Am J Cancer Res 2024;14(4):1914-1925
www.ajcr.us /ISSN:2156-6976/ajcr0156487

Original Article

Effect of metabolic dysfunction on the risk of liver-related events in patients cured of hepatitis C virus

Wei-Fan Hsu[1,2,3], Hsueh-Chou Lai[1,3], Sheng-Hung Chen[1,4], Wen-Pang Su[1], Hung-Wei Wang[1,4], Hung-Yao Chen[1], Guan-Tarn Huang[1,4], Cheng-Yuan Peng[1,4]

[1]Center for Digestive Medicine, Department of Internal Medicine, China Medical University Hospital, Taichung, Taiwan; [2]Graduate Institute of Biomedical Sciences, China Medical University, Taichung, Taiwan; [3]School of Chinese Medicine, China Medical University, Taichung, Taiwan; [4]School of Medicine, China Medical University, Taichung, Taiwan

Received March 11, 2024; Accepted April 15, 2024; Epub April 15, 2024; Published April 30, 2024

Abstract: The impact of metabolic dysfunction or metabolic dysfunction-associated fatty liver disease (MAFLD) on liver-related events (LREs) in patients with chronic hepatitis C (CHC) who had achieved a sustained virologic response (SVR) to direct-acting antiviral agents (DAAs) is unknown. A total of 924 patients with cured CHC and documented body mass index (BMI) were included in the analysis, and the data period was from September 2012 to April 2022. Hepatic steatosis was identified either through ultrasonography or blood biomarkers. Metabolic dysfunction was defined as the presence of overweight or obesity (BMI ≥ 23 kg/m^2), type 2 diabetes mellitus (DM), and metabolic dysregulation. Patients may have more than one metabolic dysfunction. Variables at 12 or 24 weeks after DAA therapy (PW12) were used to identify predictors of LREs. The median age of the 924 patients was 58 (49-65) years. Of the participants, 418 (45.2%) were male. The median BMI was 24.01 (21.78-26.73) kg/m^2, and 174 (18.8%) patients had DM. A multivariable Cox regression analysis revealed that age, male, albumin, total bilirubin, alpha-fetoprotein (AFP), metabolic dysfunction (hazard ratio: 1.709, 95% confidence interval: 1.128-2.591, P = .011), and FIB-4 > 3.25 were independent predictors of LREs. Type 2 DM and metabolic dysregulation exhibited a larger time-dependent area under the receiver operating characteristic curve for LREs than did overweight or obesity. Moreover, metabolic dysfunction was identified to be an independent predictor of hepatocellular carcinoma. Metabolic dysfunction increased the risk of LREs and HCC in patients with CHC who had achieved an SVR to DAA therapy.

Keywords: Chronic hepatitis C, direct-acting antiviral agent, hepatocellular carcinoma, liver-related event, metabolic dysfunction-associated fatty liver disease

Introduction

Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of liver conditions that ranges from steatosis to nonalcoholic steatohepatitis with or without liver fibrosis [1]. The prevalence of NAFLD ranges from 11.4% to 44.5% in Taiwan [2] and from 20% to 27% in China and Hong Kong [3]. NAFLD is becoming an increasingly common indication for liver transplantation in the United States [4], with a similar increasing trend also noted in Europe [5]. To diagnose NAFLD, other etiologies of chronic liver diseases, such as alcohol consumption, viral hepatitis, use of steroids, and metabolic liver diseases, should be excluded [6]. Recently, an international consensus panel proposed a new disease name, metabolic dysfunction-associated fatty liver disease (MAFLD), which reflects the underlying pathophysiological link between metabolic dysfunction and hepatic steatosis [7]. This new naming consensus has allowed clinicians to investigate the interactions between metabolic dysfunction and other etiologies of chronic liver disease.

The hepatitis C virus (HCV) relies on host lipid metabolism for replication during every stage of its life cycle [8]. The HCV can cause hepatic steatosis, especially in patients infected with the HCV genotype 3 [9]. The HCV also disrupts glucose hemostasis through several direct and indirect mechanisms, resulting in hepatic and extrahepatic insulin resistance (IR) [9]. Diabetes

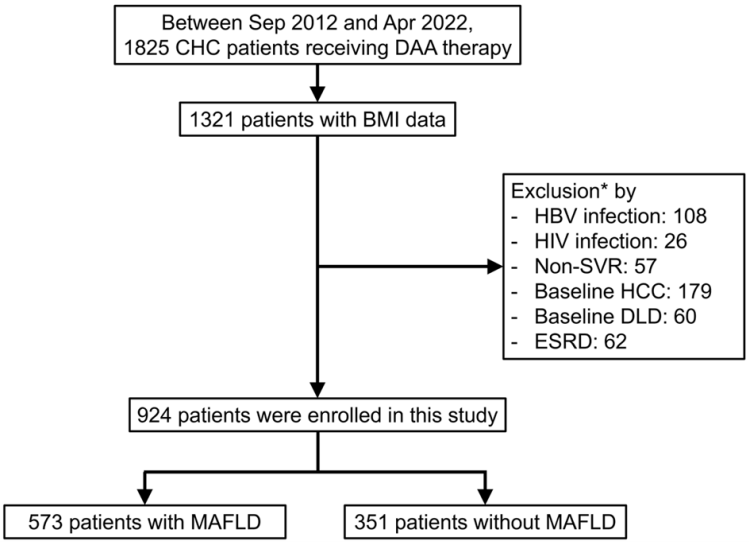


Figure 1. Flowchart of patient recruitment. BMI, body mass index; DLD, decompensated liver disease; ESRD, end-stage renal disease; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus; MAFLD, metabolic dysfunction-associated fatty liver disease; SVR, sustained virologic response.

mellitus (DM) is an extrahepatic manifestation in patients with chronic hepatitis C (CHC) [10]. Patients with CHC and DM have a higher risk of advanced liver fibrosis and liver-related events (LREs) compared with those without DM [11, 12]. Thus, HCV and DM have a complex bidirectional relationship [13].

Given the newly proposed term of MAFLD, the impact of MAFLD or metabolic dysfunction on LREs in patients with CHC who have achieved sustained virologic response (SVR) to direct-acting antiviral agents (DAAs) remains to be clarified. Accordingly, in this retrospective study, we analyzed the predictors of LREs, with a specific focus on MAFLD or metabolic dysfunction, in patients with CHC who have achieved a SVR after DAA therapy.

Patients and methods

Patients

The study included 1825 consecutive adult patients (age ≥ 18 years) with CHC who had completed DAA therapy at China Medical University Hospital between September 2012 and April 2022. CHC was defined as the presence of serum anti-HCV antibodies for > 6 months and detectable HCV RNA (COBAS Ampliprep and COBAS TaqMan HCV test, NJ, USA). We excluded patients with an unknown body mass index (BMI, *n* = 504), with hepatitis B virus coinfection (defined as positive serum hepatitis B surface antigen, HBsAg, *n* = 108), with human immunodeficiency virus coinfection (*n* = 26), without an SVR (*n* = 57), with hepatocellular carcinoma (HCC) before 12 or 24 weeks after DAA therapy (PW12, *n* = 179), with decompensated liver disease (DLD) before PW12 (*n* = 60), and with end-stage renal disease (*n* = 62). Male and female patients who drank > 30 and 20 grams of alcohol daily, respectively, had been excluded. Some patients met more than one exclusion criterion. Ultimately, 924 patients were eligible for inclusion, and 573 and 351 of these patients were with and without MAFLD, respectively (**Figure 1**).

Comorbidities were recorded at baseline, and BMI and hematologic, biochemical, and virological data were collected at baseline and at PW12. The use of metformin or statin at baseline was defined as the drug use for more than 3 months.

The study, in accordance with ethical guidelines, was approved by the Research Ethics Committee of China Medical University Hospital, Taichung, Taiwan (CMUH107-REC1-057), and was conducted in accordance with the principles outlined in the 1975 Declaration of Helsinki. Patients' identification numbers were encrypted to ensure privacy protection, and the need for informed consent was waived.

Laboratory and imaging tests

HCV genotyping was performed using the Abbott RealTime HCV Genotype II assay (Abbott Molecular, Abbott Park, IL, USA). Hematologic (Sysmex HST series, Kanagawa, Japan) and biochemical analyses (Beckman Coulter, Brea, CA, USA) were performed in the hospital's central laboratory. Liver cirrhosis (LC) was diagnosed based on unequivocal histological, clinical, and ultrasonographic data.

