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ORIGINAL ARTICLE

Does the risk of cardiovascular events differ between biopsy-proven NAFLD and MAFLD?

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Abstract

Background/purpose Cardiovascular disease (CVD) is the leading cause of death among individuals with non-alcoholic fatty liver disease (NAFLD). Recently, NAFLD was renamed metabolic-associated fatty liver disease (MAFLD). This study aimed to compare cardiovascular risk (CVR) and CVD between patients with NAFLD and MAFLD.

Methods Retrospective cross-sectional study of biopsy-proven liver steatosis performed between 2013 and 2018 at a university hospital. Cases were divided into NAFLD or MAFLD and demographic, clinical, and laboratory data were collected to assess CVR (through the atherosclerotic cardiovascular disease risk estimator and atherogenic indices) and CVD.

Results Out of 1233 liver biopsies, 171 (13.9%) presented steatosis. Of these, 109 patients met diagnostic criteria for NAFLD (63.7%) and 154 (90.1%), for MAFLD. In the NAFLD group, 78% of the cases had steatohepatitis, 24.8% had cirrhosis, and 3.7%, hepatocellular carcinoma (HCC). In the MAFLD group, 72.7% of the cases had liver inflammatory activity, 28.6% had cirrhosis, and 13.6% had HCC. In patients with MAFLD and NAFLD, CVR was intermediate/high (36.4 and 25.7%, $p=0.209$) and CVD occurred in 20.1 and 12.8% ($p=0.137$) of the cases, respectively, with no influence of liver injury severity. We observed a significant increase in high 10-year CVR ($p=0.020$) and CVD ($p=0.007$) in patients with MAFLD and concomitant viral infection (HCV and/or HBV) compared to cases with MAFLD only.

Conclusion Patients with both NAFLD and MAFLD had intermediate/high CVR, with a high rate of CVD. Patients with MAFLD and concomitant viral infection showed significantly increased CVR and CVD compared to those without viral infection.

Keywords ASCVD risk estimator · Atherogenic ratios · Cardiovascular diseases · Cardiovascular risk · Inflammatory activity · Metabolic-associated fatty liver disease · Non-alcoholic fatty liver disease · Non-fatal cardiovascular events · Steatosis · Viral infection

Abbreviations

AC	Atherogenic coefficient
ALT	Alanine aminotransferase
ASCVD	Atherosclerotic cardiovascular disease
AST	Aspartate aminotransferase
BMI	Body mass index
CVD	Cardiovascular disease
CRI	Castelli's Risk Index
CRP	C-reactive protein
CVR	Cardiovascular risk
GGT	Gamma-glutamyl transferase
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HDL	High-density lipoprotein
INR	International normalized ratio
LDL	Low-density lipoprotein
MAFLD	Metabolic-associated fatty liver disease
NAFLD	Non-alcoholic fatty liver disease

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NAS	NAFLD activity score
NASH	Non-alcoholic steatohepatitis
TC	Total cholesterol

Introduction

Non-alcoholic fatty liver disease (NAFLD) comprises a spectrum of liver diseases; it is characterized by the presence of steatosis in more than 5% of hepatocytes in the absence of excessive alcohol consumption or other causes that promote hepatic fat accumulation, such as infection by the hepatitis C virus (HCV) [1–3]. NAFLD can progress to non-alcoholic steatohepatitis (NASH), cirrhosis, and hepatocellular carcinoma (HCC) [1–3]. The prevalence of NAFLD has increased along with the worldwide growth in the number of cases of diabetes, obesity, and metabolic syndrome, although this disease can also occur in lean and non-diabetic individuals [4]. Globally, the prevalence of NAFLD is estimated to be 24%, and in South America it reaches 30.5%; particularly but not only in Asia, there is a considerable percentage of "lean NASH" patients who have a eutrophic body mass index (BMI) [5–7].

Recently, a panel of international experts from 22 countries proposed a new definition for NAFLD named "metabolic-associated fatty liver disease" (MAFLD) [8]. MAFLD, as NAFLD, is a hepatic manifestation of a heterogeneous multisystemic disorder with variable clinical presentations, influenced by interactions between genetic and environmental cues [9, 10]. The criteria for diagnosing MAFLD are based on the evidence of hepatic steatosis in patients with metabolic abnormalities (independently of alcohol consumption or the presence of comorbidities such as chronic viral hepatitis) and can be applied to patients in any clinical setting [8, 10]. Due to the worldwide increase in NAFLD, it can and frequently does coexist with other conditions such as hepatitis C and alcoholic liver disease [8, 10]. In these situations, an individual with MAFLD could present clinical consequences that are somewhat different from those experienced by people with NAFLD, and even a different response to therapeutic measures when compared to those with liver disease of a single etiology.

