Use of non-invasive scores for the estimation of mortality risk in patients with Non-Alcoholic Fatty Liver Disease

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INDICE

Introduction

Although non-alcoholic fatty liver disease (NAFLD) is rarely associated with the progression to chronic liver disease and its complications, it remains a major public health concern due to its high prevalence and its strong association with the metabolic syndrome (MetSd), with an increased risk of cardiovascular events and with a higher risk of overall and cardiovascular mortality [1-6]. In this scenario, holistic treatment of NAFLD may provide prognostic benefit through a reduction of its related overall morbidity and mortality. Hence, the stratification of a patient's individual risk based not only on the evaluation of the stage of liver disease but also on the risk of cardiovascular disease, cardiac-specific and overall mortality has a pivotal role for implementing treatment with more aggressive strategies in high-risk patients.

Even though it is widely accepted that patients with NAFLD are at highest risk of cardiovascular mortality [4], to date, there is paucity of data to guide clinicians in this risk stratification and a major proportion of NAFLD patients may be undertreated. Recently, a novel definition for NAFLD has been proposed with the aim of ameliorating its diagnosis and of identifying with more precision those patients at higher risk of cardiovascular events [7-9]. This new entity has been termed metabolic dysfunction-associated fatty liver disease (MAFLD), and is diagnosed on the basis of the co-existence of fatty liver and diabetes or obesity/overweight or fatty liver and at least two metabolic dysfunctions among: waist circumference >102/88 cm in Caucasian men and women or >90/80 cm in Asian men and women; blood pressure ≥130/85 mmHg or specific drug treatment; plasma triglycerides >150mg/dl or specific drug treatment; plasma high-density lipoprotein (HDL)/cholesterol ratio < 2.5 or plasma high sensitive C-reactive protein levels >2mg/l.

Since the proposal of this new definition, increasing data has investigated the effects of such diagnostic implementation, with controversial results. Some data have shown that patients with NAFLD and MAFLD are at higher risk for overall mortality and for liver-related and cardiovascular mortality [10,11] but robust data as to whether this terminology actually increases the detection of patients at higher risk for liver-related and cardiovascular events is required.

In the general population and in patients diagnosed with steatosis or with MAFLD no single clinical/biochemical parameter can predict cardiovascular events nor mortality, but the use of non-invasive scores might be a simple, cost-effective and useful tool for this aim.

Among liver-related parameters, fibrosis stage is well known to be associated with worse liver-related outcomes and all-cause mortality [12] and increasing research data suggest that it also predicts a higher risk of cardiovascular mortality, but evidence is still controversial [13-19]. Nonetheless, patients with a NAFLD diagnosis, independently from the stage of disease, are at increased risk of both liver-related and cardiovascular events as compared with the general population [2,16,17], hence its assessment is of pivotal importance in clinical evaluation and decision making in order to collocate patients in adequate monitoring for both liver-related (most of all, screening for hepatocellular carcinoma (HCC) and prevention of fibrosis progression and decompensation) and cardiovascular events. Among non-invasive tests for liver fibrosis, NAFLD fibrosis score (NFS), Fibrosis-4 score (FIB-4) and liver stiffness measurement with transient elastography are the most reliable makers for the non-invasive estimation of liver fibrosis and have shown an independent association with overall, cardiovascular and liver-related mortality in NAFLD patients [15,17,20-27]. The stiffness value measured

by transient elastography is the most reliable test to exclude or include advanced fibrosis and cirrhosis, and therefore to assess patients' prognosis but, to the best of our knowledge, no studies have addressed specifically the potential association between liver stiffness measurements by transient elastography and the risk of cardiovascular disease.

With regards to the most widely used cardiovascular risk scores, the Framingham risk score (FRS) [28], the QRISK2 score and the Atherosclerotic Cardiovascular Disease Score (ASCVD) [29] have been studied in NAFLD cohorts and have been associated with an increased risk of overall and cardiac-specific mortality, but definitive data on this subject are still scarce [19,29-31]. Ultimately, a new score for the assessment of the risk of major cardiovascular events, the NAFLD cardiovascular (NAFLD-CV) risk score, has recently been developed and validated in a large cohort of patient from the United Kingdom [29]. Differently from the FRS, the QRISK2 and the ASCVD score, the NAFLD-CV risk score estimates the risk of a major cardiovascular event occurrence in one year and not in 10 years.

Currently, there are no validated pharmacological treatments for patients with NAFLD, yet the interconnection between NAFLD and cardiovascular disease postulates that diabetes treatment, relevant lifestyle adjustments, and medications such as antihypertensives and lipid-lowering agents may prove useful in optimizing the clinical management of these patients. However, in real life clinical practice patients with metabolic liver disease are frequently sub-optimally treated not only because of the fear of statin prescription in patients that may have baseline altered liver enzymes, but also due to a lack in clear indications for the evaluation and management of cardiovascular risk in these patients.

In this complex and somehow heterogeneous scenario, some retrospective studies have shown the applicability of some cardiovascular risk scores to the NAFLD population with interesting results. To the best of our knowledge, the FRS, the QRISK2 score, and the ASCVD score have proven reliable in assessing the cardiovascular risk in patients with NAFLD [29,30,32-34].