Further, the fibrosis-4 score (FIB-4) was computed using the following formula [14]:

$$\text{FIB-4} = \frac{\text{Age (years)} \times \text{aspartate aminotransferase (AST) (U/L)}}{\text{Platelet count } (10^9/\text{L}) \times \sqrt{\text{alanine aminotransferase (ALT) (U/L)}}}$$

We used the cutoff of > 3.25 to define patients with advanced liver fibrosis [14].

Definition of hepatic steatosis

Hepatic steatosis was identified either through ultrasonography or blood biomarkers [15]. The diagnosis of hepatic steatosis through ultrasonography was made based on at least one of the following criteria established by the guidelines of the Asian Pacific Association for the Study of the Liver: a) higher echogenicity of the liver compared with the kidney or spleen, b) blurred vascularity, or c) signal attenuation in deeper parts of the liver [16]. The blood biomarker used to diagnose hepatic steatosis was hepatic steatosis index (HSI), which is given as follows [17]:

$$\text{HSI} = 8 \times \frac{\text{ALT}}{\text{AST}} + \text{BMI (+ 2 if type 2 DM; + 2 if female)}$$

Patients with a HSI of > 36.0 had a specificity of 92.4%-93.1% for hepatic steatosis in the original report [17]. The HSI was calculated at PW12.

Definition of metabolic dysfunction

The diagnosis of MAFLD was based on the aforementioned international expert consensus statement: hepatic steatosis and at least one of three "positive" criteria were required for diagnosis [15]. Therefore, we defined metabolic dysfunction according to three criteria: (1) overweight or obesity (BMI ≥ 23 kg/m^2); (2) type 2 DM; and (3) evidence of metabolic dysregulation. We modified the definition of metabolic dysregulation as the presence of at least two of the following metabolic risk abnormalities: a) systemic blood pressure (BP) of ≥ 130 mmHg, diastolic BP of ≥ 85 mmHg, or specific drug treatment; b) plasma triglyceride (TG) ≥ 150 mg/dL or specific drug treatment; c) plasma high-density lipoprotein (HDL) concentration of < 40 mg/dL for men or < 50 mg/dL for women, or specific drug treatment; d) prediabetes (i.e., fasting glucose levels = 100-125 mg/dL or 2-hour post-load glucose levels = 140-199 mg/dL or glycated hemoglobin = 5.7%-6.4%); and e) a homeostasis model assessment of IR score ≥ 2.5. Because of the retrospective nature of this study, we did not include the abnormalities of waist circumference and plasma high-sensitivity C-reactive protein [15]. Patients might have more than one metabolic dysfunction.

Definition of LREs

LREs, including DLD and HCC, has been defined previously [18]. In brief, DLDs included ascites, high-risk esophageal or bleeding gastric varices, hepatic encephalopathy, and hepatorenal syndrome [18]. The diagnosis of HCC was based on pathology findings or typical imaging presentations [19, 20]. The follow-up duration for each patient was calculated from the completion of DAA therapy. Data collection for a patient was stopped when any of the following occurred: the first LRE, death, loss to follow-up, or the end of follow-up (March 31, 2022).

Statistical analyses

Continuous variables are presented as medians (first to third quartile), and categorical variables are presented as frequencies (percentages). Between-group comparisons of variables were performed using the Mann-Whitney *U* test. Variables with a *P* value of < 0.20 in the univariate analysis were included in a multivariate Cox regression analysis following the conventional approach proposed previously [21]. The predictive performance of each metabolic dysfunction for 1-, 2-, and 3-year LREs was examined through a time-dependent area under the receiver operating characteristic curve (AUROC) analysis by using the DeLong test. Youden's index was used to identify the optimal alpha-fetoprotein (AFP) level cutoff for predicting LREs. A Kaplan-Meier analysis with a log-rank test was used to compare the LREs or HCC among patient subgroups. All statistical analyses were performed using SPSS (version 25.0, IBM, Armonk, NY, USA). A two-sided *P* value of < .05 was considered statistically significant.

Results

Baseline characteristics

A total of 924 patients were included in the analysis. Among the patients, 418 (45.2%)

Table 1. Demographics and baseline characteristics of patients with and without MAFLD

Variable	Total (n = 924)	MAFLD (n = 573)	Non-MAFLD (n = 351)	*P* value
Age (years)	58 (49-65)	59 (50-66)	56 (47-64)	.006
Sex (male, %)	418 (45.2)	261 (45.5)	157 (44.7)	.808
Body mass index (kg/m^2)	24.01 (21.78-26.73)	25.68 (23.50-28.42)	21.69 (20.11-23.13)	< .001
Overweight/obesity	571 (61.8)	489 (85.3)	82 (23.4)	< .001
Follow-up months	34.09 (17.31-50.20)	33.67 (17.05-50.77)	34.70 (17.50-50.03)	.900
Hemoglobin (g/dL)	14.1 (13.1-15.1)	14.1 (13.3-15.1)	13.9 (13.0-15.0)	.025
Platelet count (× 10^9/L)	171 (124-218)	170 (123-216)	172 (127-219)	.838
AST (U/L)	46 (31-80)	48 (32-84)	43 (30-73)	.086
ALT (U/L)	56 (34-102)	57 (36-105)	52 (32-95)	.075
Total bilirubin (mg/dL)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	.283
Albumin (g/dL)	4.3 (4.1-4.6)	4.3 (4.1-4.5)	4.4 (4.1-4.6)	.001
INR	1.02 (0.99-1.08)	1.02 (1.00-1.08)	1.02 (0.99-1.08)	.419
Triglyceride (mg/dL)	87 (65-119)	94 (72-133)	76 (55-99)	< .001
Total cholesterol (mg/dL)	169 (146-191)	169 (145-189)	171 (147-192)	.238
HDL (mg/dL)	46.6 (37.5-57.9)	43.9 (36.1-53.3)	53.4 (42.4-64.6)	< .001
LDL (mg/dL)	95.6 (76.3-117.0)	95.6 (77.4-117.0)	95.3 (75.4-117.4)	.964
AFP (ng/mL)	4.27 (2.78-9.19)	4.44 (2.87-9.39)	3.96 (2.69-8.19)	.124
Creatinine (mg/dL)	0.77 (0.64-0.93)	0.79 (0.65-0.95)	0.75 (0.63-0.91)	.097
Hypertension, *n* (%)	262 (28.4)	200 (34.9)	62 (17.7)	< .001
Diabetes mellitus, *n* (%)	189 (20.5)	153 (26.7)	36 (10.3)	< .001
Hepatic steatosis, *n* (%)	689 (74.6)	573 (100.0)	116 (33.0)	< .001
Liver cirrhosis, *n* (%)	156 (16.9)	108 (18.8)	48 (13.7)	.045
HCV RNA (log_{10} IU/mL)	6.64 (5.97-7.12)	6.68 (5.97-7.13)	6.57 (5.97-7.11)	.530
HCV genotype, *n* (%)				.092
1	562 (60.8)	358 (62.5)	204 (58.1)	
2	259 (28.0)	159 (27.7)	100 (28.5)	
3	15 (1.6)	8 (1.4)	7 (2.0)	
6	83 (9.0)	46 (8.0)	37 (10.5)	
Mixed genotype*	4 (0.4)	1 (0.2)	3 (0.9)	
FIB-4	2.13 (1.28-3.87)	2.17 (1.35-3.97)	2.08 (1.17-3.65)	.197

Data are presented as medians (interquartile ranges). *Three patients and one patient had genotype 1b+2 and 2+6 infection, respectively. One patient had an unclassified genotype. AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HDL, high-density lipoprotein; INR, international normalized ratio; LDL, low-density lipoprotein.