A large body of evidence has recently shown that patients with NAFLD are also at high risk of cardiovascular disease (CVD), which represents the main cause of death in these individuals (40–45% of the cases) [11]. Greater liver disease severity has been associated with an increased risk of both fatal and non-fatal CVD events such as left ventricular dysfunction, atherosclerotic CVD, cardiac arrhythmias, and ischemic stroke [11–13]. Although cardiovascular risk and CVD are expected to be equally high in MAFLD patients, specific studies in this population are still lacking. Based on the high mortality rates related to CVD, in addition to the well-known liver-related morbidity and mortality, we performed a retrospective analysis of patients, risk factors, and cardiovascular events comparing biopsy-proven NAFLD and MAFLD at a university hospital in southern Brazil.

Materials and methods

This is a retrospective cross-sectional study performed through the collection of data from electronic medical records. This study included patients over 18 years old who underwent a liver biopsy between 2013 and 2018 at a referral service of a university hospital located in southern Brazil. Cases in which liver tissue samples were considered insufficient (with less than ten portal triads) were excluded.

The definition of NAFLD adopted in this study was based on the presence of steatosis in $> 5\%$ of hepatocytes in the absence of significant ongoing or recent alcohol consumption (ethanol intake above 20 g/day for women and 30 g/day for men) and other known causes of liver disease such as hemochromatosis, Wilson's disease, and alpha-1 antitrypsin deficiency. In addition, we also excluded patients undergoing therapy with drugs known to promote liver steatosis (eg, amiodarone, tamoxifen, estrogen, or corticosteroids) [1–3]. Criteria for MAFLD diagnosis were based on the evidence of hepatic steatosis, in addition to one of the following three criteria: overweight/obesity, presence of type 2 diabetes mellitus, or evidence of metabolic dysregulation [8]. Liver tissue was subjected to histological examination and was graded as per the NASH Clinical Research Network NAFLD activity score (NAS). A diagnosis of steatohepatitis was made when NAS ≥ 4. For cases of MAFLD, the disease was described by the degree of activity according to the NAS score and the stage of fibrosis. A classification based on absence/presence was used for steatosis, inflammatory activity, fibrosis (METAVIR F1–F3), cirrhosis (METAVIR F4), and HCC regarding the histopathological characteristics described in the medical reports.

We collected clinical, laboratory, and demographic data from the patients' medical records. The biochemical data used to perform the analyses were the test results available the closest to the time of liver biopsy, considering an interval of 30 days between the procedures. Data on the occurrence of the following cardiovascular events were obtained from the patients' medical records: ischemic heart disease, myocardial infarction, atherosclerosis, aortic valve stenosis, and stroke. Cardiovascular manifestations were evaluated retrospectively from 2013 to 2018 at the referral service. For calculating cardiovascular risk, we used the atherosclerotic cardiovascular disease (ASCVD) risk estimator plus tool of the American College of Cardiology/American Heart Association [14]. Atherogenic ratios were calculated using results of the lipid profile to predict cardiovascular

risk. Such ratios were calculated using Castelli's Risk Index (CRI) (CRI-I = total cholesterol [TC]/high-density lipoprotein [HDL]; CRI-II = low-density lipoprotein [LDL]/HDL) and the Atherogenic Coefficient (AC) (AC = (TC-HDL)/HDL [15]. The following cutoff values were considered for the atherogenic indices: risk was considered low if CRI-I was higher than 3.5 for men and 3.0 for women while CRI-II and AC values were below 3.0 and 2.0, respectively, for both sexes [15, 16]. For BMI, we used the classification of eutrophic (18.5–24.9 kg/m^2), overweight (25–29.9 kg/m^2), obesity I (30–34.9 kg/m^2), obesity II (35–39.9 kg/m^2), and obesity III (over 40 kg/m^2) for adults (18–59 years), and eutrophic (22–26.9 kg/m^2) and overweight (over 27 kg/m^2) for older adults (over 60 years) [17].

This study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre, CAAE 79523617.1.0000.5327, and follows the guidelines for studies in humans.

Statistical analysis

Data symmetry was tested using the Shapiro–Wilk test. Quantitative variables were expressed as means ± standard deviations or medians and interquartile ranges (25th–75th). Categorical variables were expressed as frequencies and percentages. Mann–Whitney *U*, chi-squared, and Fisher's exact tests were performed. p values < 0.05 were considered statistically significant. Variables were analyzed using SPSS version 18.0 (SPSS Inc, Chicago, IL, USA).

Results

General characteristics of patients

Between 2013 and 2018, 1233 liver biopsies were performed for various medical indications at the institution. When reviewing the histopathological reports, we observed that 171 (13.9%) cases were diagnosed with hepatic steatosis. For the evaluation according to the NAFLD criteria, 56 (32.7%) cases were excluded due to a concomitant diagnosis of HCV and 6 (3.6%) were excluded for hepatitis B virus (HBV), totaling an evaluable sample of 109 (63.7%) patients. For the analysis according to MAFLD diagnostic criteria, among the 171 cases diagnosed with steatosis through liver biopsy, 17 (9.9%) patients were excluded for not presenting overweight/obesity, type 2 diabetes mellitus, or evidence of metabolic dysregulation, totaling a final sample of 154 (90.1%) cases.

