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ORIGINAL ARTICLE

Metabolic dysfunction outperforms ultrasonographic steatosis to stratify hepatocellular carcinoma risk in patients with advanced hepatitis C cured with direct-acting antivirals

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Abstract

Background and Aims: Metabolic dysfunction (MD)-associated fatty liver disease has been proposed to identify individuals at risk of liver events irrespectively of the contemporary presence of other liver diseases. The aim of this study was to examine the impact of MD in patients cured of chronic hepatis C (CHC).

Patients and Methods: We analysed data from a real-life cohort of 2611 Italian patients cured of CHC with direct antiviral agents and advanced liver fibrosis, without HBV/HIV, transplantation and negative for hepatocellular carcinoma (HCC) history (age 61.4 ± 11.8 years, 63.9% males, median follow-up 34, 24–40 months). Information about ultrasonographic steatosis (US) after sustained virological response was available in 1978.

Results: MD affected 58% of patients, diagnosed due to the presence of diabetes (MD-diabetes, 19%), overweight without diabetes (MD-overweight, 37%) or multiple

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metabolic abnormalities without overweight and diabetes (MD-metabolic, 2%). MD was more frequent than and not coincident with US (32% MD-only, 23% MD-US and 13% US-only). MD was associated with higher liver stiffness ($p < 0.05$), particularly in patients with MD-diabetes and MD-only subgroups, comprising older individuals with more advanced metabolic and liver disease ($p < 0.05$). At Cox proportional hazard multivariable analysis, MD was associated with increased risk of HCC (HR 1.97, 95% CI 1.27–3.04; $p = 0.0023$). Further classification according to diagnostic criteria improved risk stratification ($p < 0.0001$), with the highest risk observed in patients with MD-diabetes. Patients with MD-only appeared at highest risk since the sustained virological response achievement ($p = 0.008$), with a later catch-up of those with combined MD-US, whereas US-only was not associated with HCC.

Conclusions: MD is more prevalent than US in patients cured of CHC with advanced fibrosis and identifies more accurately individuals at risk of developing HCC.

KEYWORDS

diabetes, HCV, hepatocellular carcinoma, MAFLD, NAFLD

1 | INTRODUCTION

The metabolic dysfunction (MD)-associated fatty liver disease (MAFLD) concept has recently been proposed by an international multi-stakeholder consensus to replace non-alcoholic fatty liver disease (NAFLD) as the official definition for fatty liver disease associated with metabolic alterations and insulin resistance, the most prevalent chronic liver disorder worldwide.[1–5] MAFLD is a positive definition, which requires meeting the criteria for the presence of metabolic alterations typical of increased adiposity and insulin resistance,[1] thereby potentially identifying a more homogeneous population in term of liver disease drivers than NAFLD. Overall evidence suggests that the MAFLD definition can intercept better than NAFLD those individuals with more severe liver fibrosis and at risk of liver disease progression and HCC in the general population.[6–10]

MAFLD definition does not rule out the coexistence of other drivers of liver disease, and in the current epidemiological and clinical scenario, it may be particularly useful to unmask the contribution of metabolic dysfunction and insulin resistance in determining disease progression among individuals with chronic viral hepatitis who have cleared or suppressed viral replication. It is worth noting that hepatic fat accumulation related to metabolic co-morbidities, alcohol intake, viral variation and inherited genetic predisposition plays a major role in chronic hepatitis C (CHC) progression to cirrhosis and hepatocellular carcinoma (HCC).[11,12] Non-viral metabolic and genetic factors predisposing to fatty liver continue to affect the prognosis of CHC after viral eradication by direct-acting antivirals (DAAs).[13–15] Importantly, initial epidemiological data suggest that MAFLD may account for a much larger fraction of HCC cases than NAFLD alone,[16] but no data are yet available on the prevalence of MAFLD and on the impact on HCC and cardiovascular events (CVE) incidence in patients with advanced liver fibrosis prior to achieving pharmacological eradication of hepatitis C.

Lay Summary

Patients with liver cirrhosis cured from chronic hepatitis C remain at high risk of developing liver cancer.

Metabolic alterations associated with fatty liver, which is not always detectable by abdominal ultrasonography, remain an important risk factor for liver cancer in these patients.