patients were men, and the median age and median BMI were 58 (49-65) years and 24.01 (21.78-26.73) kg/m^2, respectively. Of the investigated patients, 262 (28.4%), 189 (20.5%), and 156 (16.9%) were diagnosed as having hypertension, DM, and LC, respectively, and 689 (74.6%) patients were identified to have with hepatic steatosis (either by ultrasonography or blood biomarkers). The baseline AST, ALT, TG, total cholesterol, HDL, and low-density lipoprotein (LDL) levels were 46 (31-80) U/L, 56 (34-102) U/L, 87 (65-119) mg/dL, 169 (146-191) mg/dL, 46.6 (37.5-57.9) mg/dL, and 95.6 (76.3-117.0) mg/dL, respectively. The median AFP was 4.27 (2.78-9.19) ng/mL. The median HCV RNA level was 6.64 (5.97-7.12) log_{10} IU/mL. In total, 562 (60.8%), 259 (28.0%), 15 (1.6%), and 83 (9.0%) patients had HCV genotypes 1, 2, 3, and 6, respectively, and 4 (0.4%) patients had mixed infections. The regimens used in the study and their treatment durations are listed in Supplementary Table 1. The median FIB-4 value was 2.13 (1.28-3.87; **Table 1**). Moreover, among the study population, 573 had MAFLD and 351 did not. Patients with MAFLD were older; had higher BMI; had higher

Table 2. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with liver-related events in all patients

Variable	Univariate analysis		Multivariable analysis 1		Multivariable analysis 2	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.049 (1.023-1.076)	< .001	1.046 (1.014-1.080)	.005	1.046 (1.013-1.079)	.006
Sex: male vs. female	1.532 (0.888-2.642)	.125	2.459 (1.336-4.524)	.004	2.316 (1.252-4.283)	.007
HCV RNA (log_{10} IU/mL)	0.901 (0.664-1.222)	.502				
Variable at 12 or 24 weeks after antiviral therapy						
ALT (U/L)	1.008 (0.997-1.018)	.159	0.999 (0.982-1.017)	.948	0.993 (0.974-1.013)	.516
Albumin (g/dL)	0.247 (0.176-0.346)	< .001	0.368 (0.215-0.627)	< .001	0.335 (0.197-0.569)	< .001
Total bilirubin (mg/dL)	1.720 (1.490-1.986)	< .001	1.333 (1.089-1.632)	.005	1.338 (1.097-1.631)	.004
AFP (ng/mL)	1.038 (1.025-1.052)	< .001	1.048 (1.033-1.063)	< .001	1.046 (1.031-1.061)	< .001
MAFLD	1.652 (0.895-3.048)	.109	1.432 (0.726-2.825)	.300	NA	
Metabolic dysfunction	1.666 (1.246-2.227)	.001	NA		1.573 (1.137-2.176)	.006
Hepatic steatosis	1.417 (0.711-2.824)	.322	NA			
FIB-4 > 3.25	1.078 (1.048-1.109)	< .001	3.545 (1.739-7.226)	< .001	3.566 (1.763-7.211)	< .001

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus; MAFLD, metabolic dysfunction-associated fatty liver disease; NA, not assessed.

hemoglobin and TG levels; had lower albumin and HDL levels; and had higher risks of overweight or obesity, hypertension, DM, hepatic steatosis, and LC than those without MAFLD (**Table 1**).

Metabolic dysfunction and FIB-4 instead of hepatic steatosis increased the risk of LREs

Our previous study demonstrated that noninvasive indices at PW12 had higher time-dependent AUROC values than did those at baseline for predicting LREs [18], and the presence of hepatic necroinflammation at baseline may affect the HSI. Therefore, we investigated the predictors of LREs or HCC at PW12. Of 924 patients, 52 patients experienced LREs during a median follow-up of 34.09 (17.31-50.20) months. The results of the univariate Cox regression analysis revealed that age, albumin, total bilirubin, AFP, FIB-4 at PW12, and metabolic dysfunction were significantly associated with LREs. Because of the collinearity between MAFLD and its components (metabolic dysfunction and hepatic steatosis), we analyzed MAFLD and its components in separate multivariable analyses. The results of the first multivariable Cox regression analysis indicated that age (hazard ratio [HR]: 1.046, 95% confidence interval [CI]: 1.014-1.080), male (HR: 2.459, 95% CI: 1.336-4.524), albumin (HR: 0.368, 95% CI: 0.215-0.627), total bilirubin (HR: 1.333, 95% CI: 1.089-1.632), AFP (HR: 1.048, 95% CI: 1.033-1.063), and FIB-4 (> 3.25, HR: 3.545, 95% CI: 1.739-7.226) were independent predictors of LREs. We used metabolic dysfunction instead of MAFLD in the second multivariable Cox regression analysis, which showed metabolic dysfunction (HR: 1.573, 95% CI: 1.137-2.176) was an independent predictor of LREs. FIB-4 > 3.25 was an independent factor of LREs in the first and second multivariable Cox regression analyses (**Table 2**). Despite the *P* value for hepatic steatosis was > 0.20 in the univariate analysis, we included hepatic steatosis in another Cox regression analysis to determine whether hepatic steatosis increased the risk of LREs. Hepatic steatosis was not identified to be a predictor of LREs in the univariate or multivariable analysis (Supplementary Table 2).

A time-dependent AUROC was used to assess the predictive performance of each type of metabolic dysfunction. The AUROCs of metabolic dysregulation for 1-, 2-, and 3-year LREs were 0.648 (95% CI: 0.613-0.682; Supplementary Figure 1A), 0.629 (95% CI: 0.590-0.667; Supplementary Figure 1B), and 0.619 (95% CI: 0.570-0.666; Supplementary Figure 1C), respectively, and the AUROC for 3-year LREs was significantly higher than those for overweight or obesity (0.515, 95% CI: 0.466-0.564; Supplementary Figure 1C). The AUROCs of type 2 DM for 1-, 2-, and 3-year LREs were 0.662 (95% CI: 0.628-0.696; Supplementary

Table 3. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with hepatocellular carcinoma in all patients

Variable	Univariate analysis		Multivariable analysis 1		Multivariable analysis 2	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.052 (1.019-1.085)	.002	1.040 (1.001-1.080)	.042	1.039 (1.001-1.079)	.046
Sex: male vs. female	1.365 (0.703-2.652)	.358				
HCV RNA (log_{10} IU/mL)	1.323 (0.853-2.051)	.211				
Variable at 12 or 24 weeks after antiviral therapy						
ALT (U/L)	1.009 (0.998-1.021)	.115	1.001 (0.983-1.020)	.877	0.996 (0.974-1.018)	.717
Albumin (g/dL)	0.456 (0.226-0.920)	.028	0.940 (0.382-2.314)	.892	0.888 (0.364-2.167)	.794
Total bilirubin (mg/dL)	1.500 (1.162-1.936)	.002	1.366 (0.966-1.932)	.078	1.367 (0.971-1.926)	.074
AFP (ng/mL)	1.048 (1.032-1.065)	< .001	1.057 (1.039-1.076)	< .001	1.056 (1.036-1.075)	< .001
MAFLD	1.509 (0.725-3.142)	.272	1.720 (0.757-3.906)	.195	NA	
Metabolic dysfunction	1.727 (1.210-2.464)	.003	NA		1.667 (1.121-2.477)	.012
Hepatic steatosis	1.125 (0.511-2.478)	.770	NA			
FIB-4 > 3.25	1.069 (1.027-1.112)	.001	3.469 (1.491-8.069)	.004	3.476 (1.509-8.006)	.003

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus; MAFLD, metabolic dysfunction-associated fatty liver disease; NA, not assessed.

Figure 1A), 0.629 (95% CI: 0.589-0.667; Supplementary Figure 1B), and 0.583 (95% CI: 0.534-0.630; Supplementary Figure 1C), respectively. No significant differences in the AUROC values were noted between type 2 DM and metabolic dysregulation.

Metformin has been shown to reduce HCC incidence after successful antiviral therapy in patients with CHC and DM [22, 23]. Moreover, atorvastatin and fluvastatin have been revealed to reduce the incidence of HCC in patients with CHC [24]. We therefore investigated the impact of metformin and statin therapy on LREs. Metformin and statin therapy were not predictors of LREs in the multivariable Cox regression analysis (Supplementary Table 3).

Metabolic dysfunction increases the risk of HCC

Of the 924 patients in this study, 35 developed incident HCC. The results of the univariate Cox regression analysis revealed that age, albumin, total bilirubin, AFP, FIB-4 at PW12, and metabolic dysfunction were significantly associated with the development of HCC. The results of the first multivariable Cox regression analysis indicated that age (HR: 1.040, 95% CI: 1.001-1.080), AFP (HR: 1.057, 95% CI: 1.039-1.076), and FIB-4 (> 3.25, HR: 3.469, 95% CI: 1.491-8.069) were independent predictors of HCCs. In the second multivariable Cox regression analysis using metabolic dysfunction instead of MAFLD, metabolic dysfunction (HR: 1.667, 95% CI: 1.121-2.477) was an independent predictor of HCCs. FIB-4 > 3.25 was an independent factor of HCC in the first and second multivariable Cox regression analyses (**Table 3**). Hepatic steatosis was included in another multivariable Cox regression analysis but was not identified as a predictor of HCC (Supplementary Table 4).