Comparison of NAFLD and MAFLD criteria

The demographic, laboratory, and clinical data of these patients according to NAFLD and MAFLD diagnoses are described in Table 1. Regarding patients with NAFLD, the median age at the time of liver biopsy was 55.0 (46.0–63.0) years, with a predominance of the female sex (58.7%) and White ethnicity (95.4%). A diagnosis of type 2 diabetes mellitus was reported in 55 (50.5%) cases and one of hypertension was observed in 67 (61.5%) cases. During the study period, 2 (1.8%) patients died, both from sepsis. According to the MAFLD diagnostic criteria, the median age at the time of biopsy was 58.0 (49.0–64.0) years, also with a predominance of the female sex (51.9%) and White ethnicity (96.7%); 82 (53.2%) patients had a diagnosis of type 2 diabetes mellitus, 96 had hypertension (62.3%), and 19 (12.3%) presented at least two metabolic risk abnormalities. During the study, 5 (3.2%) patients died, with three cases due to HCC, one due to sepsis, and the other due to acute peritonitis.

Cardiovascular risk and events in NAFLD and MAFLD

The median time between liver biopsy and the occurrence of a cardiovascular event was 1.8 (0.64–4.32) years. Data related to the prediction of the risk of developing CVD are shown in Table 2. According to the NAFLD classification for CRI-I, 31 (81.6%) men and 47 (81.0%) women were classified as having a high risk of developing CVD. Among patients who met diagnostic criteria for MAFLD, 42 (65.6%) men and 61 (82.4%) women were classified as at high risk for developing CVD according to CRI-I. Regarding CRI-II, for NAFLD and MAFLD patients, 16 (42.1 and 11.6%, respectively) men were at high cardiovascular risk. According to the NAFLD classification for AC, 36 (94.7%) men and 47 (81.0%) women were at high risk for developing CVD. Among patients who met MAFLD diagnostic criteria, 55 (84.6%) men and 61 (82.4%) women were classified as at high risk for developing CVD according to the AC.

As shown in Table 3, when stratifying NAFLD cases according to the severity of the liver injury, 2 (9.1%) patients with steatosis, 19 (31.7%) patients with inflammatory activity, and 7 (25.9%) with cirrhosis had intermediate/high risk of developing atherosclerotic cardiovascular disease in the next 10 years according to the ASCVD score. Among patients who met MAFLD diagnostic criteria, 5 (19.2%) cases with steatosis, 35 (41.7%) cases with inflammatory activity, and 16 (36.4%) with cirrhosis had an intermediate/high risk of developing atherosclerotic cardiovascular disease according to the ASCVD score. There were no significant differences, throughout different stages of liver injury, between the ASCVD scores of patients diagnosed with NAFLD and MAFLD.

The occurrence of cardiovascular events is shown in Table 3. In the study population with diagnostic criteria for NAFLD, there were reports of cardiovascular events in 14 (12.8%) cases, of which 4 (28.6%) were ischemic heart

Table 1 General characteristics of patients diagnosed with steatosis by liver biopsy according to NAFLD and MAFLD criteria