Aim of this study

With this study, we aimed to assess the cardiovascular and liver-related risk in a prospective, observational cohort of consecutive patients with NAFLD who were referred to our Unit for evaluation of liver disease. In these patients, the risk of future cardiovascular events and of liver-related events were estimated using validated risk score calculators and non-invasive tests for fibrosis. Additionally, we aimed to evaluate the potential differences in the estimated cardiovascular and liver-related risks between patients diagnosed with MAFLD and non-MAFLD patients (i.e. patients with steatosis not fulfilling the criteria for a MAFLD diagnosis).

In order to estimate the cardiovascular risk, we applied the FRS in its original and 2008 version, the QRISK2 score, the ASCVD risk score and the NAFLD-CV risk score. For the assessment of liver fibrosis, we used the reports obtained using transient elastography and validated, non-invasive laboratory tests for fibrosis such as the FIB-4 and the NFS.

Materials and methods

Study population

We prospectively enrolled all consecutive patients with a diagnosis of NAFLD at their first appointment at the specialist NAFLD clinic of the Gastroenterology Unit of Policlinico San Martino in Genoa, Italy. Enrolment began in September 1st, 2021 and ended on November 30th, 2022.

Inclusion criteria were age above 18 years and below 80 years and a NAFLD diagnosis by means of radiology [ultrasound scan (US) or magnetic resonance imaging (MRI)] or histology. We excluded patients with concomitant malignancy, human immunodeficiency virus (HIV) infection, other aetiologies of liver disease (hepatitis B virus (HBV) or hepatitis C virus (HCV) infection, autoimmune hepatitis, primary sclerosing cholangitis or primary biliary cholangitis, Wilson's disease, $Alpha_1$ anti-trypsin-deficiency, haemochromatosis, and past or current history of alcohol abuse and current alcohol intake.

Liver disease-related and cardiovascular risk scores were calculated at baseline visit and included the: FIB-4, NFS, QRISK2 score, ASCVD score, FRS and FRS 2008.

With regards to the definition of cardiovascular events, we included acute coronary syndrome, transient ischaemic attack and stroke.

NAFLD was defined by the presence of steatosis in > 5% of hepatocytes according to histological analysis, or by a proton density fat fraction (providing a rough estimation of the volume fraction of fatty material in the liver) >5.6% assessed by proton magnetic resonance spectroscopy or quantitative fat/water selective MRI, or by the presence of

steatosis at US or by the detection of steatosis with Controlled Attenuated Parameter (CAP) higher than 230 KPa.

Definition of glucose intolerance, diabetes, hypertension, dyslipidemia, overweight, obesity and metabolic syndrome were based on standard international definitions. MAFLD was defined as the presence of NAFLD and diabetes or overweight or two or more metabolic disorders as per the Expert consensus definition [7].

At the time of enrolment the following data were recorded: demographics (gender, age, ethnicity, smoking habit), anthropometric such as waits circumference and body mass index (BMI), biochemical [full blood count, mean platelet volume (MPV), fasting lipids, glucose and insulin, HbA1c, liver function tests (i.e. international normalized ratio (INR), albumin, alanine (ALT) and aspartate amino-transferase (AST), bilirubin), pharmacologic (anti-hypertensive and anti-diabetes treatment, use of aspirin and statins,), and clinical (arterial blood pressure) parameters. When available, liver stiffness and CAP, as assessed by transient elastography, was recorded; we also recorded the estimated liver disease stage using non-invasive fibrosis scores (NFS, FIB-4).

Cardiovascular risk scores

The FRS was first developed in the 1980's, it was applicable only in non-diabetic patients with no previous history of cardiovascular events aged between 18 and 75 years, and was designed to predict the 10-year risk of coronary heart disease [35]. In 2008, a modified version was validated, including more clinical parameters in order to define the 10-year risk of cardiovascular events (*i.e.*, not only coronary heart disease but also events such as: cerebrovascular and peripheral artery disease and heart failure), and

was applicable also in diabetic subjects and patients with a previous history of cardiovascular disease/events [36]. Moreover, while the original FRS results only estimated the patient's rough risk of coronary heart disease (while comparing it with the average 10-risk of myocardial infarction and death), the results of the revised 2008 version were also intended to give clinicians an indication for the optimization of lipid-lowering treatment by giving advice on target LDL cholesterol levels according to different guidelines (**Table 1**).The FRS algorithm considers age, sex, smoking status, total cholesterol, high density lipoprotein cholesterol (HDL), systolic blood pressure and current anti-hypertensive treatment. The result compares the risk of the subject with the average 10-year risk of myocardial infarction and death (Framingham Risk Score for Hard Coronary Heart Disease - MDCalc).

The FRS 2008 considers the same variables of the original version plus the presence of diabetes and history of previous cardiovascular events (such as coronary artery disease, stroke, peripheral vascular disease). The results give a 10-year risk estimation of cardiovascular events and treatment indications (**Table 1**) (Framingham Risk Score (2008) | QxMD).