Metformin and statin therapy were not predictors of HCC in another multivariable Cox regression analysis (Supplementary Table 5).

Kaplan-Meier analysis

The impact of the number of metabolic dysfunctions on the risk of LREs and HCC was investigated. Because of the small number of patients with 0, 1, 2, or 3 metabolic dysfunctions (*n* = 204, 284, 317, and 119, respectively), the patients were categorized into two groups: patients with no metabolic dysfunction or 1 metabolic dysfunction and those with 2 or 3 metabolic dysfunctions. The results of the Kaplan-Meier analysis revealed that patients with 2 or 3 metabolic dysfunctions had a lower LRE-free survival probability (**Figure 2A**) and HCC-free survival probability (**Figure 3A**). An AFP level of 4.8 ng/mL at PW12 was identified as the optimal cutoff for predicting LREs by using Youden's index. Furthermore, the proba-

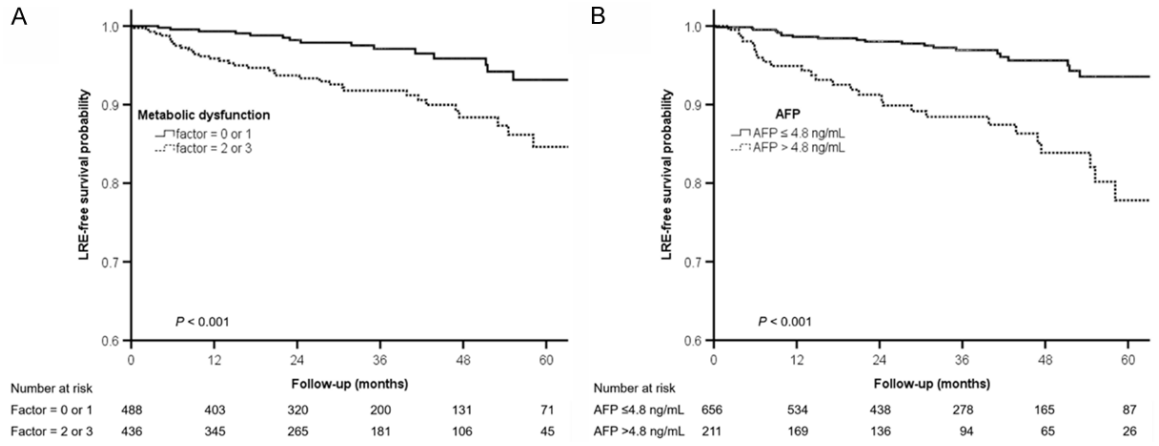


Figure 2. Kaplan-Meier analyses of LREs in patients with chronic hepatitis C. A. Patients with 0 or 1 and with 2 or 3 metabolic dysfunctions. B. Patients with an AFP of ≤ 4.8 and > 4.8 ng/mL. AFP, alpha-fetoprotein; LRE, liver-related event.

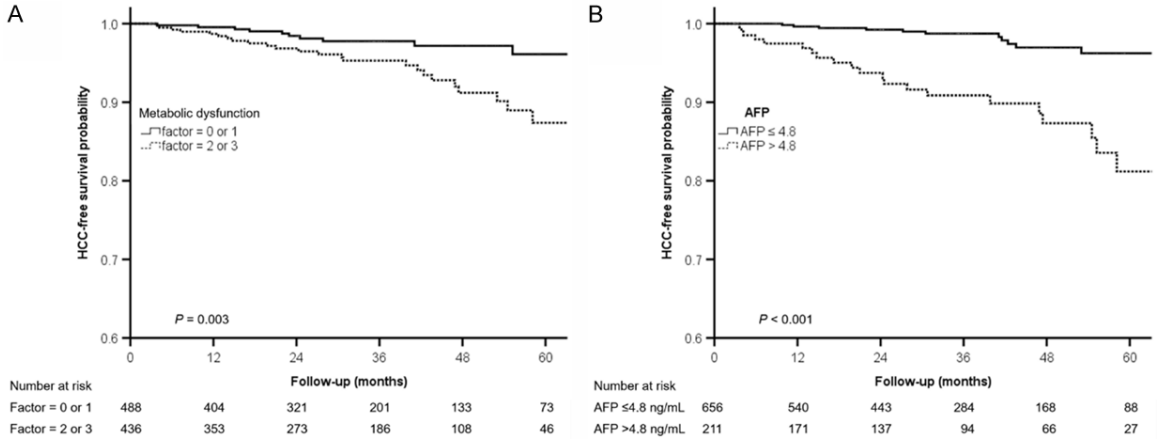


Figure 3. Kaplan-Meier analyses of HCC in patients with chronic hepatitis C. A. Patients with no metabolic dysfunction or 1 metabolic dysfunction and patients with 2 or 3 metabolic dysfunctions. B. Patients with an AFP of ≤ 4.8 and > 4.8 ng/mL. AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.

bility of LRE-free and HCC-free survival differed significantly between patients with and without an AFP level of > 4.8 ng/mL at PW12 (**Figures 2B** and **3B**).

Discussion

Metabolic dysfunction, rather than simple steatosis, increased the risk of LREs and HCC in patients with CHC who have attained an SVR to DAA therapy. Among the three metabolic dysfunctions studied, type 2 DM and metabolic dysregulation had higher predictive values for adverse outcomes than overweight or obesity.

Although the time-dependent AUROC values of metabolic dysregulation (0.648, 0.629, and 0.619 for 1-, 2-, and 3-year LREs) were higher than those of type 2 DM (0.662, 0.629, and 0.583 for 1-, 2-, and 3-year LREs), the differences between them were not significant. Therefore, further studies with a longer follow-up duration and larger number of patients are required to determine which metabolic dysfunction is a more accurate predictor of LREs and HCC.

Kurosaki et al. demonstrated that biopsy-proven hepatic steatosis (≥ 10%) is an independent risk factor for HCC in patients with CHC receiving interferon-based therapy [25]. However, their study included patients both with and without an SVR to interferon-based therapy

[25], and for the diagnosis of hepatic steatosis, the cutoff for TG accumulation in the liver parenchyma was > 5% [26]. Peleg et al. conducted a study on 515 patients with CHC and SVR to DAA therapy and revealed that ultrasonographic steatosis was associated with a higher cumulative rate of all-cause mortality and HCC than those without ultrasonographic steatosis. Furthermore, they demonstrated that advanced liver fibrosis, as defined by liver stiffness measurement ≥ 9.6 kPa obtained using Fibroscan (Echosens), was a predictor of poor outcomes (HR: 1.96) and that hepatic steatosis (HR: 9.21) combined with advanced liver fibrosis had a synergistic effect on patient outcomes (HR for both steatosis and advanced fibrosis: 17.56) [27]. Our study showed that metabolic dysfunction and FIB-4 > 3.25 instead of hepatic steatosis predicted LREs and HCC.

The replication of HCV relies on host lipid metabolism for several stages of its life cycle, including entry, replication, and assembly [8]. The HCV might directly cause lipid accumulation in hepatocytes [9, 28, 29]. HCV-induced steatosis occurs through two mechanisms: viral factors and hosts' metabolic dysfunction [30]. The degree of hepatic steatosis is highly associated with viral replication and protein expression in patients with the HCV genotype 3 infection [28, 29]. The HCV core protein and NS5A inhibit microsomal triglyceride transfer protein (MTP), leading to very-low-density lipoprotein release and the accumulation of TG in hepatocytes [31]. HCV infection also activates the sterol regulatory element-binding-protein 1c (SRBEP1c) signaling pathway and produces fatty acids and TG, thus leading to hepatic IR [32, 33] and inhibiting peroxisome proliferator-activated receptor alpha (PPARα) [34]. Conversely, in patients with HCV non-genotype 3 infection, metabolic dysfunction is the major contributor to hepatic steatosis [35], and hepatic steatosis is highly associated with hepatic and systemic IR [33]. In our study, only 15 (1.6%) patients were infected with the HCV genotype 3, and metabolic dysfunction was present in 77.9% (n = 720) of our patient cohort.

