstrated a negative association between hepatic steatosis and HBV viral activity [16]. The mechanisms have been investigated. Hepatic steatosis induced by high-fat diets reduced the serum HBeAg, HBsAg, HBcAg, and HBV DNA levels in the mouse model [32]. Steatosis inhibited HBsAg and HBV DNA secretion via the induction of endoplasmic reticulum stress in hepatocytes [33]. In a prospective study, adiponectin levels, an adipokine reducing hepatic steatosis, were increased in those with higher HBV DNA levels [34]. In HepG2-hepatitis B virus-stable cells, the viral replication was upregulated by adiponectin and downregulated by knock-down adiponectin levels [35].

Hepatic fibrosis has been found to be positively associated with the degree of hepatic steatosis based on CAP [36, 37]. Liver fibrosis is associated with nonalcoholic steatohepatitis (NASH) [18], or the existence of MAFLD by histology [19] in CHB patients. On the contrary, a recent meta-analysis did not find a significant association between steatosis and fibrosis [16]. In our study, we found that patients with MAFLD had a lower FIB-4 index and a lower prevalence of cirrhosis than those without MAFLD. One of the plausible explanations for the discrepancy between our current and previous results may be the different patient populations. Prior studies analyzed CHB patients who received liver biopsies so that they might have more advanced liver disease. In fact, MAFLD is a disease with a broad spectrum, from simple steatosis to steatohepatitis and cirrhosis [38]. Our cohort possibly includes more patients with early-stage MAFLD when they merely had steatosis but not yet developed fibrosis. As steatosis progresses to steatohepatitis, more patients may begin to have advanced fibrosis or even cirrhosis. Finally, "burnt-out NASH" occurs in patients with advanced fibrosis, and their hepatic fat gradually reduces at that stage as a natural course of NAFLD [39]. As a result, those with burnt-out NASH at enrollment might be classified as non-MAFLD group and showed a higher degree of fibrosis as well as subsequent higher HCC risks.

Viral factors have been considered the most important risk factors in HBV-related HCC, consistently demonstrated in our patients with underlying MAFLD. For example, the HBeAg positivity had an increased risk of HCC, and HBV DNA ≥ 200,000 IU/ml independently increased a 1.5-fold risk of HCC in this study. Similarly, the annual incidence of HCC in concurrent MAFLD-CHB patients of this cohort was 0.20%, which was much higher than those in patients with only fatty liver disease (0.021%) and those without liver disease (0.002%) [40].

Metabolic dysfunction is considered a risk factor for HCC development, such as overweight, obesity, and DM

[41–43]. A study using a biopsy-proven CHB cohort found MAFLD an independent risk factor of HCC occurrence [19]. We demonstrated that the accumulation of metabolic dysfunction independently increased the risk of HCC in a dose-dependent manner, suggesting that this could be used to stratify HCC risks in the CHB population. For example, those with severe metabolic dysfunction (fulfilling 2 or 3 factors) should have a more intensive monitoring strategy than those without metabolic dysfunction risk factor [11]. Furthermore, whether correcting metabolic dysfunction could reduce the risk of HCC in CHB patients warrants further investigation.

However, our study found MAFLD was associated with a reduced risk of HCC occurrence especially in those with mild metabolic dysfunction, which might be due to the presence of steatosis. According to a prospective population-based cohort study enrolling 2903 male CHB patients in Taiwan, the presence of fatty liver on ultrasonography was associated with a lower risk of HCC (HR 0.24) [41]; in the subsequent study of the same cohort, PNPLA3-148 M variant was the risk factor for HCC despite the protective effect of steatosis [44]. Another cohort study also found fatty liver as an independent protective factor for HCC in those with antiviral treatment (HR 0.21) [45]. In recent studies, the greater baseline levels of steatosis defined by higher CAP were similarly associated with lower risks of HCC occurrence in CHB patients [14, 15]. Our data confirmed steatosis was independently associated with a lower risk of HCC occurrence. We also find that MAFLD was no longer a protective factor in those with advanced fibrosis (FIB-4 > 3.25), suggesting that the protective role of MAFLD was only observed in subjects without advanced fibrosis, probably because the hepatic carcinogenesis facilitated by the late-stage steatohepatitis offset the potential effect of steatosis. On the other hand, simple steatosis seems not to adversely affect liver outcomes in non-viral liver diseases, including primary biliary cholangitis and autoimmune hepatitis [46, 47]. Despite the potential protective effect of steatosis against HCC in CHB, this study does not advocate for MAFLD being considered a favorable factor in clinical practice, since steatosis and the co-existing adverse metabolic factors are highly correlated and difficult to treat separately. In addition, metabolic dysfunction factors are associated with the presence of NASH, which increases the incidence of liver-related outcomes [18]. Moreover, MAFLD's systemic adverse impacts like cardiovascular and cancer risks undermine its permissiveness in CHB patients [38]. Instead, our findings underscore the importance of investigating the mechanisms underlying the protective effect of steatosis in CHB, as this may offer opportunities to identify potential therapeutic targets for HBV cure in the future.