Characteristics*	NAFLD ($n=109$)	MAFLD ($n=154$)	p value#
Age	55.0 (46.0–63.0)	58.0 (49.0–64.0)	0.196
Sex			
Male	45 (41.3)	74 (48.1)	0.315
Female	64 (58.7)	80 (51.9)	
Ethnicity			
White	104 (95.4)	149 (96.7)	0.745
Black	2 (1.8)	2 (1.3)	1.000
Others	3 (2.8)	3 (1.9)	0.694
Active tobacco use	6 (5.5)	20 (13.0)	0.058
T2DM	55 (50.5)	82 (53.2)	0.708
Hypertension	67 (61.5)	96 (62.3)	0.898
Staging of liver disease			
Steatosis	22 (20.2)	26 (16.9)	0.520
Inflammatory activity	85 (78.0)**	112 (72.7)	0.387
Fibrosis (METAVIR F1–F3)	65 (59.6)	98 (63.6)	0.522
Cirrhosis	27 (24.8)	44 (28.6)	0.573
HCC	4 (3.7)	21 (13.6)	0.009
NAFLD treatment			
Vitamin E	18 (16.5)	27 (17.5)	0.869
Pioglitazone	4 (3.7)	8 (5.2)	0.766
Guidance for lifestyle change	82 (75.2)	112 (72.7)	0.672
No guidance for lifestyle change	27 (24.8)	42 (27.3)	
Adult BMI			
Eutrophic	5 (10.2)	7 (6.3)	1.000
Overweight	16 (32.7)	24 (21.4)	0.864
Obesity I	11 (22.4)	14 (12.5)	0.833
Obesity II	13 (26.5)	16 (14.3)	0.695
Obesity III	4 (8.2)	5 (4.5)	1.000
Older adult BMI			
Malnutrition	0 (0.0)	1 (2.2)	1.000
Eutrophic	6 (20.0)	9 (19.5)	1.000
Overweight	24 (80.0)	36 (78.3)	0.882
Fasting glucose (mg/dL)	122 (±48.0)	114.5 (94.0–162.7)	0.089
Glycated hemoglobin	6,7 (±2.0)	6.9 (±2.0)	0.526
Triglycerides (mg/dL)	142.5 (106.5–202.5)	150.0 (108.3–208.7)	0.587
Total cholesterol (mg/dL)	187.7 (±48.7)	180 (±46.8)	0.326
HDL (mg/dL)	44.0 (±12.0)	43.9 (±11.9)	0.631
LDL (mg/dL)	108.7 (±44.4)	101.6 (±40.00)	0.160
ALT (U/I)	51.0 (35.5–75.0)	56.0 (37.5–103.5)	0.113
AST (U/I)	38.5 (26.5–55.5)	44.0 (28.0–67.5)	0.153
GGT (U/I)	72.0 (39.0–148.0)	94.5 (48.7–186.5)	0.080
Alkaline phosphatase (U/I)	85.0 (64.5–114.0)	94.0 (69.5–144.5)	0.122
CRP	6.7 (2.7–16.6)	6.8 (3.1–18.0)	0.663
Direct bilirubin (mg/dL)	0.2 (0.1–0.3)	0.2 (0.1–0.3)	0.161
Total bilirubin (mg/dL)	0.5 (0.3–0.8)	0.6 (0.4–0.9)	0.222
Albumin (g/dL)	4.4 (±0.5)	4.3 (±0.5)	0.168
Ferritin (ng/mL)	380.0 (152.9–649.5)	380.0 (153.0–668.0)	0.972
Platelets ($\times 10^3/\mu L$)	207.0 (±72.0)	190.0 (±83.9)	0.075
INR	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.736

ALT alanine aminotransferase, *AST* aspartate aminotransferase, *BMI* body mass index, *CRP* C-reactive protein, *GGT* gamma-glutamyl transferase, *HCC* hepatocellular carcinoma, *HDL* high-density lipoprotein, *INR* international normalized ratio, *LDL* low-density lipoprotein, *MAFLD* metabolic-associated fatty liver dis-

Table 1 (continued)

ease, *NAFLD* non-alcoholic fatty liver disease, and *T2DM* type 2 diabetes mellitus

*Variables described by frequency (%), mean (± standard deviation), or median (25th–75th percentiles). **Steatohepatitis. $^{\#}p \leq 0.05$ was considered statistically significant. Reference values for laboratory parameters: fasting glucose: normal levels < 100 mg/dL; glycated hemoglobin: 0.1–0.7 mg/dL; triglycerides: < 175 mg/dL; total cholesterol: < 190 mg/dL; HDL: > 40 mg/dL; ALT: < 55 U/L; AST: 5–34 U/L; GGT: 11–59 U/L for men and 8–33 U/L for women; alkaline phosphatase: 40–129 U/L for men and 35–104 U/L for women; direct bilirubin: up to 0.5 mg/dL; total bilirubin: 0.3–1.2 mg/dL; albumin: 3.5–5.2 g/dL for adults 20–60 years of age and 3.2–4.6 g/dL for adults 60–90 years of age; ferritin: 30–400 ng/mL; platelets: $150–400 \times 10^3/\mu L$.

Table 2 Cardiovascular risk according to NAFLD and MAFLD criteria

Variables*	Risk for CVD/NAFLD ($n = 109$)	Risk for CVD/MAFLD ($n = 154$)	p value$^{\#}$
Current 10-year ASCVD risk ($n = 51$ and $n = 85$)			
Low	18 (35.3)	23 (27.0)	0.206
Borderline	5 (9.8)	6 (7.1)	0.396
Intermediary	24 (47.1)	46 (54.1)	0.507
High	4 (7.8)	10 (11.8)	0.338
CRI-I			
Both sexes ($n = 96$ and $n = 138$)	4.4 (± 1.4)	4.3 (± 1.4)	0.510
Men ($n = 38$ and $n = 65$)	4.6 (± 1.4)	4.3 (± 1.5)	0.332
Low risk	7 (18.4)	23 (34.4)	0.052
High risk	31 (81.6)	42 (65.6)	
Women ($n = 58$ and $n = 74$)	4.3 (± 1.4)	4.2 (± 1.3)	0.677
Low risk	11 (19.0)	13 (17.6)	0.506
High risk	47 (81.0)	61 (82.4)	
CRI-II	95 (87.2)	138 (89.6)	
Both sexes	2.6 (± 1.0)	2.4 (± 1.0)	0.207
Men ($n = 38$ and $n = 65$)	2.9 (± 1.0)	2.4 (± 1.0)	0.320
Low risk	22 (57.9)	49 (35.5)	0.052
High risk	16 (42.1)	16 (11.6)	
Women ($n = 57$ and $n = 73$)	2.4 (± 1.0)	2.4 (± 0.9)	0.851
Low risk	42 (73.7)	55 (39.9)	0.493
High risk	15 (26.3)	18 (13.0)	
AC	96 (88.1)	139 (90.3)	
Both sexes	3.4 (± 1.4)	3.4 (± 1.5)	0.822
Men ($n = 38$ and $n = 65$)	3.6 (± 1.4)	3.4 (± 1.7)	0.326
Low risk	2 (5.3)	10 (15.4)	0.107
High risk	36 (94.7)	55 (84.6)	
Women ($n = 58$ and $n = 74$)	3.3 (± 1.4)	3.3 (± 1.3)	0.502
Low risk	11 (19.0)	13 (17.6)	0.506
High risk	47 (81.0)	61 (82.4)	