Studies have demonstrated that patients with CHC have a higher prevalence of hepatic steatosis and dyslipidemia than healthy individuals and patients with chronic hepatitis B [9, 36]. Hepatic steatosis has been implicated in hepatocellular injury, including liver fibrosis [37] and necroinflammation, in patients with CHC [38]. Afsari et al. revealed that patients with any degree of hepatic steatosis, as determined using the Brunt scale, had a 1.6 times higher odds ratio of advanced liver fibrosis or cirrhosis than those without hepatic steatosis [37]. HCV core protein exacerbated hepatic steatosis by activating SRBEP1c, conferring hepatic IR, and inhibiting PPARα. These mechanisms, in combination with other factors, resulted in hepatic oxidative stress and necroinflammation [38].

The successful eradication of HCV through DAA therapy was shown to reduce hepatic steatosis, defined by the controlled attenuation parameter (CAP), in patients with baseline CAP > 220 dB/m [39]. HCV eradication through DAA therapy can also lead to an increase in serum TG and LDL levels [39, 40], which could be partially explained by HCV-induced MTP inhibition [31]. Recent studies have observed decreased IR and improved glycemic control following HCV eradication with DAA therapy in patients with or without DM [41]. Despite the short-term improvement in IR in response to HCV eradication, the impact of systemic metabolic dysfunction on LREs in patients with CHC after successful viral eradication is unknown. The present study demonstrated that metabolic dysfunction, rather than simple steatosis alone, was the main factor that influenced the progression of hepatic fibrosis and hepatocarcinogenesis after an SVR. Based on the study findings, we suggest that metabolic dysfunction rather than hepatic steatosis should be considered a more suitable predictor for LREs and HCC in patients with CHC who have achieved an SVR to DAA therapy. The definition of hepatic steatosis used in this study differs from that of others [25, 27]. Therefore, additional studies involving a larger sample size are required to confirm these findings.

Noninvasive indices, such as FIB-4 score and the AST to platelet ratio index measured at the time of an SVR, were able to minimize the impact of HCV-induced hepatic necroinflammation [42]. Our previous findings indicate that noninvasive indices at PW12 had higher time-dependent AUROC values than those at baseline [18]. Therefore, we used variables at PW12 to investigate the predictors of LREs and HCC. In the present study, we demonstrated that

metabolic dysfunction and FIB-4 > 3.25 were predictors of LREs and HCC. The patients with 2 or 3 metabolic dysfunctions had a higher median FIB-4 score at PW12 than those with no metabolic dysfunctions or 1 metabolic dysfunction [1.83 (1.21-2.79) vs. 1.15 (1.04-2.63), P = .029, Supplementary Table 6]. Thus, patients with a larger number of metabolic dysfunctions at the time of SVR had more severe hepatic fibrosis and a higher risk of further disease progression despite achieving an SVR.

The T-COACH conducted in Taiwan, which involved a large-scale multicenter cohort, revealed that metformin reduced HCC risk after successful viral eradication in patients with DM and CHC [23]. Similarly, an analysis of the Electronically Retrieved Cohort of HCV Infected Veterans database revealed that atorvastatin and fluvastatin reduced the incidence of HCC among patients with HCV infection [24]. Because of the limited number of enrolled patients in this study, we could not verify whether metformin and statin mitigate the risk of LREs and HCC. Patients who used metformin appeared to have a higher risk of LREs (Supplementary Table 3) and HCC (Supplementary Table 5) in the univariate Cox regression analysis. However, this result may be attributed to confounding by indication [43].

This study has several limitations that should be acknowledged. First, the sample size was relatively small, with only 924 patients included in this single-center retrospective study. Therefore, the weight of different combinations of metabolic dysfunctions (such as DM plus overweight or obesity, DM plus metabolic dysregulation, et al.) could not be assessed. Instead, the number of metabolic dysfunctions was summed to indicate the severity of metabolic abnormality. Second, patients could have been lost to follow-up after achieving an SVR, which may have affected the median follow-up duration [34.09 (17.31-50.20) months]. Third, the exclusion of patients with missing BMI data at baseline or PW12 may have affected the representation of patients with MAFLD or the estimation of HRs. Finally, this study did not determine the patterns of lipid profile, glycemic control, and IR kinetics over time following the eradication of HCV or investigate how changes in metabolic factors modulate the long-term risks of HCC and LREs.

Upon submitting the manuscript, a new fatty liver disease nomenclature, “steatotic liver disease” (SLD), was proposed, and the definition of metabolic dysfunction-associated SLD (MASLD) is different from MAFLD. The definition of MASLD contains five cardiometabolic criteria instead of overweight or obesity, type 2 DM, or metabolic dysregulation [44]. Despite this evolution, the definition of MAFLD has still been used in several guidelines [45] and clinical practices [46, 47]. Researchers also called for more flexible conduct rather than abruptly adopting only the new MASLD nomenclature [48]. Therefore, metabolic dysfunctions defined by MAFLD could still be used for risk stratification in CHC patients with SVR to DAA therapy.

In conclusion, metabolic dysfunction increased the risk of LREs and HCC in patients with CHC who had achieved an SVR to DAA therapy.

Acknowledgements

We thank Yi-Chune Guo, Hung-Yu Kuo, and Yi-Ting Lin for their assistance with data collection, and we also thank secretary help from Fatty Liver Special Interest Group of Taiwan Association for the Study of the Liver. This study was supported by a grant (DMR-113-174) from China Medical University Hospital, Taichung, Taiwan.

Disclosure of conflict of interest

Cheng-Yuan Peng has served as an advisory committee member for AbbVie, Bristol-Myers Squibb, Gilead, and Merck Sharp & Dohme.

Address correspondence to: Dr. Cheng-Yuan Peng, Center for Digestive Medicine, Department of Internal Medicine, China Medical University Hospital, No. 2, Yuh-Der Road, Taichung 40447, Taiwan. Tel: +886-4-22052121 Ext. 12260; Fax: +886-4-22071600; E-mail: 010456@tool.caaumed.org.tw

References

[1] Spengler EK and Loomba R. Recommendations for diagnosis, referral for liver biopsy, and treatment of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Mayo Clin Proc 2015; 90: 1233-1246.

[2] Huang JF, Tsai PC, Yeh ML, Huang CF, Huang CI, Hsieh MH, Dai CY, Yang JF, Chen SC, Yu ML, Chuang WL and Chang WY. Risk stratification of non-alcoholic fatty liver disease across body