The ASCVD Risk Calculator is similar to the FRS 2008 version as it was designed to estimate a patient's 10-year ASCVD risk at an initial visit to establish a reference point [37,38]. The American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommend the use of this estimate as an important starting point for decision making in primary prevention of ASCVD [39]. According to the estimated risk, the ASCVD score results are given with indications on statin initiation and dosage (**Table 2**). The ASCVD score risk calculator takes into account the following parameters: age, sex, smoking status, BMI, total cholesterol/ HDL ratio, hypertension, concomitant

chronic rheumatoid arthritis, atrial fibrillation, diabetes mellitus and family history for cardiovascular event. In patients with borderline or intermediate risk, the following risk enhancers are taken into account in order to better define the indication for pharmacological treatment: family history of early ASCVD (men <55 years old, women <65), current high cholesterol (i.e. low density lipoprotein (LDL) cholesterol 160-189mg/dl; non-HDL cholesterol 190-219mg/dL), MetSd, chronic kidney disease, chronic inflammatory conditions (i.e., rheumatoid arthritis, psoriasis, HIV), history of pre-eclampsia or early menopause, high-risk ethnicity (*e.g.*, South Asian Ancestry), high lipid biomarkers, triglycerides ≥175 mg/dL, high-sensitivity C-reactive protein (*i.e.*, ≥2.0 mg/dL), elevated lipoprotein (a) (i.e. ≥50 mg/dL or ≥125 nmol/L), elevated apolipoprotein B (≥130 mg/dL), ankle-brachial index <0.9. To calculate this parameter we used the following calculator 2018 Prevention Guidelines Tool CV Risk Calculator (heart.org). Based on the estimated risk, the ASCVD score results are given with indications on statin initiation and dosage (**Table 2**).

The QRISK2 is a well-established cardiovascular disease risk score, in use across the United Kingdom since 2009, which is designed to identify people at high risk of developing cardiovascular disease who need to be recalled and assessed in more detail to reduce their risk of developing cardiovascular events. Similar to the FRS and the ASCVD score, the QRISK2 score estimates the risk of a person developing cardiovascular disease over the next 10 years and compares it with the score of a healthy person of the same age, sex, and ethnic group [*i.e.*, with no adverse clinical indicators and a cholesterol ratio of 4.0, systolic blood pressure of 125 and a BMI of 25] [40,41].The QRISK2 score algorithm considers the following variables: age, sex, smoking status, BMI, total

cholesterol/HDL ratio, hypertension, concomitant chronic rheumatoid arthritis, atrial fibrillation, diabetes mellitus and family history for cardiovascular events (QRISK2-2017).

The NAFLD CV risk score was elaborated and validated in the UK in 2020, it considers MPV, age and history of diabetes, and gives an estimate on the 1-year risk of major cardiovascular events. Cardiovascular death, acute coronary syndrome, stroke, and transient ischaemic attack were defined as major acute cardiovascular events). Acute coronary syndrome was defined as a diagnosis of ST-myocardial infarction, type 1, Non-ST myocardial infarction and/or unstable angina (LDEye (ld-eye.com)) [29].

Non-invasive tests for fibrosis

Simple non-invasive panels such as the NFS and FIB-4 are recommended by the European Association for the Study of the Liver (EASL) – European Association for the Study of Diabetes (EASD) - European Association for the Study of Obesity (EASO) Clinical Practice Guidelines as part of the diagnostic regimen for ruling out advanced fibrosis [42]. The guidelines further recommend the use of NFS and FIB-4 as prognostic markers to rule out progression to severe disease, including liver-related and all-cause mortality.

The FIB-4 score was created to estimate the presence of advanced fibrosis originally for HCV-related hepatitis, but it was subsequently approved also for its use in NAFLD. The algorithm considers age, AST, ALT levels and platelet count. Using a lower cut-off value of 1.45, a FIB-4 score <1.45 has a negative predictive value of 90% for advanced fibrosis (Ishak fibrosis score 4-6 which includes early bridging fibrosis to cirrhosis). In contrast, a FIB-4 >3.25 has a 97% specificity and a positive predictive value of 65% for advanced fibrosis [43].

The NFS formula is another accurate non-invasive test for the estimation of liver fibrosis. Once the above formula is calculated the resultant score is interpreted after the following: <-1.455: indicates the absence of significant fibrosis (F0-F2 fibrosis); ≤-1.455 to ≤0.675: an indeterminate score; >0.675: indicates the presence of significant fibrosis (F3-F4 fibrosis) [44].

Transient elastography is the most reliable non-invasive tool for the assessment of liver fibrosis [45]. We considered the following cut-offs for defining the different grades of fibrosis: no fibrosis 0 (F0) if stiffness values < 5 KPa; low fibrosis (F1) if ≥ 5 KPa and ≤ 8 KPa; moderate fibrosis (F2) if ≥ 8 KPa and ≤ 10 KPa; advanced fibrosis (F3) if ≥ 10 KPa and < 14 KPa and cirrhosis if ≥ 14 KPa. For the assessment of steatosis, we considered absence of steatosis if CAP values were below 230 KPa, low grade steatosis if CAP values were between 231 and 260 KPa, moderate steatosis for CAP values between 261-280 KPa and severe steatosis for CAP values higher than 280 KPa.