The information between parentheses in the variables column identifies the number of cases evaluated for the respective item

AC atherogenic coefficient, *ASCVD* atherosclerotic cardiovascular disease, *CVD* cardiovascular diseases, *CRI* Castelli's Risk Index, *MAFLD* metabolic-associated fatty liver disease, and *NAFLD* non-alcoholic fatty liver disease

*Variables described by frequency (%) or mean (± standard deviation). $^{\#}p \leq 0.05$ was considered statistically significant

disease, 3 (21.4%) were myocardial infarction, 3 (21.4%) were atherosclerosis, 2 (14.3%) were aortic valve stenosis, and 2 (14.3%) were stroke. Regarding patients with MAFLD diagnostic criteria, 31 (20.1%) cases had reports of cardiovascular events, with 8 (25.8%) patients having myocardial infarction, 7 (22.6%) having ischemic heart

Table 3 ASCVD risk and cardiovascular events in patients with NAFLD and MAFLD according to the severity of liver injury

Variables*	Overall		*p* value#	Steatosis		*p* value#	Inflammatory activity		*p* value#	Cirrhosis		*p* value#
	NAFLD ($n=109$)	MAFLD ($n=154$)		NAFLD ($n=22$)	MAFLD ($n=26$)		NAFLD** ($n=60$)	MAFLD ($n=84$)		NAFLD ($n=27$)	MAFLD ($n=44$)	
Current 10-year ASCVD risk												
Low + borderline	23 (21.1)	29 (18.8)	0.209	3 (13.6)	4 (15.4)	1.000	14 (23.3)	15 (17.9)	0.347	6 (22.2)	10 (22.7)	0.736
Intermediary + high	28 (25.7)	56 (36.4)		2 (9.1)	5 (19.2)		19 (31.7)	35 (41.7)		7 (25.9)	16 (36.4)	
Cardiovascular events												
Yes	14 (12.8)	31 (20.1)	0.137	3 (13.6)	9 (34.6)	0.180	8 (13.3)	14 (16.7)	0.645	3 (11.1)	8 (18.2)	0.515
No	95 (87.2)	123 (79.9)		19 (86.4)	17 (65.4)		52 (86.7)	70 (83.3)		24 (88.9)	36 (81.8)	

ASCVD atherosclerotic cardiovascular disease, *MAFLD* metabolic-associated fatty liver disease, and *NAFLD* non-alcoholic fatty liver disease

*Variables described by frequency (%). **Steatohepatitis. #$p \leq 0.05$ was considered statistically significant

disease, 6 (19.4%) presenting atherosclerosis, 6 (19.4%) presenting aortic valve stenosis, and 4 (12.9%) presenting stroke. However, there were no significant differences in the occurrence of cardiovascular events throughout different stages of liver injury between patients diagnosed by the NAFLD and MAFLD classifications.

Cardiovascular risk and events in the MAFLD group in the presence of viral infection

Table 4 shows the stratification of events and cardiovascular risk in cases that met the MAFLD diagnostic criteria, with or without concomitant viral infection. In the evaluated population, 92 (59.7%) patients presented MAFLD without concomitant HCV and/or HBV infection, while 62 (40.3%) had MAFLD and a concomitant diagnosis of viral infection (HCV and/or HBV). Of these, 57 (91.9%) cases had HCV, 4 (6.5%) had HBV, and 1 (1.6%) had concomitant HCV and HBV infection at the time of biopsy. The median viral load of patients with HCV was 728,013 Ul/mL (80,529–2,942,475), and of these, 40 (78.4%) cases received treatment and reached sustained virological response. For patients infected with HBV, the median viral load at the time of liver biopsy was 9103 Ul/mL (200–11,028) and these cases did not undergo treatment for the infection. There was a significant increase ($p=0.020$) in the number of patients at high risk of developing atherosclerotic cardiovascular disease in the next 10 years (according to the ASCVD score) when comparing those with MAFLD and viral infection to those without concomitant viral infection. When comparing atherogenic indices, patients with MAFLD and concomitant viral infection had significant increases in CRI-II ($p=0.029$) and AC ($p=0.042$) when compared to those with MAFLD but no diagnosis of viral infection.