Within this context, the aim of this study was to examine the prevalence, relationship with metabolic alterations and ultrasonographic steatosis (US) and clinical utility of the MAFLD definition, as captured by the presence of MD, to predict major clinical events (HCC and CVE incidence) in a large real-life cohort of patients with advanced liver fibrosis treated with DAAs for CHC (NAVIGATORE-Lombardia). This cohort was previously collected to study the impact of metabolic co-morbidities and treatments on clinical outcomes.[15] The overall goal would be to examine what would be the clinical implications of adopting the MAFLD definition in patients with advanced CHC remaining at high risk of both liver-related and cardiovascular events despite viral eradication.[13,15] Here, the MAFLD definition may also be useful because it recognizes the impact of progression to advanced liver fibrosis on the extinction of hepatic fat accumulation and disease activity,[17] and it does not require the demonstration of steatosis in patients with cirrhosis in the presence of metabolic dysfunction.[1] In this clinical setting, where liver biopsy has been replaced by non-invasive assessment of liver damage, US is not sensitive enough to detect altered hepatic lipid metabolism. Moreover, since advanced fibrosis leads to extinction of fat accumulation, this setting offers the unique opportunity to test the superiority of the MAFLD over NAFLD definition to predict liver-related events.

2 | PATIENTS AND METHODS

2.1 | Study cohort

The study cohort was derived from the NAVIGATORE-Lombardia study database.[15] Briefly, data of all CHC patients treated with DAA in the Lombardy region in Northern Italy starting from December 2014 to December 2018 in 48 different clinical centres were collected through the NAVIGATORE-Lombardia Network web-based platform.[18] Liver fibrosis was staged in all patients before DAA treatment, either by liver biopsy (METAVIR stage) or non-invasively by transient elastography. Liver stiffness measurement (LSM) thresholds were previously reported[18]; LSM threshold for F3 was set at ≥10 kPa, for F4 at ≥13 kPa. Patients with decompensated cirrhosis were allocated to fibrosis stage F4.[19] After data retrieval and revision of the database, 8740 patients for whom age, sex, anthropometric features, fibrosis staging, metabolic co-morbidities and pharmacological history were available, were selected.

After exclusion of patients without cirrhosis (stage F0–F2, $n=3086$), who previously underwent liver transplant ($n=64$), were positive for HBsAg ($n=77$) or HIV ($n=903$), had a previous diagnosis of HCC ($n=324$), who did not achieve a sustained virological response after treatment ($n=96$) or without at least 6 months of follow-up ($n=1581$), we selected 2611 patients (study cohort). Median follow-up was 34, interquartile range 24–40 months. MD was defined according to criteria adapted from the MAFLD definition,[1] in the presence of overweight/obesity, of diabetes or at least two among the following features of metabolic dysfunction: arterial hypertension, hypertriglyceridemia and impaired fasting glucose. Alterations in circulating cholesterol were not considered due to the impact of cirrhosis on cholesterol synthesis. Since detailed biochemical evaluation after sustained virological response was not available, we arbitrarily considered the presence of treatment for hypertension, dyslipidaemia, and hyperglycaemia. We also analysed the impact of MD subtypes, based on the diagnostic criterium met, on the main study outcomes. MD-diabetes was diagnosed in patients with diabetes, MD-overweight in those without diabetes with BMI > 25 Kg/m2, MD-metabolic in those without diabetes and with normal BMI, but with multiple metabolic alterations.

As only 136 patients were classified as having fibrosis stage F3 (5.2%), we considered them together with cirrhosis for the analyses. Information (retrospectively collected by the individual centres) about the presence of US was available for 1978 (75.8%) patients with cirrhosis after the achievement of sustained virological response.[15]

The clinical features of the study cohort stratified according to the presence of MD are shown in Table 1.

All patients underwent regular HCC surveillance and HCC was diagnosed according to the EASL guidelines.[20] CVE were defined as stroke, myocardial infarction, hospitalization due to ischaemic heart disease or heart failure and sudden death.

Informed consent was obtained from each patient and the registry was approved by the Ethical committees and Review Boards of the participating centres and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study analysis plan was approved by the Ethics Committee of the University of Milan (on 23 October 2018).

2.2 | Statistical analysis

For descriptive statistics, categorical variables are shown as number and proportion, while continuous variables are shown as mean and standard deviation (SD) or median and interquartile range (IQR), as appropriate. The impact of MD on clinical events and survival was assessed by log-rank test, whereas the independent predictors of clinical events by multivariable Cox regression proportional hazard models. As one of the main study aim was to examine the impact of MD on the clinical outcomes, we included as covariates in multivariable models demographic features (age, sex), BMI, presence of diabetes, use of drugs potentially affecting outcomes (statins and metformin, when significant at univariate analysis), the main clinical risk factors associated with the specific outcomes at univariate analysis (detailed in the results section and tables) and variables significantly associated with the outcome at univariate analysis.

Statistical analysis was carried out using the JMP 16.0 Pro Statistical Analysis Software (SAS Institute, Cary, NC), and R statistical analysis software version 4.1 (http://www.R-project.org/). $p<0.05$ (two-tailed) were considered significant.