mass index in a community basis. J Formos Med Assoc 2020; 119: 89-96.
[3] Farrell GC, Wong VW and Chitturi S. NAFLD in Asia–as common and important as in the West. Nat Rev Gastroenterol Hepatol 2013; 10: 307-318.
[4] Younossi ZM, Stepanova M, Ong J, Trimble G, AlQahtani S, Younossi I, Ahmed A, Racila A and Henry L. Nonalcoholic steatohepatitis is the most rapidly increasing indication for liver transplantation in the United States. Clin Gastroenterol Hepatol 2021; 19: 580-589, e585.
[5] Terrault NA and Pageaux GP. A changing landscape of liver transplantation: king HCV is dethroned, ALD and NAFLD take over! J Hepatol 2018; 69: 767-768.
[6] Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, Kleiner DE and Loomba R. AASLD practice guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 2023; 77: 1797-1835.
[7] Eslam M, Sanyal AJ and George J; International Consensus Panel. MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease. Gastroenterology 2020; 158: 1999-2014, e1.
[8] Schaefer EA and Chung RT. HCV and host lipids: an intimate connection. Semin Liver Dis 2013; 33: 358-368.
[9] Bugianesi E, Salamone F and Negro F. The interaction of metabolic factors with HCV infection: does it matter? J Hepatol 2012; 56 Suppl 1: S56-65.
[10] El-Serag HB, Hampel H, Yeh C and Rabeneck L. Extrahepatic manifestations of hepatitis C among United States male veterans. Hepatology 2002; 36: 1439-1445.
[11] Patel S, Jinjuvadia R, Patel R and Liangpunsakul S. Insulin resistance is associated with significant liver fibrosis in chronic hepatitis C patients: a systemic review and meta-analysis. J Clin Gastroenterol 2016; 50: 80-84.
[12] Yen YH, Kuo FY, Kee KM, Chang KC, Tsai MC, Hu TH, Lu SN, Wang JH, Hung CH and Chen CH. Diabetes is associated with advanced fibrosis and fibrosis progression in non-genotype 3 chronic hepatitis C patients. Dig Liver Dis 2019; 51: 142-148.
[13] Ciardullo S, Mantovani A, Ciaccio A, Carbone M, Invernizzi P and Perseghin G. Hepatitis C virus infection and diabetes: a complex bidirectional relationship. Diabetes Res Clin Pract 2022; 187: 109870.
[14] Sterling RK, Lissen E, Clumeck N, Sola R, Correa MC, Montaner J, S Sulkowski M, Torriani FJ, Dieterich DT, Thomas DL, Messinger D and Nelson M; APRICOT Clinical Investigators. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. Hepatology 2006; 43: 1317-1325.
[15] Eslam M, Newsome PN, Sarin SK, Anstee QM, Targher G, Romero-Gomez M, Zelber-Sagi S, Wai-Sun Wong V, Dufour JF, Schattenberg JM, Kawaguchi T, Arrese M, Valenti L, Shiha G, Tiribelli C, Yki-Jarvinen H, Fan JG, Gronbaek H, Yilmaz Y, Cortez-Pinto H, Oliveira CP, Bedossa P, Adams LA, Zheng MH, Fouad Y, Chan WK, Mendez-Sanchez N, Ahn SH, Castera L, Bugianesi E, Ratziu V and George J. A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement. J Hepatol 2020; 73: 202-209.
[16] Farrell GC, Chitturi S, Lau GK and Sollano JD; Asia-Pacific Working Party on NAFLD. Guidelines for the assessment and management of non-alcoholic fatty liver disease in the Asia-Pacific region: executive summary. J Gastroenterol Hepatol 2007; 22: 775-777.
[17] Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW and Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. Dig Liver Dis 2010; 42: 503-508.
[18] Hsu WF, Lai HC, Chuang PH, Su WP, Chen SH, Chen HY, Wang HW, Huang GT and Peng CY. Posttreatment nonalcoholic fatty liver disease fibrosis scores for predicting liver-related complications in patients with chronic hepatitis C receiving direct-acting antiviral agents. J Viral Hepat 2022; 29: 785-794.
[19] Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, Roberts LR and Heimbach JK. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American Association for the Study of Liver Diseases. Hepatology 2018; 68: 723-750.
[20] European Association for the Study of the Liver. Electronic address: easloffice@easloffice.eu; European Association for the Study of the Liver. EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol 2018; 69: 182-236.
[21] Maldonado G and Greenland S. Simulation study of confounder-selection strategies. Am J Epidemiol 1993; 138: 923-936.
[22] Valenti L, Pelusi S, Aghemo A, Gritti S, Pasulo L, Bianco C, Iegri C, Cologni G, Degasperi E, D'Ambrosio R, Del Poggio P, Soria A, Puoti M, Carderi I, Pigozzi MG, Carriero C, Spinetti A, Zuccaro V, Memoli M, Giorgini A, Vigano M, Rumi MG, Re T, Spinelli O, Colombo MC, Quirino T, Menzaghi B, Lorini G, Pan A, D'Arminio Monforte A, Buscarini E, Autolitano A, Bonfanti P, Terreni N, Aimo G, Mendeni M, Prati D, Lampertico P, Colombo M and Fagiuoli S; NAVIGA-

TORE-Lombardia Network. Dysmetabolism, diabetes and clinical outcomes in patients cured of chronic hepatitis C: a real-life cohort study. Hepatol Commun 2022; 6: 867-877.

[23] Tsai PC, Kuo HT, Hung CH, Tseng KC, Lai HC, Peng CY, Wang JH, Chen JJ, Lee PL, Chien RN, Yang CC, Lo GH, Kao JH, Liu CJ, Liu CH, Yan SL, Bair MJ, Lin CY, Su WW, Chu CH, Chen CJ, Tung SY, Tai CM, Lin CW, Lo CC, Cheng PN, Chiu YC, Wang CC, Cheng JS, Tsai WL, Lin HC, Huang YH, Yeh ML, Huang CF, Hsieh MH, Huang JF, Dai CY, Chung WL, Chen CY and Yu ML; T-COACH Study Group. Metformin reduces hepatocellular carcinoma incidence after successful antiviral therapy in patients with diabetes and chronic hepatitis C in Taiwan. J Hepatol 2023; 78: 281-292.

[24] Simon TG, Bonilla H, Yan P, Chung RT and Butt AA. Atorvastatin and fluvastatin are associated with dose-dependent reductions in cirrhosis and hepatocellular carcinoma, among patients with hepatitis C virus: results from ERCHIVES. Hepatology 2016; 64: 47-57.

[25] Kurosaki M, Hosokawa T, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, Tamaki N, Ueda K, Tsuchiya K, Kuzuya T, Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Enomoto N and Izumi N. Hepatic steatosis in chronic hepatitis C is a significant risk factor for developing hepatocellular carcinoma independent of age, sex, obesity, fibrosis stage and response to interferon therapy. Hepatol Res 2010; 40: 870-877.

[26] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA and Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999; 94: 2467-2474.

[27] Peleg N, Issachar A, Sneh Arbib O, Cohen-Naftaly M, Harif Y, Oxtrud E, Braun M, Leshno M, Barsheshet A and Shlomai A. Liver steatosis is a major predictor of poor outcomes in chronic hepatitis C patients with sustained virological response. J Viral Hepat 2019; 26: 1257-1265.

[28] Kumar D, Farrell GC, Fung C and George J. Hepatitis C virus genotype 3 is cytopathic to hepatocytes: reversal of hepatic steatosis after sustained therapeutic response. Hepatology 2002; 36: 1266-1272.

[29] Lonardo A, Adinolfi LE, Loria P, Carulli N, Ruggiero G and Day CP. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology 2004; 126: 586-597.

[30] Modaresi Esfeh J and Ansari-Gilani K. Steatosis and hepatitis C. Gastroenterol Rep (Oxf) 2016; 4: 24-29.

[31] Asselah T, Rubbia-Brandt L, Marcellin P and Negro F. Steatosis in chronic hepatitis C: why does it really matter? Gut 2006; 55: 123-130.

[32] Khan M, Jahan S, Khaliq S, Ijaz B, Ahmad W, Samreen B and Hassan S. Interaction of the hepatitis C virus (HCV) core with cellular genes in the development of HCV-induced steatosis. Arch Virol 2010; 155: 1735-1753.

[33] Ipsen DH, Lykkesfeldt J and Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. Cell Mol Life Sci 2018; 75: 3313-3327.

[34] de Gottardi A, Pazienza V, Pugnale P, Bruttin F, Rubbia-Brandt L, Juge-Aubry CE, Meier CA, Hadengue A and Negro F. Peroxisome proliferator-activated receptor-alpha and -gamma mRNA levels are reduced in chronic hepatitis C with steatosis and genotype 3 infection. Aliment Pharmacol Ther 2006; 23: 107-114.

[35] Vidali M, Tripodi MF, Ivaldi A, Zampino R, Occhino G, Restivo L, Sutti S, Marrone A, Ruggiero G, Albano E and Adinolfi LE. Interplay between oxidative stress and hepatic steatosis in the progression of chronic hepatitis C. J Hepatol 2008; 48: 399-406.

[36] Machado MV and Cortez-Pinto H. Insulin resistance and steatosis in chronic hepatitis C. Ann Hepatol 2009; 8 Suppl 1: S67-75.

[37] Afsari A, Lee E, Shokrani B, Boortalary T, Sherif ZA, Nouraie M, Laiyemo AO, Alkhalloufi K, Brim H and Ashktorab H. Clinical and pathological risk factors associated with liver fibrosis and steatosis in African-Americans with chronic hepatitis C. Dig Dis Sci 2017; 62: 2159-2165.

[38] Kukla M, Piotrowski D, Waluga M and Hartleb M. Insulin resistance and its consequences in chronic hepatitis C. Clin Exp Hepatol 2015; 1: 17-29.

[39] Kawagishi N, Suda G, Nakamura A, Kimura M, Maehara O, Suzuki K, Nakamura A, Ohara M, Izumi T, Umemura M, Nakai M, Sho T, Natsuizaka M, Morikawa K, Ogawa K, Kudo Y, Nishida M, Miyoshi H and Sakamoto N. Liver steatosis and dyslipidemia after HCV eradication by direct acting antiviral agents are synergistic risks of atherosclerosis. PLoS One 2018; 13: e0209615.

