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COORDINATOR: PROF. GIOVANNI SQUADRITO

Prevalence of NAFLD and cardiovascular risk assessment in patients with newly diagnosed familial combined hyperlipidemia.

Ph.D. Candidate:

Valentina Cairo, MD

Supervisor

Prof. Giovanni Squadrito, MD

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ABSTRACT

Background: Nonalcoholic fatty liver disease (NAFLD), generally linked to obesity and metabolic syndrome, is the most common cause of liver disease worldwide. Patients with NAFLD compared to those without have higher risk of both cardiovascular and neoplastic disease and therefore have worse life expectation. NAFLD, defined as liver steatosis not caused by other known causes of liver disease, is characterized by a wide spectrum of clinic manifestation ranging from simple steatosis to nonalcoholic steatohepatitis, liver fibrosis, liver cirrhosis and hepatocellular carcinoma. NAFLD patients are often affected by other metabolic disorders such as diabetes mellitus and/or dyslipidemia. Dyslipidemias are disorders of lipid metabolism due to lipid accumulation in blood vessel, phenotypically classifiable into hypercholesterolemia, hypertriglyceridemia, and mixed forms. The most important complications of dyslipidemias are atherosclerosis and cardiovascular (CV) disease. No sufficient data are available on the prevalence and clinical significance of NAFLD in patients with new diagnosis of dyslipidemia.

Aim: Aim of the study was to evaluate the prevalence of NAFLD and to assess CV risk in patients with new diagnosis of familial combined hyperlipemia.

Materials and methods: We enrolled 80 patients [mean age 52.5 ±9.45 SD; median 53.5 (range 18-75 years); 37 males/43 females] referred by general practitioners to the Department of Internal Medicine of Messina University Hospital. All patients had dyslipidemia that was defined as follows: total cholesterol higher than 240 mg/dl and/or LDL higher than 160 mg/dl, HDL lower than 40 mg/dl (man) or 50 mg/dl (women), and ApoB >120 mg/dl. Steatosis was assessed by both hepatic steatosis index (HSI) and abdomen ultrasound (US). Liver fibrosis was non-invasively assessed by transient elastography (TE) and by fibrosis 4 score (FIB4). Presence of atherosclerosis was assessed by carotid ultrasound to identify carotid intima media thickness (c-IMT) and presence/absence of plaque.

Results: Liver steatosis was found in 56/80 patients (70%) by US examination. According to HSI, liver steatosis was diagnosed in 34 patients (42.5%), absent in 8 patients (10%), inconclusive in 38 patients (47.5%). US examination identified liver steatosis in 22 patients in whom HSI did not reveal steatosis, whereas 4 patients had HSI diagnostic for steatosis, but they had no US steatosis. We therefore analyzed two group of patients: a) the steatosis group (subjects with steatosis diagnosed by either US or HSI [n=60, 75%]) and b) a group without steatosis, where both US and HSI excluded steatosis (n= 20, 25%). We found that patients with

steatosis had a significantly higher BMI compared to those without ($p < 0.05$). Liver steatosis correlated with fasting insulin ($p < 0.05$), liver stiffness ($p < 0.05$), BMI ($p < 0.001$), and inversely correlated with HDL-cholesterol ($p < 0.05$). Liver fibrosis assessed by TE was significantly associated with BMI ($p < 0.001$) and c-IMT ($p< 0.05$), and fibrosis assessed by FIB4 was significantly associated with sex ($p < 0.05$), c-IMT ($p < 0.05$) and atherosclerotic plaque ($p < 0.05$). The presence of any grade of liver fibrosis was significantly associated with atherosclerotic plaque (OR 4.760, $p < 0.05$), independently from arterial hypertension, sex and smoke habit (OR 4.624, $p=0.008$ from the multivariable model).

Conclusion:

In our cohort of patients with newly diagnosed familial combined hyperlipemia we found a high prevalence of hepatic steatosis. Indeed, the risk of atherosclerotic plaque increased in patients with liver fibrosis, making possible to speculate a possible connection between liver disease and CV damage in dyslipidemic patients.

Introduction

Nonalcoholic fatty liver disease

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of liver disease worldwide and its prevalence overlaps with that of obesity and metabolic syndrome [1-5]. Patients with NAFLD have high risk of developing both cardiovascular disease (CV) and malignancies, significantly reducing life expectancy as compared to non-NAFLD individuals [6]. NAFLD is defined by the presence in the liver of fat (steatosis), not related to other known causes of liver disease [1-3]. Patients with NAFLD may suffer from metabolic disorders such as type 2 diabetes mellitus (T2DM), arterial hypertension or dyslipidemia [7, 8]. Indeed, in 2020 a consensus of international expert proposed to change name disease from NAFLD to MAFLD (metabolic associated fatty liver disease) identifying the following diagnostic criteria:

- hepatic steatosis and at least one of:
- -overweight/obesity;
- - Type 2 Diabetes Mellitus;
- - or in lean people presence of two or more metabolic disorder between
    - a) abnormal waist circumference,
    - b) arterial hypertension,
    - c) hypertriglyceridemia,
    - d) low HDL cholesterol,
    - e) prediabetes,
    - f) insulin resistance,
    - g) abnormal levels of high sensitivity C reactive protein [9, 10].