3 | RESULTS

3.1 | Metabolic dysfunction prevalence

The prevalence of MD in patients stratified by the definition criteria is shown in Figure 1A. Of the overall cohort, 58% of patients had MD, 19% with diabetes, 37% with increased adiposity but no diabetes, and only 2% with multiple metabolic alterations without neither diabetes nor increased adiposity.

The clinical features of the study cohort stratified by the presence of MD is shown in Table 1. According to the inclusion criteria, patients with MD had higher body mass, prevalence of diabetes and of hypertension and dyslipidaemia ($p<0.0001$) than those without MD. However, the prevalence of infection with genotype 3 and of positive history of alcohol intake was not significantly different between patients with and without MD. Patients with MD had higher LSM than those without ($p<0.0001$), confirming that MD is on average associated with more severe liver disease also in patients with advanced fibrosis cured of CHC.

The clinical features of the study cohort stratified by the presence of and inclusion criteria for MD are shown in Table 2. The sex distribution was not significantly different, but patients with MD-overweight tended to be younger, whereas those with MD-metabolic older than patients with MD-diabetes and without MD. By definition, MD-overweight patients had the highest BMI, followed by patients with

TABLE 1 Clinical features of 2611 patients with advanced liver fibrosis cured of CHC from the NAVIGATORE-Lombardia real-life cohort study stratified by the presence of metabolic dysfunction (MD).

	MD				
	Yes		No		
	n/mean	%/SD	*n*/mean	%/SD	*p*-value
n=	1505	57.6%	1106	42.4%	
Sex					
F	491	32.7%	469	42.4%	<0.0001
Age					
years	61.4	11.7	61.6	11.9	0.66
BMI					
Kg/m^2	27.6	3.8	22.3	2.0	<0.0001
BMI class					
Underweight	20	1.3%	144	13.0%	<0.0001
Normoweight	253	16.8%	962	36.8%	
Overweight	909	60.4%	0	0	
Obese	323	21.4%	0	0	
Diabetes					
Diabetes, insulin	166	12.3%	0	0	<0.0001
Diabetes, metformin	317	21.1%	0	0	
No diabetes	1022	67.9%	1106	100%	
Hypertension					
Yes	534	35.5%	267	24.1%	<0.0001
Dyslipidemia					
Yes	61	4.0%	15	1.4%	<0.0001
G3					
Yes	196	13.0%	156	14.1%	0.42
Alcohol					
Positive history	261	17.3%	188	17.0%	0.47
Cirrhosis					
Yes	1437	95.5%	1038	93.9%	0.065
LSM					
kPa	23.4	12.0	21.4	10.2	<0.0001

MD-diabetes, MD-metabolic and no MD. Hypertension and dyslipidaemia were most prevalent in patients with MD-metabolic, followed by MD-diabetes and MD-overweight. LSM was higher in patients with MD-diabetes versus those without MD ($p < 0.05$).

Among the 1978 patients with cirrhosis for whom information was available, expectedly the presence of MD did not perfectly match that of US (Figure 1B). However, the prevalence of US was higher in patients with MD-diabetes and in those with MD-overweight (39.8% and 44.6% respectively), than in patients with MD-metabolic and in those without MD (20.0% and 29.0% respectively; $p < 0.0001$).

3.2 | Metabolic dysfunction and US identify different patients subsets

We therefore next looked at the combined prevalence of MD according to demonstration of US (available in 1978 patients with cirrhosis), to examine whether these definitions were superimposable or identified specific subsets of patients with different risk of liver disease (Figure 1C). About one third of patients met the MD diagnosis without US evidence of US (MD-only, 32%), 23% had combined MD-US, 13% US not meeting MD criteria and another third of patients no evidence of neither MD nor US. When stratifying patients according to both the definition criteria for MD and the presence of US, the proportion of those with evidence of US was not significantly different according to the MD subgroup (p = NS; Figure 1D).

The clinical features of the study cohort stratified by the combined MD and US presence are shown in Table 3. Patients with either MD or US were more frequently males than those without, whereas those with US, either alone or associated with MD, were younger and reported more frequently a history of drinking than those with MD alone or without MD/US ($p < 0.05$ for all). According to the definition, patients with MD alone or MD/US had