The strength of this study is the large sample size with detailed clinical information. However, there are some limitations as well. First, this is a retrospective study in which missing data could not be avoided, although multiple imputation was performed in the sensitivity analysis. Second, the fatty liver was defined qualitatively rather than quantitatively based on ultrasonography rather than histology, and the presence of NASH was hard to identify and analyze; however, we chose those with persistent MAFLD in the study to reduce the classification bias; additionally, we further examined the accuracy of ultrasonography for detection of steatosis as described in the method. Third, those with burnt-out NASH and cirrhosis at enrollment could be classified as non-MAFLD group and were possibly associated with a higher risk of HCC. Fourth, the study included Asians only, and the results need to be validated in Western populations.

In conclusion, concurrent hepatic steatosis is independently associated with a lower risk of HCC occurrence in untreated CHB patients; however, a higher burden of metabolic dysfunction increases the risk of HCC. Future studies are needed to clarify the molecular interaction between MAFLD and HBV.

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Data availability The data supporting this study's findings are not publicly available due to patients' privacy but are available from a reasonable request and approved by the Institutional Review Boards.

Declarations

Conflict of interest T-HS received a research Grant from Gilead Sciences, and was on speaker's bureaus for Abbvie, Bayer, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp and Dohme, and Takeda. J-HK has served as a consultant for Abbvie, Abbott, Gilead Sciences, Roche, and Sysmex and on speaker's bureaus for Abbvie, Bristol-Myers Squibb, Gilead Sciences, Merck Sharp, and Dohme, and Sysmex. S-CH, T-CT, C-LC, S-JH, S-HL, C-MH, C-HL, T-YL, H-CY, C-JL, P-JC declare no conflict of interest.

Ethical approval This study was approved by the Institutional Review Board of National Taiwan University Hospital (202104086RIND) and conformed to the ethical principles for medical research involving human subjects of the Declaration of Helsinki updated in 2013. The informed consent was waived because this is a retrospective study conducted by a review of medical records only.