Among patients who met the MAFLD diagnostic criteria and did not have concomitant HCV and/or HBV infection, 12 (13.0%) had some cardiovascular event between 2013 and 2018: 4 (33.3%) had a myocardial infarction, 2 (16.7%) had a stroke, and 6 (50.0%) had other conditions that included ischemic heart disease and aortic valve stenosis. On the other hand, among patients with MAFLD and concomitant HCV and/or HBV infection, 19 (30.6%) individuals presented some cardiovascular event in the evaluated period: 6 (31.6%) presented atherosclerosis, 5 (26.3%) had a myocardial infarction, 4 (21.1%) had ischemic heart disease, 2 (10.5%) had a stroke, and 2 (10.5%) had aortic valve stenosis. In the evaluated population, there was a significant increase ($p=0.007$) in reports of non-fatal cardiovascular events among patients with MAFLD and viral infection (HCV and/or HBV) compared to cases with MAFLD but without a diagnosis of concomitant viral infection.

Table 4 Evaluation of cardiovascular risk in patients who met the MAFLD diagnostic criteria and whether they had concomitant viral infections (HCV and/or HBV)

Variables*	MAFLD HBV and/or HCV negative ($n=92$)	MAFLD HBV and/or HCV positive ($n=62$)	p value[#]
Current 10-year ASCVD risk ($n=47$ and $n=38$)			
Low	16 (34.0)	7 (18.4)	0.085
Borderline	5 (10.6)	1 (2.6)	0.158
Intermediary	24 (51.1)	22 (57.9)	0.341
High	2 (4.3)	8 (21.1)	**0.020**
CRI-I			
Both sexes ($n=55$ and $n=83$)	4.4 (±1.3)	4.0 (±1.5)	0.326
Men ($n=30$ and $n=34$)	3.9 (±1.2)	4.2 (±1.7)	0.877
Low risk	9 (30.0)	13 (38.2)	0.335
High risk	21 (70.0)	21 (61.8)	
Women ($n=53$ and $n=21$)	4.5 (±1.4)	3.6 (±1.1)	0.102
Low risk	9 (17.0)	4 (19.0)	0.536
High risk	44 (83.0)	17 (81.0)	
CRI-II			
Both sexes ($n=56$ and $n=82$)	2.0 (±0.9)	2.4 (±1.0)	**0.029**
Men ($n=35$ and $n=30$)	2.1 (±1.0)	2.4 (±1.0)	0.063
Low risk	21 (70.0)	28 (80.0)	0.256
High risk	9 (30.0)	7 (20.0)	
Women ($n=52$ and $n=21$)	2.0 (±0.7)	2.4 (±1.0)	0.192
Low risk	37 (71.2)	18 (85.7)	0.157
High risk	15 (28.8)	3 (14.3)	
AC			
Both sexes ($n=55$ and $n=84$)	2.4 (±1.2)	3.0 (±1.5)	**0.042**
Men ($n=32$ and $n=34$)	2.2 (±1.0)	3.2 (±1.7)	0.015
Low risk	2 (6.5)	8 (23.5)	0.057
High risk	29 (93.5)	26 (76.5)	
Women ($n=54$ and $n=21$)	2.5 (±1.4)	2.6 (±1.1)	0.831
Low risk	9 (17.0)	4 (19.0)	0.538
High risk	44 (83.0)	17 (81.0)	
History of CVD			
Yes	12 (13.0)	19 (30.6)	**0.007**
No	80 (87.0)	43 (69.4)	

The information between parentheses in the variables column identifies the number of cases evaluated for the respective item

The items that are highlighted in bold are the variables that showed the significant difference in the statistical analysis performed

AC atherogenic coefficient, *ASCVD* atherosclerotic cardiovascular disease, *CVD* cardiovascular disease, *CRI* Castelli's Risk Index, *HCV* hepatitis C virus, *HBV* hepatitis B virus, and *MAFLD* metabolic-associated fatty liver disease

*Variables described by frequency (%) or mean (±standard deviation). [#]$p \leq 0.05$ was considered statistically significant