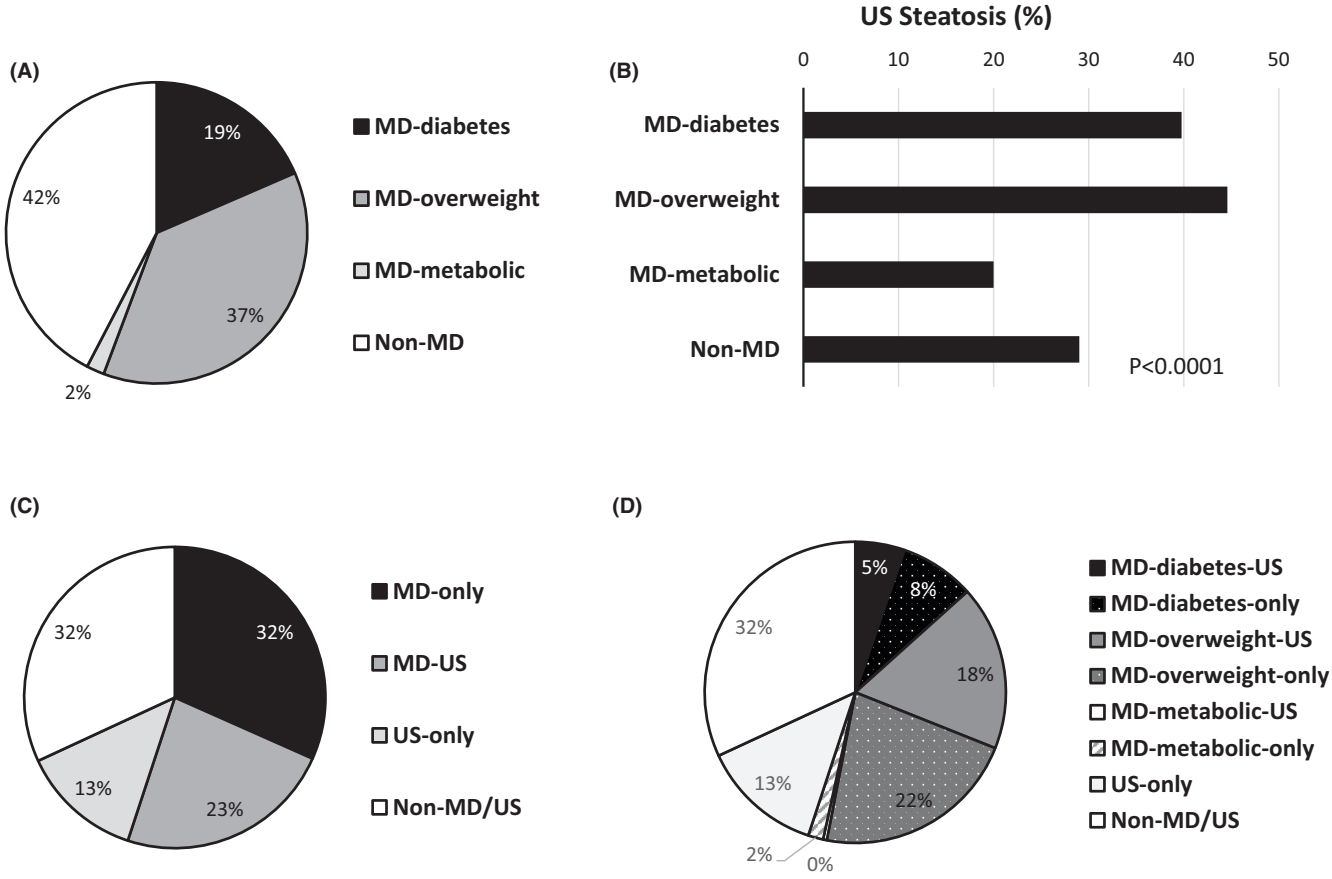

FIGURE 1 Prevalence of metabolic dysfunction (MD) in patients cured of CHC with advanced fibrosis. (A) Prevalence of MD subtypes, defined according to the diagnostic criterium met ($n=2611$). (B) Prevalence of ultrasonographically detected steatosis (US) in CHC according to the presence and diagnostic criteria for MD. (C) Combined prevalence of MD and US (n = 1978). (D) Combined prevalence of MD, further classified according to the diagnostic criteria and US ($n=1978$).

higher BMI, prevalence of diabetes, dyslipidaemia and arterial hypertension than those without ($p<0.05$). In this subset of patients with cirrhosis, LSM was higher in patients with MD, either with or without US, as compared than in those with US alone or without MD/US ($p<0.05$).

3.3 | Impact on clinical outcomes

The impact of MD diagnosis on the risk of development of de novo HCC and CVE is shown in Figure 2. Number of individuals at risk are shown in Table S1. Meeting the MD criteria was associated with an increased risk of HCC (p = 0.001; Figure 2A). Classification of MD according to the criteria leading to the diagnosis further improved risk stratification ($p<0.0001$; Figure 1B). The subset at highest risk was that of patients with MD-diabetes, followed by MD-overweight and MD-metabolic (Figure 2B). When considering MD together with demonstration of US in the subset where information was available, patients with MD alone appeared at highest risk of HCC since the sustained virological response achievement, with a later catch-up of those with combined MD-US diagnosis (Figure 2C).