These scores have been demonstrated to predict not only fibrosis stage but also the risk of liver-related events and of mortality [15,17,20-27]. Based on a study by Angulo et al., based on the NFS, patients in the intermediate-risk and high-risk groups, compared with the low-risk group, have a 7.7-fold and 34.2-fold higher risk of developing liver related events, respectively. Based on the Fib-4 score, only the high risk group show a significantly higher risk of liver-related events, with an adjusted Hazard Ratio (aHR) of 6.9 (95% Confidence Interval (CI) 2.3–20.4, p = 0.001). The aHRs for death or liver transplantation in the intermediate-risk and high-risk groups compared with the low-risk group are 4.2 (95% CI: 1.3–13.8) and 9.8 (95% CI: 2.7–35.3), respectively, based on the NFS. Based on the FIB-4 score, only the high-risk group has a greater risk of death or liver transplantation (aHR = 6.6; 95% CI: 2.3–20.4)[15].

Based on transient elastography, a liver stiffness ≥ 15 KPa is associated with a significant risk of developing liver-related events. As shown in a recent study, patients with liver stiffness measurement > 15 show significantly higher incidence of HCC than those with liver stiffness between 10–15 KPa or those with liver stiffness <10 KPa (13.4%, 9.7%, and 2.1%, respectively, $p < 0.001$). Moreover, a liver stiffness >15 KPa showed a significantly higher incidence of hepatic decompensation than a liver stiffness between 10–15 KPa or < 10 KPa (7.6%, 2.1%, and 0.2%, respectively, $p < 0.001$) [27].

This research was fully approved by the Ethics Committee.

Statistical analysis

Statistical analysis was performed using SPSS version 20.0 for Windows (IBM, New York).

Descriptive statistics were computed for all variables, with continuous variables expressed as means and standard deviation (SD), and categorical variables expressed as relative frequencies and percentages.

RESULTS

Demographic characteristics

Over the study period, 106 patients were consecutively admitted at the NAFLD specialist clinic. Baseline characteristics are shown in **Table 3**. There were 56 (52.4%) female and 50 (47.6%) men. Median age was 60 (+/-18) years. NAFLD diagnosis was made by means of ultrasound scan in most cases (82.1%), while only approximately one-fifth of cases (21 patients) had a histological diagnosis. Mean CAP was 284 kPa (±51.5). In the subgroup of patients who underwent liver biopsy, 85.7% had histologic features of NASH and 35% had cirrhosis at diagnosis, a finding that was in accordance with what estimated by transient elastography. Overall, advanced fibrosis and cirrhosis were present in approximately one-third of the study population (34.5 %), while 65.6% subjects had no or moderate fibrosis as assessed by transient elastography. All patients with cirrhosis had compensated liver disease.

Approximately one third of the study population had diabetes (34 subjects) whereas 6 (6%) subjects had impaired glucose tolerance. The prevalence of hypertension was 54.7% while that of obesity and overweight were 28.3 % and 36.8 %, respectively. Mean BMI was 27.5 kg/m^2 (±4.6). Metabolic syndrome was present in 55 subjects (51.9%) while a diagnosis of MAFLD was applicable to 73 (68.9%) of the study subjects. Most of the population were non-smokers (85.7%) and only 14.3% of the subjects were actively smoking. Overall, 7 patients had experienced a previous cardiovascular event and only 1 patient had a history of chronic kidney disease.

Patients with MAFLD had higher prevalence of advanced fibrosis as assessed by transient elastography (p = 0.038) and cirrhosis (p= 0.001). 0.001Hypertension (68.5%),

obesity (39.7 %), overweight (49.3 %), diabetes (46.6 %), and MetSd (74.0 %) were highly prevalent in these patients. The BMI was higher in MAFLD patients (30.1 ± 5.1 vs 23.0 ± 1.9; p = 0.001), as were triglycerides (median 131 vs 100 mg/dL, p = 0.001) and CAP values (312.0 KPa vs 261 KPa, p = 0.008). Median albumin levels were overall in the normal range in both patients with and without MAFLD, although they were significantly lower in the former group (4.3 vs 4.5 g/L, p= 0.038); contrariwise, AST (25 IU/ml vs 30 IU/ml) and ALT (30 IU/ml vs 38 IU/ml) levels were lower in the MAFLD group (p = 0.03 and 0.009, respectively), but were within normal range. Lastly, the NFS was significantly higher in the MAFLD group (**Table 4**).

Cardiovascular risk scores

As defined by the QRISK2 score, 19 (17.5%) of the study population showed same cardiovascular risk as compared with that of a healthy person of same age, sex and ethnic group. 24 (22.7%) subjects showed a lower risk and 63 (59.8%) a higher risk of cardiovascular events. Overall, the cohort showed a 1.5 – fold higher risk of cardiovascular events as compared with healthy individuals of same age and sex and with no adverse clinical indicators and a cholesterol ratio of 4.0, systolic blood pressure of 125 and BMI of 25 (as defined by the QRISK2 algorithm). Among patients with high QRISK2 score risk, as much as 87.9% had MAFLD (p = 0.001), while 72.4 % had MetSd (p = 0.001). At univariate analysis, the variables that predicted a higher QRISK2score were BMI (p = 0.006), cholesterol/HDL ratio (p=0.001), presence of diabetes (p=0.001), triglycerides(p = 0.001), presence of MetSd and MAFLD (both p=0.001), smoking status (p = 0.001), the presence of advanced liver fibrosis/cirrhosis at transient elastography, and a clinical diagnosis of cirrhosis (p = 0.02 and 0.001, respectively). When considering non-invasive scores for the estimation of liver fibrosis, only the NFS resulted significantly

associated with a higher QRISK2 score. At multivariate analysis, only the presence of MetSd was still statistically significant (p = 0.016).