Discussion

In this study, we observed a significant increase in cardiovascular events in patients with MAFLD and a concomitant diagnosis of viral infection (HCV and/or HBV) compared to cases with MAFLD but no viral infection. In addition, patients with MAFLD and viral infection had a significant increase in the risk of developing cardiovascular disease according to atherogenic indices and the ASCVD score. In the past 10 years, it has become evident that NAFLD is not just a broad-spectrum clinicopathological condition with high potential for progression, but a systemic disease capable of affecting extrahepatic systems, including the heart and the vascular system. This clinical condition should be

considered an independent and significant risk factor for the development of clinical and subclinical CVD. In this sense, cardiometabolic risk conditions must be carefully and routinely evaluated in this population. The fact that CVD is the main cause of death in patients with NAFLD it is not surprising as metabolic syndrome, endothelial dysfunction, and chronic inflammation are present in most cases. However, the definition of NAFLD excludes some comorbidities, such as viral hepatitis and alcohol abuse, that can be themselves related to CVD. Considering that MAFLD criteria do not exclude these comorbidities, CVD is expected to be more frequent in this population than in patients with NAFLD. In this study, among 109 cases diagnosed with liver steatosis by biopsy according to NAFLD criteria, 12.8% had some cardiovascular event; when performing this evaluation according to the MAFLD diagnostic criteria, 20.1% of the patients presented a non-fatal cardiovascular event between 2013 and 2018 at a referral service. To the best of our knowledge, this is the first report of a comparison of cardiovascular risk and CVD between patients with NAFLD and MAFLD.

Routine biochemical tests such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), and gamma-glutamyl transferase (GGT), as well as the lipid profile and C-reactive protein, can be useful in evaluating a patient with NAFLD [18, 19]. In this study, we observed a small change in serum levels of AST and ALT. Although elevated levels of serum transaminases are observed in this population, normal results for this test can occur in more than 70% of patients with NAFLD [18]. Moreover, we observed a slight increase in median serum GGT levels, and studies have reported that they can be considered an independent risk factor and a long-term predictor of CVD in patients with NAFLD [18–21].

Among the risk factors associated with the development of CVD, the presence of dyslipidemia is an important predictor [22, 23]. Predicting CVD is not trivial and several scoring systems have been described for cardiovascular risk management, such as the ASCVD score, Framingham Risk Score, atherogenic indices, QRisk2, and SCORE [24]. Atherogenic indices (obtained through lipid parameters) or predictive risk scores (such as ASCVD) are alternatives that can be used to stratify these patients according to the future risk of developing CVD. It is suggested that CVD increases with liver disease severity. In this study, no significant differences were observed between the severity of liver injury according to the NAFLD and MAFLD classification and the ASCVD score. Golabi et al. assessed the ASCVD risk score in patients with NAFLD. The authors reported that among 1262 individuals with NAFLD, the prevalence of a high risk for CVD was 55.9%; this was associated with a higher risk of overall and cardiac-specific mortality [25]. These data are superior to those observed in our study, in which 25.7 and 36.4% of patients with NAFLD and MAFLD, respectively, presented an intermediate/high risk of developing CVD according to the ASCVD score. Other studies corroborate our data, showing that predictive models such as the ASCVD risk score can provide an easy tool to assess 10-year and lifetime risk for cardiovascular events in patients with this clinical condition [26–28]. In addition, atherogenic dyslipidemia is known to play a critical role in the development of CVD in patients with NAFLD. In a cross-sectional study aimed at assessing dyslipidemia using lipid ratios and atherogenic indices in participants recruited from semi-urban communities in Nigeria, CRI-I, CRI-II, and AC predicted a prevalence of cardiovascular risk of 22.5, 15.9, and 11.2%, respectively. The authors concluded that these atherogenic indices could be used to assess cardiovascular risks even when lipid profiles were apparently normal [29]. In comparison, we reported a higher prevalence of cardiovascular risk in the sample assessed using these atherogenic indices. To date, few studies have investigated the relationship between atherogenic indices and liver disease [30–32]. Recently, the relationship between the atherogenic index of plasma and NAFLD was analyzed in Chinese and Japanese non-obese individuals, and the study concluded that this index could be used as a new screening indicator for non-obese people with NAFLD in different nations [33].

Among the 109 patients included in this study according to NAFLD diagnostic criteria, 12.8% had some non-fatal cardiovascular event. According to MAFLD diagnostic criteria, 20.1% of the evaluated population had some cardiovascular manifestation. Considering that this is a retrospective study, our results should be interpreted with caution. A meta-analysis evidenced a 64% increase in the risk of developing fatal and/or non-fatal cardiovascular disease in individuals with NAFLD in comparison with those without the disease [34]. However, the observational design of the studies included in this meta-analysis did not allow the authors to draw definitive causal inferences; moreover, in most situations, the diagnosis of NAFLD was established by ultrasound or computed tomography, which are only able to diagnose simple steatosis and not advanced stages of the disease [34]. In a study that included cases with histologically confirmed NAFLD, researchers demonstrated that advanced liver fibrosis was related to a greater risk of developing CVD, although limitations regarding sample size and a restricted selected population should be taken into account [35]. In parallel, a recent study showed that regardless of BMI, metabolically unhealthy individuals were at higher risk of developing coronary heart disease compared to healthy people [36]. On the other hand, overweight and obese people were more likely to develop coronary heart disease compared to eutrophic individuals [36]. The link between NAFLD/MAFLD and cardiovascular risk seems intuitive, and specific management for the prevention of these clinical conditions and the associated mortality, in

addition to lifestyle advice, are not mentioned in the current guidelines for CVD and should be the subject of debate among specialists [37, 38].