On the other hand, MD diagnosis was not associated with CVE incidence, although in this cohort of patients with cirrhosis, CVE incidence was lower than that of HCC (Figure 2D,E).

At Cox proportional hazard model, MD was associated with higher HCC incidence independently of age, sex and infection with genotype 3-HCV (HR 1.97, 95%CI 1.27–3.04; $p=0.0023$; Table 4, upper panel). The risk was mostly increased in patients with MD-diabetes ($p<0.0001$; Table 4, middle panel), and in those with MD-only ($p=0.0064$; Table 4, bottom panel).

The impact of MD on HCC risk was also independent of BMI (included in the model as a continuous variable: HR 2.39, 95%CI 1.42–4.03 and $p=0.001$).

4 | DISCUSSION

In this study, we examined the consequences of applying the MD diagnostic criteria derived from the MAFLD definition in a real-life cohort of patients with advanced hepatitis C who were cured with direct antiviral agents. We took advantage of the NAVIGATORE-Lombardia cohort, a real-world cohort representative of a large

TABLE 2 Clinical features of 2611 patients with advanced liver fibrosis cured of CHC from the NAVIGATORE-Lombardia real-life cohort study stratified by the presence and inclusion criteria for metabolic dysfunction (MD).

	MD-diabetes		MD-overweight		MD-metabolic		Non MD	
	n/mean	%/SD	*n*/mean	%/SD	*n*/mean	%/SD	*n*/mean	%/SD
n=	483	18.5%	972	37.42%	50	1.9%	1106	42.4%
Sex								
F	160	33.1%	309*	31.8%	28	44.0%	469	42.4%
Age								
years	63.6	11.2	59.8*	11.6	70.9*	12.3	61.6	11.9
BMI								
Kg/m^2	26.1*	4.2	28.6*	6.1	22.5*	1.6	22.3	2.0
Hypertension								
Yes	171	52.9%	314*	32.3%	49*	98%	267	24.1%
Dyslipidaemia								
Yes	19	5.9%	30*	3.1%	12	24%	15	1.4%
G3								
Yes	49	10.1%	145	14.9%	2*	4.0%	156	14.1%
Alcohol								
Positive history	72	14.9%	181	18.6%	8	16.0%	188	17.0%
Cirrhosis								
Yes	466	96.5%	925	95.5%	46	92.0%	1038	93.9%
LSM								
kPa	24.3*	12.5	23.7	9.8	–	–	21.4	10.2

*$p < 0.05$ versus non-MD.

European region, where data related to metabolic co-morbidities were collected in order to assess their impact on the main clinical events after antiviral treatment, namely HCC and CVE.[15] In this cohort, we confirmed a role of older age and male sex in predisposing to the development of de novo HCC after achieving a sustained virological response, as we previously reported in the overall population, including patients with less advanced fibrosis, co-infected with HCV/HIV or transplanted.[15] Furthermore, in line with previous data, among the single metabolic abnormalities defining MD the presence of diabetes remained independently and robustly associated with a marked threefold increase in the risk of developing HCC.[13,15] Among the viral cofactors, it is notable that previous infection with HCV genotype 3 was associated with HCC risk, in line with other recent studies.[21–25]

The first noteworthy finding of our study was that in the setting of patients advanced CHC that we explored, MD and US diagnoses were coincident in only about one third of patients (34.3%), with MD being much more prevalent than US. This observation suggests that the prevalence of MAFLD, which in patients with cirrhosis does not require the demonstration of increased hepatic fat accumulation (e.g., US) when a positive history is reported, may be higher than previously expected. This hypothesis is consistent with evidence that the ability to accumulate lipids within intracellular lipid droplets resulting in hepatic fat accumulation decreases progressively with the progression of liver fibrosis in individuals with FLD, while lipotoxicity leading to the clinical complications of liver disease does not.[17] Indeed, hepatic fat content progressively returns into the normal range in those with most advanced liver disease.[26] The term 'burnt-out nonalcoholic steatohepatitis' has been coined to specifically define this condition. Alterations in hepatic vasculature and mitochondrial metabolism, reduced exposure to insulin and stimulation of catabolic pathways[27] and increased levels of adiponectin, the insulin-sensitizing adipokine which is excreted by the liver,[28] together with the progressive accumulation of somatic mutations that alters lipid metabolism,[29] can account for this phenomenon. Despite the progressive reduction in hepatic fat, more severe fibrosis is associated with an increased incidence of diabetes.[30,31] In fact, the presence of diabetes in 18% and overweight without diabetes in 37% were the most frequent criteria leading to MD diagnosis respectively. Furthermore, ultrasonography have a low sensitivity for detecting mild steatosis,[32] which would particularly affect the ability of this approach to rule out fatty liver disease in patients with cirrhosis when hepatic fat content begins to decrease. However, we cannot rule out that in some patients with MD-diabetes without US, the development of hyperglycaemia may have represented a consequence of advanced liver fibrosis rather than the evolution of advanced MAFLD.