With regards to the ASCVD score, 26.3% cases showed high risk values, 32.5% showed intermediate risk whereas 25% showed a low risk and 16.3% showed a borderline risk (between low and intermediate). Among patients with intermediate-high ASCVD, nearly 91.5% had MAFLD (p = 0.001?) and 72.3 % had MetSd (p = 0.001). At univariate analysis, BMI (p = 0.021), total cholesterol (p = 0.036), total cholesterol/HDL ratio (p = 0.037), INR (p = 0.001), age (p = 0.001), platelet count (p = 0.001), LDL (p = 0.031), Fib-4 (p = 0.001), NFS (p = 0.001), advanced fibrosis (F3-F4) at transient elastography (p = 0.005), CAP value (p= 0.048) ,clinical diagnosis of cirrhosis (p=0.01), diabetes (p = 0.007), obesity (p = 0.043), hypertension (p = 0.002), MetSd (p = 0.001), and MAFLD (p = 0.001) were significantly associated with high ASCVD risk score. At multivariate analysis, none of the variables resulted still significantly associated with high ASCVD score.

The figure for the original Framingham risk score (FRS), which was applicable only for the non-diabetic population, was different, since as much as 87.1% of cases showed a lower risk of cardiovascular events as compared with that estimated for the general population by the FRS calculator and only 6.3% cases showed a high-risk profile. Conversely, when the FRS 2008 version was used, 43.1% cases showed a high-risk profile, 25.5% a moderate risk and 31.4% a low-risk profile. Among patients with moderate-high FRS 2008, 80% were diagnosed with MAFLD (chi square p = 0,022), while 62.9% presented MetSd (p = 0.001). Variables associated with a high risk as per FRS 2008 at univariate analysis were: advanced fibrosis or cirrhosis at transient elastography (F3-F4) (p= 0.003), clinical diagnosis of cirrhosis (p = 0.003), diabetes (p = 0.038),

hypertension (p= 0.001), MetSd (p = 0.022), MAFLD (p = 0.006), and NFS (p = 0.014). At multivariate analysis, none of the variables resulted statistically significant.

With regards the NAFLD-CV risk score, almost a third of the study population (28.3%) showed a high risk of developing a major cardiovascular event in one year. The risk factors for a higher NAFLD-CV risk score were, as expected, diabetes (p= 0.020), age (P= 0.001), mean platelet volume (p=0.001), platelet count (p= 0.008), basal glucose (p=0.008), hypertension (p= 0.018), advanced fibrosis and cirrhosis at transient elastography (p= 0.021), and clinical diagnosis of cirrhosis (p = 0.011). Also the non-invasive tests NFS (p = 0.02) and FIB-4 (p= 0.01) were significantly associated with high NAFLD-CV risk score, while no statistically significant association was found for MetSd and MAFLD even though the proportion of patients presenting high NAFLD CV risk score and MAFLD or MetSd was quite twice that of patients with high NAFLD CV risk score without of MetSd (36.4% vs 19.6%) and tended to be higher for those with MAFLD (32.9% vs 18.2%). At multivariate analysis, no single variable was associated with a higher risk of NAFLD CV risk score.

Differences in CV events between MAFLD and non-MAFLD patients

Patients with MAFLD had a higher prevalence of moderate and high cardiovascular risk as estimated by the QRISK2 score (74.2% vs 29.0%, p = 0.001), by the ASCVD score (71.2% vs 23.8 %, p = 0.001) and the FRS 2008 (78.6% vs 46.9% p = 0.003). Conversely, the 1-year risk of major cardiovascular events, as assessed by the NAFLD CV risk score, was not statistically different (Table 4).

Non-invasive tests for liver fibrosis

Mean transient elastography stiffness values was 9.67 kPa (± 9.60). Almost a fifth of patients (n. 17, 18.3%) showed absent fibrosis, nearly 39% (n. 36) showed fibrosis stage 1, whilst 8 (8.6%) had fibrosis stage 2, 9 (9.7%) fibrosis stage 3 and 23 (24.8%) had cirrhosis at stiffness values measurements.

Mean FIB-4 was 1.81 (± 1.81) whereas mean NFS was -0.903 (± 1.826), which both are indeterminate for the estimation of liver fibrosis. Overall, 61 (57.5%), 26 (24.5 %) and 19 (17.9%) of patients showed FIB-4 values indicative for excluding advanced fibrosis, indeterminate and probable advanced fibrosis, respectively. The figure for NFS results was similar as 43 (40.6%) subjects had NFS values suggestive of low grades of fibrosis, 39 (36.8%) subjects presented indeterminate values and 24 (22.6%) had NFS suggestive of high fibrosis. Therefore, based on the evidence by Angulo et. al, based on NFS 59.4% patients had a high risk of liver-related events in a mean FU of 8 years. Based on Fib-4, 17.9 % patients had a higher risk of liver-related events.