NAFLD prevalence varies due to the different diagnostic technique used to detect it and due to geographical areas. In fact, it ranges from 17% to 46%, being the highest in South America (30%), Middle East (31%), and Europe (24%), Italy comprised (20-25%) and the lower prevalence in Africa (13.5%) [4, 5]. NAFLD is responsible for 6.3% of liver cirrhosis in Italy [11].

NAFLD hits differently according to gender, being women more preserved during reproductive period, but loosing this protection after menopause [12]. Age is associated with risk of NAFLD,

the highest prevalence for men is between 50 and 60 years (29,3%), and over 60 years for women (25.4%) [13, 14]

NAFLD prevalence varies according to different ethnicity with the highest prevalence in Hispanic, non-Hispanic whites and lowest prevalence in African Americans [15, 16].

Ethnicity ascendancy and heritability of NAFLD suggest an important role of genetic predisposition in disease developing. In fact, the following genes have been associated with steatosis: PNPLA3, TM6SF2, NCAN, PPP1R3B, MBOAT7 [17-19]. A recent meta-analysis showed that PNPLA3 mutation is linked not only with steatosis but also with fibrosis progression, regardless of the presence of obesity or diabetes mellitus [20]. Most common PNPLA3 polymorphism is a missense mutation [Ile148 → Met148 (I148M)]; the gene product is a protein involved in lipolysis and lipogenesis, and this SNP is more frequent in Hispanic [19, 21]. Genetic mutation in TM6SF2 is also associated to higher risk of liver steatosis and fibrosis independently from age, obesity,T2DM and PNPLA3 genotype [22, 23].

NAFLD is characterized by a wide spectrum of clinic manifestations ranging from simple steatosis, to steatohepatitis eventually leading to liver cirrhosis, hepatocellular carcinoma (HCC) and end stage liver disease or liver transplantation (Figure 1). Fibrosis progression is however faster in patients with NASH than NAFLD patients [24]. HCC arises in liver cirrhosis and in NASH also with lower degree of fibrosis, but it has also been established it can arise in NAFL [25]. Progression to end stage liver diseases is 14 % among patients with F2 fibrosis over 13 years, rising from 25% in those with F3 fibrosis [26]. Obesity and T2DM can trigger and sustain inflammation and fibrosis and are risk factors for progression of liver damage [17, 27-29], but liver fibrosis itself is the major risk factor for the progression of fibrosis [30]; in fact liver fibrosis is characterized by excessive amount of extracellular matrix (ECM), ECM is mainly produced by hepatic stellates cells HSCs, on liver injury, as excessive fat accumulation, HSCs once activated, start deposition of ECM; many cells are involved in activation and maintenance of HSCs such as hepatocytes, liver sinusoidal endothelial cells, and macrophages [31].

It is universally accepted that steatosis should be documented whenever NAFLD is suspected as primary disease or as a coexisting condition [1]. The initial diagnostic workup should include a non-invasive imaging examination and general liver biochemistry [1-3, 32].

US is considered the first-line imaging technique due to its low cost, and its broad availability. The low sensitivity among obese patients (BMI>40) represents the main intrinsic limit of US; moreover the diagnosis could be missed when liver fat is lower than 20% [33, 34].

Transient elastography (TE) is an ultrasound-based technology that have some application in NAFLD screening; indeed, it allows to simultaneously measure liver steatosis with continuous attenuation parameters (CAP), and liver fibrosis. CAP shows a good sensitivity; however TE is not used in clinical practice so far, since it has not been extensively validated [1, 35, 36]. Further studies are needed to assess its sensitivity, as compared with magnetic resonance and also in different histological patterns [32]