Secondly, at baseline, patients with MD had more severe liver disease, as detected by LSM, than those without, confirming the known association between metabolic co-morbidities and

TABLE 3 Clinical features of 1978 patients with advanced liver fibrosis cured of CHC from the NAVIGATORE-Lombardia real-life cohort study stratified by the presence of metabolic dysfunction (MD) and ultrasonographic steatosis (US).

	MD-only		MD-US		US-only		Non-MD/US		
	n/mean	%/SD	*n*/mean	%/SD	*n*/mean	%/SD	*n*/mean	%/SD	*p*-value
n=	626	31.7%	463	23.4%	258	13.0%	631	31.9%	
Sex									
F	210*	33.6%	162*	35.0%	100*	38.8%	283	44.9%	0.0002
Age									
Years	62.1**	11.9	59.7*	11.4	59.5*	11.1	62.7**	12.3	<0.0001
BMI									
Kg/m^2	27.2*,**	3.5	28.6*	3.7	22.4**	2.0	22.3	2.0	<0.0001
BMI class									
Underweight	8*	1.3%	0*	0	33	12.8%	78	12.4%	<0.0001
Normoweight	108*	17.3%	42*	9.1%	225**	87.2%	553	87.6%	
Overweight	407*	65.0%	285*	61.6%	0**	0	0	0	
Obese	103*,**	16.5%	136*	29.3%	0**	0	0	0	
Diabetes									
Diabetes, insulin	85*	13.5%	48*	10.4%	0**	0	0	0	<0.0001
Diabetes, metformin	74*	11.8%	57*	12.3%	0**	0	0	0	
No diabetes	467*	74.6%	358*	77.3%	631	100%	258	100%	
Hypertension									
Yes	265*	42.3%	176*	38.0%	51*	19.8%	165	26.1%	<0.0001
Dyslipidemia									
Yes	29*	4.6%	19*	4.1%	7	2.7%	3	0.5%	<0.0001
G3									
Yes	72	11.5%	66	14.2%	38	14.7%	87	13.8%	0.44
Alcohol									
Positive history	91	14.5%	98*	21.2%	55*	21.3%	96	15.2%	0.0001
LSM									
kPa	23.9*	11.7	23.8*	11.7	21.3**	9.5	22.4**	10.8	0.0058

*$p < 0.05$ versus non-MD/US; **$p < 0.05$ versus MD-US (excluding 'non-MD/US').

progression of liver disease, even in patients with CHC.[13,33] This is consistent with evidence emerging from both the general population and clinical cohorts of patients without advanced viral hepatitis that the MAFLD definition identifies better than NAFLD individuals at risk of liver disease.[6–10] Among patients with MD, those diagnosed due to the presence of diabetes were at a more advanced disease stage compared to those diagnosed due to overweight, who were younger at an earlier stage of liver disease. Only a few patients met MD criteria due to the copresence of metabolic alterations without diabetes and overweight, and these were mostly older individuals affected by hypertension and dyslipidaemia. These data are in line with those showing that diabetes is a main determinant of liver disease severity in patients with advanced CHC cured with DAA.[15]

On the other hand, despite the limitations related to the possible under-reporting and lack of quantitative information, alcohol intake was associated more closely with US, identifying patients with less advanced liver disease than MD. Possible advantages of the application of the MAFLD definition in this setting include the avoidance of stigmatization related to the use of the term 'alcohol', the increase in patients' understanding of the cause of the disease and of their adherence to therapeutic strategies against metabolic triggers of the disease.[5,34] While alcohol history is often unreliable and thresholds for 'safe' (if ever) alcohol use are arbitrary and affected by interindividual variability, there is a synergic multiplicative effect between dietary alcohol intake and insulin resistance in determining liver disease, including in CHC patients.[35–37] From a pathophysiological point of view, alcoholic and non-alcoholic fatty liver are similar, if