[40] Meissner EG, Lee YJ, Osinusi A, Sims Z, Qin J, Sturdevant D, McHutchison J, Subramanian M, Sampson M, Naggie S, Patel K, Remaley AT, Masur H and Kottilil S. Effect of sofosbuvir and ribavirin treatment on peripheral and hepatic lipid metabolism in chronic hepatitis C virus, genotype 1-infected patients. Hepatology 2015; 61: 790-801.

[41] Ciancio A, Bosio R, Bo S, Pellegrini M, Sacco M, Vogliotti E, Fassio G, Bianco Mauthe Degerfeld AGF, Gallo M, Giordanino C, Terzi di Bergamo L, Ribaldone D, Bugianesi E, Smedile A, Rizzetto M and Saracco GM. Significant improvement of glycemic control in diabetic patients with HCV infection responding to direct-acting antiviral agents. J Med Virol 2018; 90: 320-327.

[42] Hsu WF, Lai HC, Su WP, Lin CH, Chuang PH, Chen SH, Chen HY, Wang HW, Huang GT and Peng CY. Rapid decline of noninvasive fibrosis index values in patients with hepatitis C receiving treatment with direct-acting antiviral agents. BMC Gastroenterol 2019; 19: 63.

[43] Golozar A, Liu S, Lin JA, Peairs K and Yeh HC. Does metformin reduce cancer risks? Methodologic considerations. Curr Diab Rep 2016; 16: 4.

[44] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, Romero D, Abdelmalek MF, Anstee QM, Arab JP, Arrese M, Bataller R, Beuers U, Boursier J, Bugianesi E, Byrne CD, Castro Narro GE, Chowdhury A, Cortez-Pinto H, Cryer DR, Cusi K, El-Kassas M, Klein S, Eskridge W, Fan J, Gawrieh S, Guy CD, Harrison SA, Kim SU, Koot BG, Korenjak M, Kowdley KV, Lacaille F, Loomba R, Mitchell-Thain R, Morgan TR, Powell EE, Roden M, Romero-Gomez M, Silva M, Singh SP, Sookoian SC, Spearman CW, Tiniakos D, Valenti L, Vos MB, Wong VW, Xanthakos S, Yilmaz Y, Younossi Z, Hobbs A, Villota-Rivas M and Newsome PN; NAFLD nomenclature consensus group. A multisociety Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 2023; 78: 1966-1986.

[45] Cheng PN, Chen WJ, Hou CJ, Lin CL, Chang ML, Wang CC, Chang WT, Wang CY, Lin CY, Hung CL, Peng CY, Yu ML, Chao TH, Huang JF, Huang YH, Chen CY, Chiang CE, Lin HC, Li YH, Lin TH, Kao JH, Wang TD, Liu PY, Wu YW and Liu CJ. Taiwan Association for the Study of the Liver-Taiwan Society of Cardiology Taiwan position statement for the management of metabolic dysfunction-associated fatty liver disease and cardiovascular diseases. Clin Mol Hepatol 2024; 30: 16-36.

[46] Suzuki Y, Kaneko H, Okada A, Ohno R, Yokota I, Fujiu K, Jo T, Takeda N, Morita H, Node K, Yasunaga H and Komuro I. Comparison of SGLT2 inhibitors vs. DPP4 inhibitors for patients with metabolic dysfunction associated fatty liver disease and diabetes mellitus. J Endocrinol Invest 2023; [Epub ahead of print].

[47] Huang CF, Liang PC, Tsai PC, Wei YJ, Huang CI, Wang CW, Jang TY, Yeh ML, Hsu PY, Hsieh MY, Lin YH, Dai CY, Chuang WL, Huang JF and Yu ML. The interplay of metabolic dysfunction-associated fatty liver disease and viral hepatitis on liver disease severity: a large community-based study in a viral endemic area. J Gastroenterol Hepatol 2024; 39: 193-201.

[48] Lonardo A, Bril F, Caldwell SH, Eslam M, Fan JG, Gish RG, Gronbaek H, Sanal MG, Stefan N, Suzuki A, Targher G, Tilg H, Yu ML, Zheng MH and George J. Researchers call for more flexible editorial conduct rather than abruptly adopting only the new MASLD nomenclature. J Hepatol 2024; 80: e192-e194.

Supplementary Table 1. Treatment regimens, with duration in weeks, used in this study

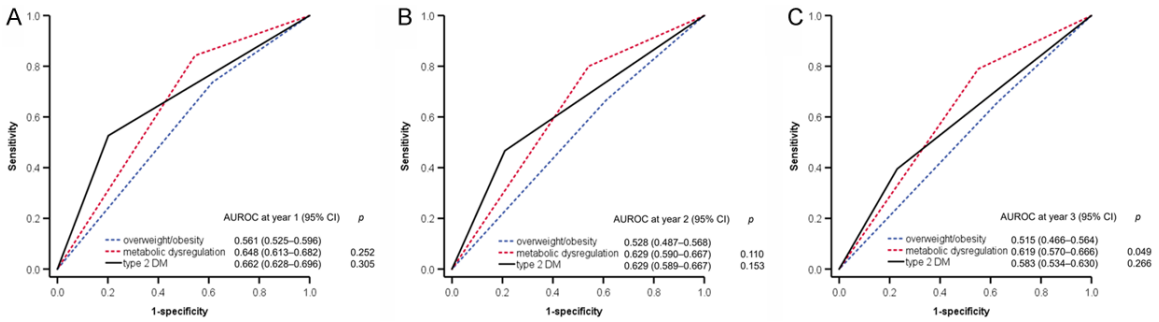
Regimens (n = 924)	Duration (weeks)	n (%)
GZR + EBR	12-16	120 (13.0)
GZR + EBR + RBV	12-16	11 (1.2)
DCV + ASV	24	55 (6.0)
DCV + ASV + RBV	24	5 (0.5)
SOF + LDV	12	145 (15.7)
SOF + LDV + RBV	12	35 (3.8)
SOF + DCV	12	15 (1.6)
SOF + DCV + RBV	12	1 (0.1)
SOF + VEL	12	144 (15.6)
SOF + VEL + RBV	12	6 (0.6)
SOF + RBV	12	50 (5.4)
PrOD	12	77 (8.3)
PrOD + RBV	12	7 (0.8)
G/P	8-12	253 (27.4)
Regimens including RBV	12-24	115 (12.4)

ASV, asunaprevir; DCV, daclatasvir; EBR, elbasvir; G/P, glecaprevir plus pibrentasvir; GZR, grazoprevir; LDV, ledipasvir; PrOD, paritaprevir/ritonavir/ombitasvir plus dasabuvir; RBV, ribavirin; SOF, sofosbuvir; VEL, velpatasvir.

Supplementary Table 2. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with liver-related events in all patients

Variable	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.049 (1.023-1.076)	< .001	1.046 (1.013-1.079)	.006
Sex: male vs. female	1.532 (0.888-2.642)	.125	2.315 (1.250-4.286)	.008
HCV RNA (log_{10} IU/mL)	0.901 (0.664-1.222)	.502		
Variables at 3 or 6 months after therapy				
ALT (U/L)	1.008 (0.997-1.018)	.159	0.993 (0.974-1.013)	.506
Albumin (g/dL)	0.247 (0.176-0.346)	< .001	0.331 (0.194-0.566)	< .001
Total bilirubin (mg/dL)	1.720 (1.490-1.986)	< .001	1.338 (1.098-1.632)	.004
AFP (ng/mL)	1.038 (1.025-1.052)	< .001	1.046 (1.031-1.061)	< .001
Metabolic dysfunction	1.666 (1.246-2.227)	.001	1.595 (1.132-2.246)	.008
Hepatic steatosis	1.417 (0.711-2.824)	.322	0.900 (0.398-2.037)	.801
FIB-4 > 3.25	1.078 (1.048-1.109)	< .001	3.588 (1.771-7.265)	< .001

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus.



Supplementary Figure 1. Time-dependent AUROCs representing the predictive performance of each metabolic dysfunction for LREs. A. AUROCs at year 1. B. AUROCs at year 2. C. AUROCs at year 3. AUROC, area under the receiver operating characteristic curve; DM, diabetes mellitus; LREs, liver-related events.