With regards to transient elastography, 17 (18.3%) had no fibrosis, 36 (38.7%) had low grade fibrosis, 8 (8.6%) had moderate fibrosis, 9 (9.7%) had advanced fibrosis and 23 (24.8%) had cirrhosis.

Differences in NITs for MAFLD and non-MAFLD patients

Patients with MAFLD showed higher rates of high NFS as compared with the non-MAFLD counterpart (31.5% vs 3,1%), a finding that was in line with the transient elastography findings. Indeed, patients with MAFLD had higher prevalence of stiffness values suggestive of advanced fibrosis and cirrhosis, as compared with non-MAFLD. Contrariwise, no statistically significant difference was shown with regards to the categories of Fib-4 (Table 4).

CV risk based on grades of fibrosis as assessed by transient elastography

We compared the CV risk in subgroups of patients with low to moderate fibrosis and with advanced fibrosis and cirrhosis as by transient elastography measurements. Patients with advanced fibrosis/cirrhosis had a significantly higher risk of cardiovascular events as estimated by all the cardiovascular risk scores. Indeed, around 70-80% patients with advanced fibrosis and cirrhosis showed high risk by QRISK2 ($p = 0.014$), by ASCVD score ($p = 0.005$) and by FRS 2008 ($p = 0.018$). Notably, patients with low to moderate grades of fibrosis had a high risk of cardiovascular events, but still the proportion of patients presenting a high risk was half that of patients with advanced fibrosis and cirrhosis (Table 5). When we assessed the distribution of the cardiovascular risk in the subgroups of patients by NFS or Fib-4, we found that only patients with high Fib-4 had a significantly higher CV risk as per FRS2008 and QRISK2, whereas both intermediate and high-Fib4 had significantly higher CV risk as per ASCVD.

Discussion

NAFLD is a growing global health concern, and its damaging effects in terms of cardiovascular risk are becoming increasingly more apparent. NAFLD is estimated to affect around one quarter of the world population and is often comorbid with other metabolic disorders including diabetes mellitus, hypertension, coronary artery disease, and metabolic syndrome. NAFLD has a causative relationship with metabolic syndromes [3,4,17] and a wealth of evidence supports the link between NAFLD and non-alcoholic steatohepatitis (NASH) and atherosclerosis and major cardiovascular events [4,5,46,47].

With this study, we aimed to assess the baseline liver-related and cardiovascular risk of a real-life cohort of NAFLD patients admitted at the NAFLD specialist clinic of the Gastroenterology Department of a secondary referral centre in Genoa, Italy. Most patients were admitted after an indication from their general practitioner, while a minor proportion were identified in the course of specialistic evaluation.

Overall, we found that a high proportion of patients, corresponding to approximately two-thirds of the study cohort, showed at least a moderate or high risk of cardiovascular morbidity at 10-year, as estimated by the application of the ASCVD score, the QRISK2 score and the FRS in its 2008 version. Moreover, almost one-third of patients showed a high 1-year risk of MACE at the NAFLD CV risk score estimation.

Assessment of these scores is recommended in clinical practice as part of a strategy aimed at primary prevention for the general population, with the objective to identify those patients who deserve more intense preventive treatments so as to minimize their risk of cardiovascular events by reducing, or ideally eliminating, the main risk factors (*i.e.*, hypertension, diabetes, overweight, LDL levels) with lifestyle coaching

and the prescription of drugs, when necessary. The results from this study were somewhat expected as it is well known that patients affected by steatosis carry an inherently higher risk of cardiovascular events, independently from the presence of other metabolic disorders, and that this risk further increases in patients with one or more comorbid metabolic dysfunctions [4,16,48-51].

In our study cohort, MetSd and MAFLD were highly prevalent as 51.9% subjects showed the MetSd and 68.9% could have been defined as MAFLD-patients. Therefore, the finding of an overall increased risk of cardiovascular disease was not surprising. However, we did not find an independent association between MAFLD and an increased cardiovascular risk. Even if the comparison of subjects diagnosed with MAFLD with those without MAFLD showed a significantly higher proportion of patients with a high cardiovascular risk in the MAFLD cohort (around 80% vs 28-40%) as shown in Table 4, when we looked for the variables that predicted a higher cardiovascular risk in the overall cohort, the only variable that was significantly associated with a higher cardiovascular risk was the presence of MetSd and not MAFLD, at least as estimated by the QRISK2 score. Indeed, in the overall cohort, nearly 60% patients showed an increased QRISK2 score and at the univariate analysis, the MetSd, its features and MAFLD were all significantly associated with an increased QRISK2. However, when the variables were analysed in binary logistic regression, only the MetSd remained significantly associated with a high cardiovascular risk. We expected to find similar results when analysing the variables associated with a moderate-high ASCVD score, as 57.5% subjects showed an intermediate or high-risk profile and metabolic dysfunctions, MAFLD and MetSdd were associated with a higher cardiovascular risk in the univariate

analysis but in multivariate analysis, no single variable was still predictive of a higher ASCVD score. Similar results were observed for the FRS 2008.