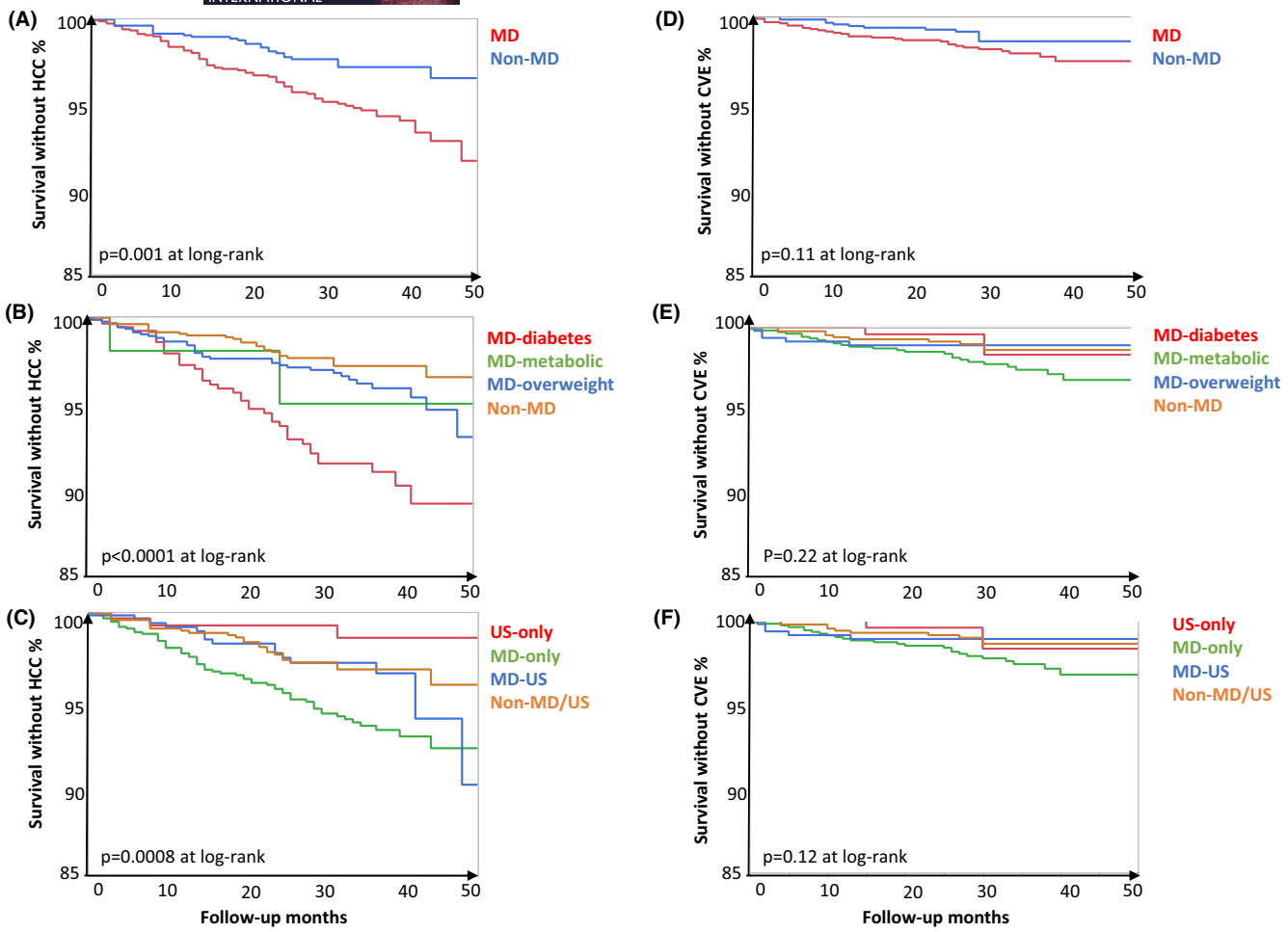

FIGURE 2 Impact of metabolic dysfunction (MD) on the most frequent clinical events, namely development of de novo hepatocellular carcinoma (HCC) and cardiovascular events (CVE) in patients with available follow-up ($n = 2611$). (A) Impact of MD on HCC. (B) Impact of MD stratified according to the diagnostic criteria on HCC. (C) Impact of combined MD-US on HCC ($n = 1978$). (D) Impact of MD on CVE. (E) Impact of MD stratified according to the diagnostic criteria on CVE. (F) Impact of combined MD-US on CVE ($n = 1978$).

not the same disorder,[38] and share the same genetic determinants,[39] being also endogenous alcohol production by the intestinal microbiota likely involved in NAFLD pathogenesis.[40]

Expectedly, in this cohort liver disease was more severe in patients with MD-diabetes as compared to MD-overweight, as these were slightly older and likely at a more advanced disease stage. Accordingly, liver fibrosis was more severe in those with MD alone as compared to MD-US, because again this category identified a group of older patients with declining adiposity but higher prevalence of diabetes, suggestive of a more advanced stage of both metabolic and hepatic disease.