Supplementary Table 3. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with liver-related events in all patients

Variable	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.049 (1.023-1.076)	< .001	1.045 (1.012-1.078)	.007
Sex: male vs. female	1.532 (0.888-2.642)	.125	2.425 (1.307-4.501)	.005
HCV RNA (log_{10} IU/mL)	0.901 (0.664-1.222)	.502		
Metformin	2.904 (1.627-5.185)	< .001	1.774 (0.836-3.768)	.136
Statin	0.888 (0.353-2.234)	.801		
Variables at 3 or 6 months after therapy				
ALT (U/L)	1.008 (0.997-1.018)	.159	0.994 (0.974-1.014)	.527
Albumin (g/dL)	0.247 (0.176-0.346)	< .001	0.327 (0.192-0.555)	< .001
Total bilirubin (mg/dL)	1.720 (1.490-1.986)	< .001	1.356 (1.108-1.659)	.003
AFP (ng/mL)	1.038 (1.025-1.052)	< .001	1.047 (1.032-1.063)	< .001
Metabolic dysfunction	1.666 (1.246-2.227)	.001	1.354 (0.922-1.987)	.122
Hepatic steatosis	1.417 (0.711-2.824)	.322		
FIB-4 > 3.25	1.078 (1.048-1.109)	< .001	3.502 (1.725-7.107)	< .001

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus; MAFLD, metabolic dysfunction-associated fatty liver disease.

Supplementary Table 4. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with hepatocellular carcinoma in all patients

Variable	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.052 (1.019-1.085)	.002	1.039 (1.001-1.078)	.047
Sex: male vs. female	1.365 (0.703-2.652)	.358		
HCV RNA (log_{10} IU/mL)	1.323 (0.853-2.051)	.211		
Variables at 3 or 6 months after therapy				
ALT (U/L)	1.009 (0.998-1.021)	.115	0.996 (0.974-1.018)	.705
Albumin (g/dL)	0.456 (0.226-0.920)	.028	0.873 (0.357-2.135)	.766
Total bilirubin (mg/dL)	1.500 (1.162-1.936)	.002	1.366 (0.968-1.926)	.076
AFP (ng/mL)	1.048 (1.032-1.065)	< .001	1.055 (1.036-1.076)	< .001
Metabolic dysfunction	1.727 (1.210-2.464)	.003	1.699 (1.117-2.583)	.013
Hepatic steatosis	1.125 (0.511-2.478)	.770	0.876 (0.348-2.203)	.778
FIB-4 > 3.25	1.069 (1.027-1.112)	.001	3.468 (1.5070-7.980)	.003

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus.

Supplementary Table 5. Univariate and multivariable Cox regression analyses of factors at 3 or 6 months after therapy associated with hepatocellular carcinoma in all patients

Variable	Univariate analysis		Multivariable analysis	
	Hazard ratio (95% CI)	*P* value	Hazard ratio (95% CI)	*P* value
Age (years)	1.052 (1.019-1.085)	.002	1.039 (1.001-1.079)	.046
Sex: male vs. female	1.365 (0.703-2.652)	.358		
HCV RNA (log_{10} IU/mL)	1.323 (0.853-2.051)	.211		
Metformin	2.617 (1.282-5.346)	.008	0.975 (0.398-2.392)	.957
Statin	1.068 (0.377-3.027)	.902		
Variables at 3 or 6 months after therapy				
ALT (U/L)	1.009 (0.998-1.021)	.115	0.996 (0.974-1.018)	.715
Albumin (g/dL)	0.456 (0.226-0.920)	.028	0.888 (0.364-2.169)	.794
Total bilirubin (mg/dL)	1.500 (1.162-1.936)	.002	1.366 (0.970-1.926)	.074
AFP (ng/mL)	1.048 (1.032-1.065)	< .001	1.056 (1.036-1.075)	< .001
Metabolic dysfunction	1.727 (1.210-2.464)	.003	1.678 (1.052-2.678)	.030
Hepatic steatosis	1.125 (0.511-2.478)	.770		
FIB-4 > 3.25	1.069 (1.027-1.112)	.001	3.484 (1.505-8.065)	.004

AFP, alpha-fetoprotein; ALT, alanine aminotransferase; FIB-4, fibrosis-4 score; HCV, hepatitis C virus; MAFLD, metabolic dysfunction-associated fatty liver disease.

Supplementary Table 6. Demographics and baseline characteristics of patients with 0 or 1 metabolic dysfunction and of patients with 2 or 3 metabolic dysfunctions

Variable	Total (n = 924)	With 0 or 1 metabolic dysfunction (n = 488)	With 2 or 3 metabolic dysfunctions (n = 436)	*P* value
Age (years)	58 (49-65)	56 (46-64)	59 (52-66)	< .001
Sex (male, %)	418 (45.2)	207 (42.4)	211 (48.4)	.069
Body mass index (kg/m^2)	24.01 (21.78-26.73)	22.50 (20.83-24.64)	25.68 (23.74-28.37)	< .001
Overweight/obesity, *n* (%)	571 (61.8)	194 (39.8)	377 (86.5)	< .001
Follow-up months	34.09 (17.31-50.20)	34.23 (17.98-50.91)	33.90 (16.63-49.80)	.767
Hemoglobin (g/dL)	14.1 (13.1-15.1)	13.9 (13.1-15.0)	14.1 (13.1-15.1)	.406
Platelet count (× 10^9/L)	171 (124-218)	178 (130-224)	162 (119-208)	.004
AST (U/L)	46 (31-80)	40 (29-69)	53 (34-91)	< .001
ALT (U/L)	56 (34-102)	51 (31-89)	64 (39-117)	< .001
Total bilirubin (mg/dL)	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.9 (0.6-1.1)	.015
Albumin (g/dL)	4.3 (4.1-4.6)	4.4 (4.1-4.6)	4.3 (4.1-4.5)	.001
INR	1.02 (0.99-1.08)	1.02 (0.99-1.08)	1.03 (1.00-1.08)	.075
Triglyceride (mg/dL)	87 (65-119)	74 (56-97)	102 (78-143)	< .001
Total cholesterol (mg/dL)	169 (146-191)	173 (150-197)	166 (143-186)	< .001
HDL (mg/dL)	46.6 (37.5-57.9)	54.6 (44.4-63.8)	40.7 (33.8-49.4)	< .001
LDL (mg/dL)	95.6 (76.3-117.0)	97.0 (76.1-117.7)	94.9 (76.4-117.0)	.425
AFP (ng/mL)	4.27 (2.78-9.19)	3.88 (2.62-7.00)	4.77 (2.99-10.57)	< .001
Creatinine (mg/dL)	0.77 (0.64-0.93)	0.75 (0.63-0.88)	0.81 (0.66-0.98)	< .001
Hypertension, *n* (%)	262 (28.4)	76 (15.6)	186 (42.7)	< .001
Diabetes mellitus, *n* (%)	189 (20.5)	4 (0.8)	185 (42.4)	< .001
Hepatic steatosis, *n* (%)	689 (74.6)	320 (65.6)	369 (84.6)	< .001
Liver cirrhosis, *n* (%)	156 (16.9)	64 (13.2)	92 (21.1)	.001
HCV RNA ($\log_{10}$ IU/mL)	6.64 (5.97-7.12)	6.61 (5.96-7.11)	6.66 (5.99-7.15)	.690
HCV genotype, *n* (%)				
1	562 (60.8)	292 (59.8)	270 (61.9)	
2	259 (28.0)	139 (28.5)	120 (27.5)	
3	15 (1.6)	12 (2.5)	3 (0.7)	
6	83 (9.0)	42 (8.6)	41 (9.4)	
Mixed genotype*	4 (0.4)	2 (0.4)	2 (0.5)	
Baseline FIB-4	2.13 (1.28-3.87)	1.95 (1.12-3.49)	2.42 (1.45-4.23)	< .001
FIB-4 at PW12	1.75 (1.13-2.70)	1.15 (1.04-2.63)	1.83 (1.21-2.79)	.029
Incident HCC, n (%)	35 (3.8)	10 (2.0)	25 (5.7)	< .001
LRE, n (%)	52 (5.6)	15 (3.1)	37 (8.5)	< .001

Data are presented as medians (interquartile ranges). *Three patients and one patient had genotype 1b+2 and 2+6 infection, respectively. One patient had an unclassified genotype. AFP, alpha-fetoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; LRE, liver-related events; PW12, 12 or 24 weeks after DAA therapy.