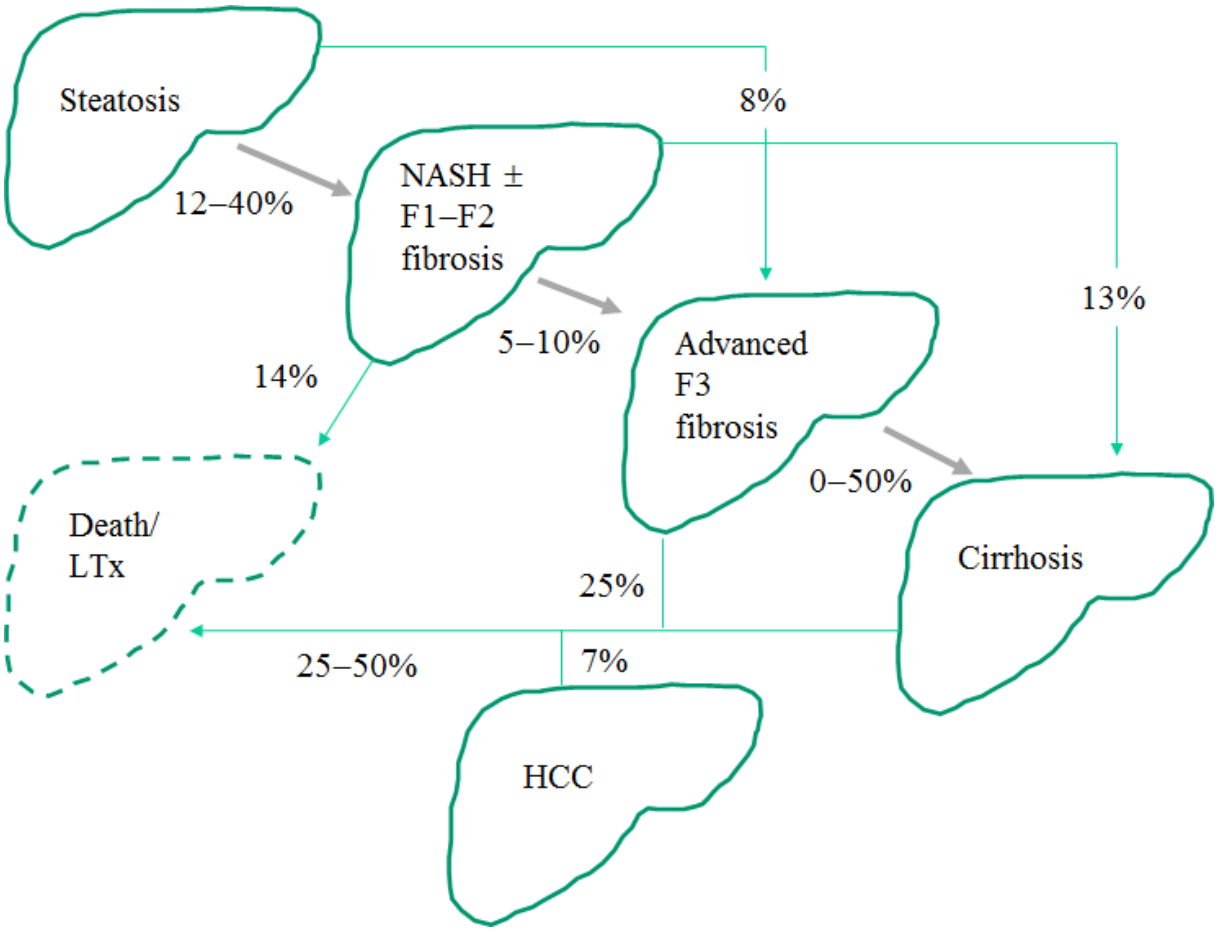

FIGURE 1 NAFLD NATURAL HISTORY ADAPTED FROM: DE ALWIS, NON-ALCOHOLIC FATTY LIVER DISEASE: THE MIST GRADUALLY CLEARS. J HEPATOLOGY. 2008 [37]

On the other hand, TE is validated as screening tool to assess liver stiffness; its cut-off has been established to 9.9 KPa for advanced fibrosis (≥ F3) with 95% sensitivity and 77% specificity [38], while at 7.9 KPa cut off is useful as screening test to exclude advanced fibrosis with

sensitivity of 91%, specificity of 75% and negative predictive values of 97% [32, 39]. Data suggest that combination of TE and biochemical scores performs better than either method alone [40]. The main shortcoming of TE is the unreliability in the presence of high BMI (although XL probes have been developed and used in some tertiary referral centres) and/or thoracic fold thickness.

Magnetic resonance imaging (MRI), either by proton density fat fraction (H-MRS) or spectroscopy, remains the non-invasive gold standard in assessing percentage of fat accumulation, detecting as low as 5% -10%. Magnetic resonance elastography performs better than TE in identifying different degrees of liver fibrosis in NAFLD patients [41]. Nevertheless, they have the same predictive value for advanced fibrosis stages [2, 32]. However, in clinical practice MRI is limited by high cost and poor availability.