References

1. Li J, Zou B, Yeo YH, Feng Y, Xie X, Lee DH, et al. Prevalence, incidence, and outcome of non-alcoholic fatty liver disease in Asia, 1999–2019: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol. 2019;4:389–398
2. Fan JG, Kim SU, Wong VW. New trends on obesity and NAFLD in Asia. J Hepatol. 2017;67:862–873
3. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15:11–20
4. Orci LA, Sanduzzi-Zamparelli M, Caballol B, Sapena V, Colucci N, Torres F, et al. Incidence of hepatocellular carcinoma in patients with nonalcoholic fatty liver disease: a systematic review, meta-analysis, and meta-regression. Clin Gastroenterol Hepatol. 2022;20:283-292.e210
5. Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, et al. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol. 2020;73:202–209
6. Lin S, Huang J, Wang M, Kumar R, Liu Y, Liu S, et al. Comparison of MAFLD and NAFLD diagnostic criteria in real world. Liver Int. 2020;40:2082–2089
7. Huang SC, Su HJ, Kao JH, Tseng TC, Yang HC, Su TH, et al. Clinical and histologic features of patients with biopsy-proven metabolic dysfunction-associated fatty liver disease. Gut Liver. 2021;15:451–458
8. Nguyen VH, Le MH, Cheung RC, Nguyen MH. Differential clinical characteristics and mortality outcomes in persons with NAFLD and/or MAFLD. Clin Gastroenterol Hepatol. 2021;19:2172-2181.e2176
9. Su HJ, Kao JH, Tseng TC, Yang HC, Su TH, Chen PJ, et al. Pathologic findings of patients with nonalcoholic fatty liver disease and the impact of concurrent hepatitis B virus infection in Taiwan. J Formos Med Assoc. 2020;119:1476–1482
10. Yang M, Wei L. Impact of NAFLD on the outcome of patients with chronic hepatitis B in Asia. Liver Int. 2022;42:1981–1990
11. Huang SC, Kao JH. Metabolic dysfunction-associated fatty liver disease and chronic hepatitis B. J Formos Med Assoc. 2022;121:2148–2151
12. Joo EJ, Chang Y, Yeom JS, Ryu S. Hepatitis B virus infection and decreased risk of nonalcoholic fatty liver disease: a cohort study. Hepatology. 2017;65:828–835
13. Shi JP, Fan JG, Wu R, Gao XQ, Zhang L, Wang H, et al. Prevalence and risk factors of hepatic steatosis and its impact on liver injury in Chinese patients with chronic hepatitis B infection. J Gastroenterol Hepatol. 2008;23:1419–1425
14. Mak LY, Hui RW, Fung J, Liu F, Wong DK, Li B, et al. Reduced hepatic steatosis is associated with higher risk of hepatocellular carcinoma in chronic hepatitis B infection. Hepatol Int. 2021;15:901–911
15. Oh JH, Lee HW, Sinn DH, Park JY, Kim BK, Kim SU, et al. Controlled attenuation parameter value and the risk of hepatocellular carcinoma in chronic hepatitis B patients under antiviral therapy. Hepatol Int. 2021;15:892–900
16. Zheng Q, Zou B, Wu Y, Yeo Y, Wu H, Stave CD, et al. Systematic review with meta-analysis: prevalence of hepatic steatosis, fibrosis and associated factors in chronic hepatitis B. Aliment Pharmacol Ther. 2021;54:1100–1109
17. Chan AW, Wong GL, Chan HY, Tong JH, Yu YH, Choi PC, et al. Concurrent fatty liver increases risk of hepatocellular carcinoma among patients with chronic hepatitis B. J Gastroenterol Hepatol. 2017;32:667–676
18. Choi HSJ, Brouwer WP, Zanjir WMR, de Man RA, Feld JJ, Hansen BE, et al. Nonalcoholic steatohepatitis is associated with liver-related outcomes and all-cause mortality in chronic hepatitis B. Hepatology. 2020;71:539–548
19. van Kleef LA, Choi HSJ, Brouwer WP, Hansen BE, Patel K, de Man RA, et al. Metabolic dysfunction-associated fatty liver disease increases risk of adverse outcomes in patients with chronic hepatitis B. JHEP Rep. 2021;3: 100350
20. Lee YC, Chao YT, Lin PJ, Yang YY, Yang YC, Chu CC, et al. Quality assurance of integrative big data for medical research within a multihospital system. J Formos Med Assoc. 2022;121:1728–1738
21. Tchelepi H, Ralls PW, Radin R, Grant E. Sonography of diffuse liver disease. J Ultrasound Med. 2002;21:1023–1032 (**quiz 1033-1024**)
22. Sarin SK, Kumar M, Lau GK, Abbas Z, Chan HL, Chen CJ, et al. Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update. Hepatol Int. 2016;10:1–98
23. EASL. Clinical practice guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;2017(67):370–398
24. Terrault NA, Lok ASF, McMahon BJ, Chang KM, Hwang JP, Jonas MM, et al. Update on prevention, diagnosis, and treatment of chronic hepatitis B: AASLD 2018 hepatitis B guidance. Hepatology. 2018;67:1560–1599
25. Su TH, Liao CH, Liu CH, Huang KW, Tseng TC, Yang HC, et al. Acoustic radiation force impulse US imaging: liver stiffness in patients with chronic hepatitis B with and without antiviral therapy. Radiology. 2018;288:293–299
26. Bruix J, Sherman M. American association for the study of liver D: management of hepatocellular carcinoma: an update. Hepatology. 2011;53:1020–1022
27. van Buuren S, Groothuis-Oudshoorn K. Mice: multivariate imputation by chained equations in R. J Stat Softw. 2011;45:1–67
28. Austin PC. The use of propensity score methods with survival or time-to-event outcomes: reporting measures of effect similar to those used in randomized experiments. Stat Med. 2014;33:1242–1258