The finding of an increased cardiovascular risk only for patients with MetSd and not for those with MAFLD at multivariate analysis, as estimated by the QRISK2 score, was unexpected given the fact that MAFLD definition was validated not only with the aim of ameliorating the identification of patients with steatosis but also with that of implementing the identification of those at higher risk of advanced liver disease, cardiovascular disease and mortality [7]. Moreover, previous studies have reported that MAFLD identifies with higher accuracy patients at higher cardiovascular risk than standard NAFLD definition [10,52-54]. However, these findings are derived by heterogeneous cohorts from retrospective studies and, to the best of our knowledge, no prospective studies investigating this issue have been published yet.

Our findings may be explained by the small numerosity of the study cohort or may actually indicate that the usefulness of the MAFLD definition for the estimation of cardiovascular risk deserves further research. We could argue that MAFLD diagnostic criteria may be useful to augment the diagnosis of NAFLD and to better identify patients at higher risk of liver-related events, but may not be effective in the risk stratification of cardiovascular prognosis. For this prognostic purpose, the most important risk factor for cardiovascular disease may rely on the presence of MetSd rather than solely on a MAFLD diagnosis. Undoubtedly, patients with MAFLD are at higher risk of cardiovascular events as compared with non-MAFLD. However, considering the fact that MAFLD is diagnosed on the basis of the presence of steatosis together with diabetes or obesity or at least two metabolic dysfunctions [7], all patients with steatosis and metabolic syndrome can be diagnosed as MAFLD-patients. Hence, even if apparently MAFLD definition seems to

globally better identify the NAFLD patients at higher cardiovascular risk, even among patients with MAFLD probably the highest risk is conferred by being diagnosed with MAFLD and MetSd and not solely on the basis of being MAFLD-patients. Therefore, our results suggest that patients with MAFLD and MetSd are those at higher cardiovascular risk and who deserve intense cardiovascular screening and aggressive prevention.

Among patients diagnosed with MAFLD we found a significantly higher proportion of cirrhosis. This finding indicates that patients with MAFLD may be at highest risk of fibrosis progression and this is in line with extensive literature showing that diabetes, obesity and MetSd increase the risk of cirrhosis [10,55-59]. Indeed, diabetes ($p = 0.001$), obesity ($p = 0,01$), hypertension ($p=0.02$), MetSd ($p = 0.001$) which were all more prevalent in MAFLD, and MAFLD ($p= 0.001$) were all independently associated with the presence of cirrhosis in our study.

When we looked for the variables associated with a higher ASCVD score, FRS 2008 and a higher NAFLD CV risk score, at multivariate analysis no single variable resulted predictive but this finding may be reflective of the small numerosity of the study cohort.

A significant finding of this study is that according to the treatment indications based on these risk calculators, a relevant proportion of patients were actually undertreated. Indeed, if we consider the recommendations given by the AHA/ACC guidelines which are based on the results of the ASCVD score, the vast majority of patients with high ASCVD score (who should be on lipid lowering agents) were not receiving statins. In fact, 26.3% patients showed a high ASCVD score and should therefore have been on statin treatment, yet only 30% of them were actually taking the

drug. Moreover, only half of the patients who were taking the medication had target LDL levels. We found a similar figure when we considered the results from the indications as per the FRS 2008 and the QRISK2 score.

These data indicate that a significant proportion of patients with NAFLD who are at high risk of cardiovascular events are undertreated as their cardiovascular risk is not routinely assessed. Moreover, these findings may reflect that not only raising the awareness of the higher cardiovascular risk in this population is needed but also that more informative campaign regarding the safety and efficacy of statin treatment in NAFLD [60] is required. Indeed, not only statins are safe in patients with fatty liver but they may also reduce fibrosis progression [61] and the risk of cardiovascular disease [62]. Moreover, statins may reduce the risk of HCC [63]. Hence, in light of the potential pleiotropic effects of these drugs, statins should be used with less fear in patients diagnosed with NAFLD or MAFLD.

This study has the limit of a small numerosity and that NAFLD diagnosis was mainly based on imaging criteria. Indeed, only 17.9% of our patients were diagnosed based on liver histology but this is reflective of real-life clinical practice as biopsy is usually performed only in a minority of cases as it is invasive and more expensive. However, in real life clinical practice only a minority of patients with a NAFLD diagnosis undergoes a confirmatory liver biopsy and staging relies mostly on non-invasive tests. Furthermore, even if only a minority of patients had a histological diagnosis, the presence of steatosis in our study was assessed both by ultrasound scan or MRI and in all our subjects CAP values were greater than 230 KPa. Another limit of the study is that we may have lost some patients with MAFLD as we did not have the plasma C-reactive protein levels for most patients nor the HOMA-index. However, the strengths of our

study are that it is representative of the real life clinical practice, that it only included patients with a diagnosis of steatosis who did not have any other potential aetiology of liver disease, and that it has a prospective design. Moreover, to the best of our knowledge, this is the first real-life cohort study which gives a picture of the baseline cardiovascular risk in an NAFLD cohort by the utilisation of the ASCVD, the QRISK2, the FRS, the FRS 2008 and the NAFLD-CV risk score. Additionally, our results give the clinicians the important message that most of the NAFLD/MAFLD patients are not adequately assessed for their cardiovascular risk and are not therefore optimally treated with lipid lowering agents as recommended by the EASL guidelines.