Thirdly, patients with MD were at about two-fold higher risk of developing de novo HCC. This risk was particularly increased in those with diabetes (about three-fold), whereas the difference was not statistically significant in those with overweight alone. Again, consistently with the considerations expressed above regarding the older age and more severe liver damage at baseline, the risk was higher in those with MD alone as compared to the MD-US group, although at the end of the 4-year follow-up, there was a tendency for the MD-US group to catch up. These data are in line with evidence gathered in a large Korean cohort of patients with chronic hepatitis B, where the MAFLD status predicted HCC incidence independently of cirrhosis and of antiviral treatment.[41]

All in all, evidence suggests that in patients with advanced HCV cured with DAAs, HCC risk stratification based on MD and even more in the presence of diabetes, rather than US, should be used together with additional non-invasive biomarkers[14] to guide reinforced HCC surveillance by, for example, magnetic resonance imaging, including with abbreviated protocols, in high-risk patients.[42,43] In addition, pharmacological approaches targeting metabolic comorbidities, such as metformin in patients with diabetes or fasting hyperglycaemia but without decompensated cirrhosis, may decrease HCC incidence.[15,44]

Limitations of this study are related to the real-life design of the NAVIGATORE-Lombardia cohort, enabling to collect a large number of cases, even if the retrospective analysis did not allow a systematic assessment of hepatic fat content in the cohort by a uniform approach. Furthermore, ultrasonography is known to have a limited sensitivity to detect mild steatosis,[32] which is particularly true in patients with cirrhosis. In addition, clinical

TABLE 4 Impact of metabolic dysfunction (MD) diagnosis on the incidence of de novo HCC in 2611 Italian patients with advanced liver fibrosis cured of CHC.

	HR	95%CI	*p* value*
Age, years	1.04	1.02–1.06	0.0002
Sex, F	0.64	0.41–1.00	0.0526
HCV, G3	1.96	1.14–3.38	0.0147
MD, yes	1.97	1.27–3.04	0.0023
Non-MD	Reference		
MD-diabetes, yes	3.03	1.86–4.95	<0.0001
MD-overweight, yes	1.46	0.89–2.49	0.13
MD-metabolic, yes	1.37	0.32–5.81	0.69
Non-MD	Reference		
MD-only, yes	1.92	1.20–3.07	0.0064
MD-US, yes	1.25	0.66–2.34	0.49
US-only, yes	0.43	0.13–1.42	0.17
Non-MD/US	Reference		

Abbreviation: HR, hazard ratio.

*At multivariable Cox proportional regression models. Alternative models are shown including instead of the presence of MAFLD (upper panel), the subtype of MAFLD (middle panel) or the combined evaluation of the presence of MAFLD and FLD (bottom panel).

criteria for MD were simplified due to the lack of detailed biochemical information on patients after the achievement of the sustained virological response, and we could not confirm a MAFLD diagnosis due to the lack of systematic assessment of a history of liver steatosis in all patients. For the same reason, we could not assess the impact of genetic variants influencing MAFLD/NAFLD susceptibility[14,45,46] on their prevalence in this cohort and on the risk of developing HCC. Finally, the present results were likely influenced by the ethnicity and specific epidemiological feature of the NAVIGATORE-Lombardia cohort and may not be representative of other regions and of younger patients with less severe fibrosis. Therefore, they will require further independent validation.

In conclusion, by applying the MD criteria in a real-life cohort of patients cured of CHC with advanced fibrosis, we found that MD is more prevalent than US and identifies more accurately individuals with advanced liver disease at risk of developing de novo HCC. MD appears more useful than US to stratify the risk of HCC in patients cured of CHC with advanced fibrosis.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest relevant to this study. LV has received speaking fees from MSD, Gilead, AlfaSigma and AbbVie, served as a consultant for Gilead, Pfizer, AstraZeneca, Novo Nordisk, Intercept, Diatech Pharmacogenetics, Ionis Pharmaceuticals, Boeringher Ingelheim and received research grants from Gilead. DP has received advisory boards, travel or research grants, speaking and teaching fees from Macopharma, Ortho Clinical Diagnostics, Grifols, Terumo, Immucor, Diamed and Diatech Pharmacogenetics. SF: Advisory board and speaker's bureau for Gilead, Abbvie, Kedrion, Intecept, Novartis, MSD, Eisai, Bayer, Roche and Novo Nordisk.

ETHICAL APPROVAL

Informed consent was obtained from each patient and the clinical registry implemented by the Lombardia regional healthcare system was approved by the Ethical committees and Review Boards of the participating centres and conforms to the ethical guidelines of the 1975 Declaration of Helsinki. The study analysis plan was approved by the Ethics Committee of the University of Milan (on 23 October 2018).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX

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