29. Hui RWH, Seto WK, Cheung KS, Mak LY, Liu KSH, Fung J, et al. Inverse relationship between hepatic steatosis and hepatitis B viremia: results of a large case-control study. J Viral Hepat. 2018;25:97–104
30. Wang MM, Wang GS, Shen F, Chen GY, Pan Q, Fan JG. Hepatic steatosis is highly prevalent in hepatitis B patients and negatively associated with virological factors. Dig Dis Sci. 2014;59:2571–2579
31. Yu MW, Lin CL, Liu CJ, Huang YW, Hu JT, Wu WJ, et al. Hepatic steatosis and development of type 2 diabetes: impact of chronic hepatitis B and viral specific factors. J Formos Med Assoc. 2022;121:1478–1487
32. Hu D, Wang H, Wang H, Wang Y, Wan X, Yan W, et al. Non-alcoholic hepatic steatosis attenuates hepatitis B virus replication in an HBV-immunocompetent mouse model. Hepatol Int. 2018;12:438–446
33. Liu Q, Mu M, Chen H, Zhang G, Yang Y, Chu J, et al. Hepatocyte steatosis inhibits hepatitis B virus secretion via induction of endoplasmic reticulum stress. Mol Cell Biochem. 2022;477:2481–2491
34. Wong VW, Wong GL, Yu J, Choi PC, Chan AW, Chan HY, et al. Interaction of adipokines and hepatitis B virus on histological liver injury in the Chinese. Am J Gastroenterol. 2010;105:132–138
35. Yoon S, Jung J, Kim T, Park S, Chwae YJ, Shin HJ, et al. Adiponectin, a downstream target gene of peroxisome proliferator-activated receptor γ, controls hepatitis B virus replication. Virology. 2011;409:290–298
36. Seto WK, Hui RWH, Mak LY, Fung J, Cheung KS, Liu KSH, et al. Association between hepatic steatosis, measured by controlled attenuation parameter, and fibrosis burden in chronic hepatitis B. Clin Gastroenterol Hepatol. 2018;16:575-583.e572
37. Mak LY, Hui RW, Fung J, Liu F, Wong DK, Cheung KS, et al. Diverse effects of hepatic steatosis on fibrosis progression and functional cure in virologically quiescent chronic hepatitis B. J Hepatol. 2020;73:800–806
38. Huang SC, Liu CJ. Chronic hepatitis B with concurrent metabolic dysfunction-associated fatty liver disease: challenges and perspectives. Clin Mol Hepatol. 2023;29(2):320–331
39. van der Poorten D, Samer CF, Ramezani-Moghadam M, Coulter S, Kacevska M, Schrijnders D, et al. Hepatic fat loss in advanced nonalcoholic steatohepatitis: are alterations in serum adiponectin the cause? Hepatology. 2013;57:2180–2188
40. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, et al. Risk of hepatocellular cancer in patients with non-alcoholic fatty liver disease. Gastroenterology. 2018;155:1828-1837.e1822
41. Yu MW, Shih WL, Lin CL, Liu CJ, Jian JW, Tsai KS, et al. Body-mass index and progression of hepatitis B: a population-based cohort study in men. J Clin Oncol. 2008;26:5576–5582
42. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. Gut. 2005;54:533–539
43. Chen CL, Yang HI, Yang WS, Liu CJ, Chen PJ, You SL, et al. Metabolic factors and risk of hepatocellular carcinoma by chronic hepatitis B/C infection: a follow-up study in Taiwan. Gastroenterology. 2008;135:111–121
44. Hsueh RC, Wu WJ, Lin CL, Liu CJ, Huang YW, Hu JT, et al. Impact of PNPLA3 p.I148M and hepatic steatosis on long-term outcomes for hepatocellular carcinoma and HBsAg seroclearance in chronic hepatitis B. J Hepatocell Carcinoma. 2022;9:301–313
45. Li J, Yang HI, Yeh ML, Le MH, Le AK, Yeo YH, et al. Association between fatty liver and cirrhosis, hepatocellular carcinoma, and hepatitis B surface antigen seroclearance in chronic hepatitis B. J Infect Dis. 2021;224:294–302
46. Minuk GY, Iliant V, Zhou N, Kaita KD, Wong SG, Peretz D, et al. Concomitant nonalcoholic fatty liver disease does not alter the activity, severity or course of primary biliary cholangitis. Liver Int. 2018;38:1110–1116
47. De Luca-Johnson J, Wangensteen KJ, Hanson J, Krawitt E, Wilcox R. Natural history of patients presenting with autoimmune hepatitis and coincident nonalcoholic fatty liver disease. Dig Dis Sci. 2016;61:2710–2720

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