In conclusion, we feel that more research with larger cohorts is needed to validate the applicability of cardiovascular risk scores in NAFLD. Moreover, due to the finding of an increased cardiovascular risk in patients with advanced fibrosis and cirrhosis, it should be assessed whether adding steatosis and/or fibrosis stage to the algorithms may help in further identifying patients with a higher cardiovascular risk. . Finally, this study confirms that patients with a MAFLD diagnosis, especially if comorbid with metabolic syndrome, have a significantly higher risk of cardiovascular events and are at higher risk of liver-related events as well and should therefore be regularly monitored at both liver diseases and cardiology specialist clinics.

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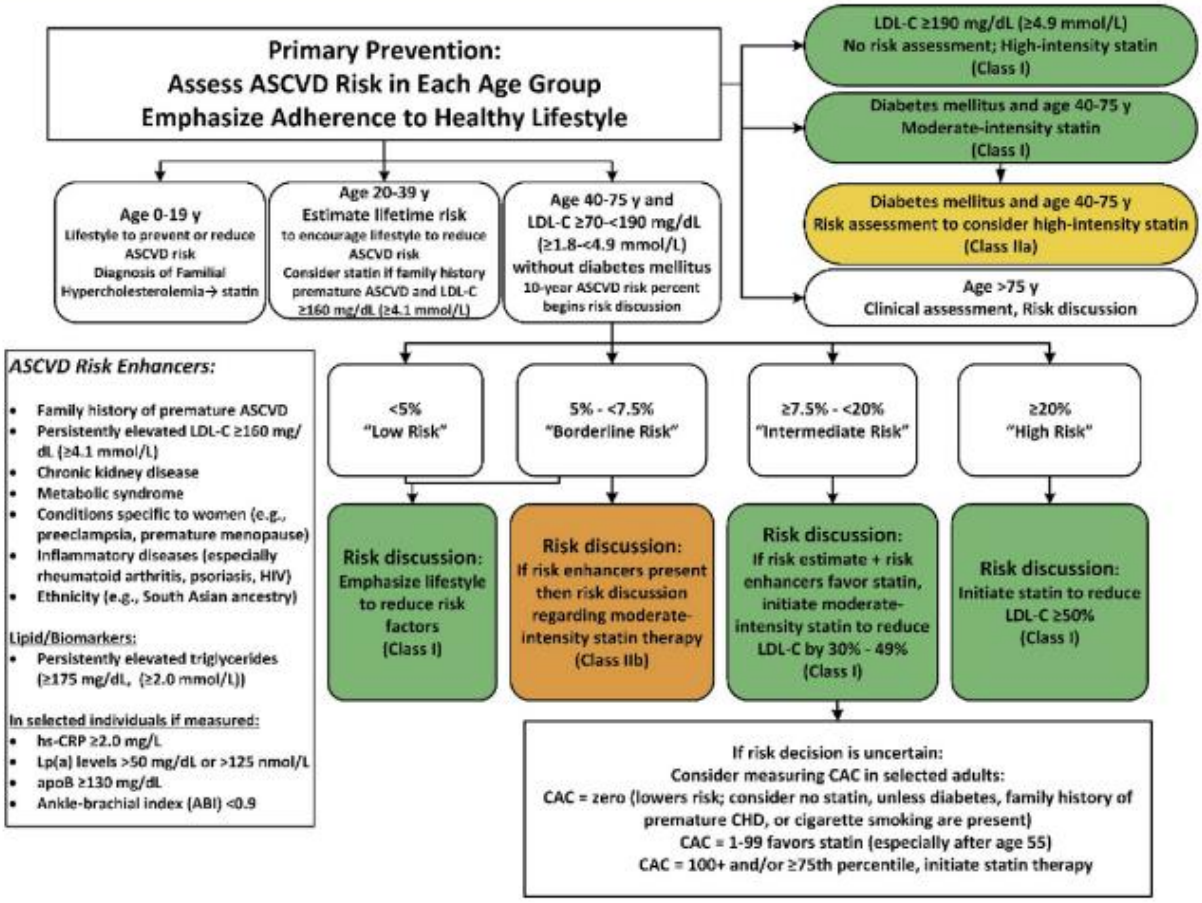
FIGURE 3 Primary Prevention



Colors correspond to Class of Recommendation in Table 1. ABI indicates ankle-brachial index; apoB, apolipoprotein B; ASCVD, atherosclerotic cardiovascular disease; CAC, coronary artery calcium; CHD, coronary heart disease; HIV, human immunodeficiency virus; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; and Lp(a), lipoprotein (a). Reproduced with permission from Grundy et al. (S4.3-1). Copyright © 2018, American Heart Association, Inc., and American College of Cardiology Foundation.