In this study, we observed a significant increase in non-fatal cardiovascular events in patients who met diagnostic criteria for MAFLD and had concomitant chronic viral hepatitis compared to those with MAFLD and no viral infection. Additionally, we reported a significant increase in the number of patients at high risk of developing atherosclerotic cardiovascular disease. This is original information, and our results regarding cardiovascular risk and the occurrence of cardiovascular events in this population should be further explored. The role of HCV infection in the development of CVD is related to its interference in glucose and lipid metabolism (resulting in metabolic syndrome) or to its participation in the activation of mechanisms that facilitate chronic inflammation and/or endothelial dysfunction [39]. Studies have addressed the systemic and cardio-metabolic risks associated with fatty liver; for example, patients with "virus-associated fatty liver disease" have an increased risk of developing CVD and type 2 diabetes mellitus. However, in clinical practice, different medical expertise is involved in the diagnosis and management of cardiovascular risk in this population [40]. The association of HCV infection with CVD is still an issue of controversy. Some reports corroborate how our data demonstrated a high risk of developing CVD in HCV-infected persons, whereas others have not reported such an association [40–42].

In this real-life study, most patients did not receive any specific pharmacological therapy for NAFLD. Currently, there are no approved drugs for NAFLD, but phase II and III clinical studies are evaluating new therapies. In addition, efforts are being made to discover new therapeutic strategies that can promote both liver and heart benefits in this multimorbid population with several metabolic risk factors [43–46]. Despite the complexity of this disease, many cases of hepatic steatosis can be reversible if the causal factor is eliminated. Vilar-Gomez et al. prospectively evaluated a group of patients diagnosed with NASH who were submitted to a low-calorie diet combined with physical exercise. Significant changes in histological characteristics related to the disease were reported after 12 months [47]. In cases of MAFLD, the treatment of comorbidities such as viral hepatitis or alcoholic liver disease will certainly lower the cardiovascular risk [8, 10].

This study explores the differences between NAFLD and MAFLD regarding cardiovascular events in an unprecedented way. All patients were diagnosed with steatosis through liver biopsy, most of them with NASH or, in the case of MAFLD, with liver inflammatory activity. These findings may explain why we did not observe a difference between cardiovascular outcomes of MAFLD and NAFLD cases in our population, since liver inflammation alone can be associated with the induction of systemic inflammation. The findings of this study justify that the cardiovascular health of patients with NAFLD and MAFLD should be observed, and those with MAFLD and viral hepatitis may deserve greater surveillance. Some limitations warrant mention. This is a retrospective cross-sectional study, which limits our ability to infer temporal or causal relationships. It is a population study performed in a single center, therefore, the number of evaluated cases is limited. Clinical and laboratory data were collected from medical records and some information could have not been obtained due to the retrospective nature of the study. In addition, the fact that patients with HCC were included can also be considered a limitation.

In conclusion, in this study we observed an intermediate/high cardiovascular risk in individuals diagnosed with NAFLD or MAFLD, but there was a significant increase in non-fatal cardiovascular events in patients with MAFLD and concomitant HCV and/or HBV infection in comparison to other cases of MAFLD. Based on the important risk of cardiovascular events in this population, NAFLD/MAFLD management should focus on modifying lifestyle and risk factors, not only reducing the risk of progression of the liver disease but also providing benefits in reducing possible cardiac complications. Strict monitoring must be guaranteed to assess adherence, tolerability, and the impact of interventions in the treatment of these patients.

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Author contributions GTSG: methodology, formal analysis, investigation, data curation writing—original draft and writing—review and editing visualization; LL: conceptualization, methodology, formal analysis, investigation, data curation writing—original draft and writing—review and editing visualization; MAF: conceptualization, methodology and review and editing visualization; VEGDS: formal analysis, investigation; MRÁ: conceptualization, methodology, data curation writing—original draft and writing—review and editing visualization, visualization, supervision and project administration.

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Data availability The data will be made available.

Compliance with ethical standards

Conflict of interest Gabriel Tayguara Silveira Guerreiro, Larisse Longo, Mariana Alves Fonseca, Valessa Emanoele Gabriel de Souza and Mário Reis Álvares-da-Silva declare that they have no conflict of interest.

Consent to Participate This study was approved by the Ethics Committee of Hospital de Clínicas de Porto Alegre, CAAE

79523617.1.0000.5327, and follows the guidelines for studies in humans. As this is a Retrospective cross-sectional study, it is not necessary to apply the consent form.

Consent to Publish Authors consent to the publication of this manuscript.

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