Liver biopsy is the gold standard for the diagnosis of NASH because neither diagnostic imaging, nor non-invasive score can detect inflammation in NAFLD. In fact, histology provides the correct diagnosis based on the presence of steatosis, ballooning and lobular or portal inflammation [1] (figure 2). Other histological features can be seen in NASH but are not necessary for the diagnosis such as portal inflammation, polymorphonuclear infiltrates, Mallory-Denk bodies, apoptotic bodies, clear vacuolated nuclei, microvacuolar steatosis and megamitochondria [1]. The steatosis activity fibrosis (SAF) score is a score based on histological specimens used to assess disease activity; it was created including the semiquantitative scoring of steatosis, activity, and fibrosis. Fibrosis staging is based on simplified Kleiner classification [42].

Due to both its high costs and invasiveness, International guidelines recommend to use liver biopsy only in uncertain diagnosis and suspected NAFLD-related advanced liver disease [1].

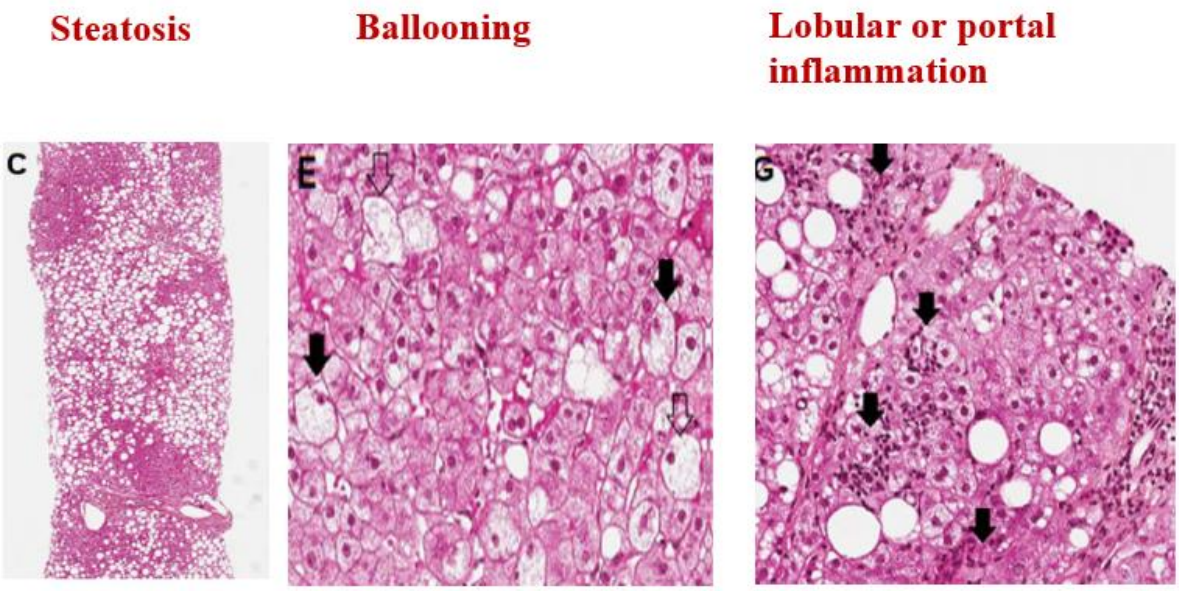

FIGURE 2: HISTOLOGICAL SPECIMEN OF STEATOSIS, BALLOONING, LOBULAR PORTAL INFLAMMATION [43]

Fatty liver index (FLI), NAFLD liver fat score (NAFLD-LFS) and hepatic steatosis index (HSI) are the most validated biochemical scores used to non-invasively assess steatosis. FLI is based on waist circumference, BMI, Triglycerides and gamma-GT [44]; NAFLD-LFS is based on the presence of metabolic syndrome, T2DM, fasting insulin and serum transaminases [45]; HSI is based on age, gender, BMI, and serum transaminases [46, 47].

NAFLD fibrosis score (NFS) and FIB4 are the most common biochemical scores used to assess liver fibrosis. NFS is based on age, BMI, serum transaminases, albumin, platelets, and presence of impaired fasting glucose or T2DM [48]. FIB4 uses age, serum transaminases and platelets [1, 47, 49]. FIB4 test predicts CV mortality, overall mortality and liver-related mortality [1], with higher cut–off of 2.67 has a positive predictive value of 80% to assess advanced fibrosis, a lower cut off of 1.30 has a negative predictive value of 90% to exclude advanced fibrosis [47, 49, 50].

A large number of studies demonstrated the accuracy of one or a combination of non-invasive scores in assessing liver fibrosis and can be adopted in clinical practice to guide surveillance and therapeutic approaches [50-52].

Dyslipidemias

CVD causes more than 4 million/years of death in Europe in 2015. Deaths are higher in women (2.2 million) than man (1.8 million) but this is inverse in individuals younger than 65 years old being deaths more common in men [53, 54].

Atherosclerotic cardiovascular disease (ASCVD) is the major component of CVD. Many studies have demonstrated the pivotal role of LDL cholesterol. The retention of LDL-C and other cholesterol-rich apolipoprotein B (APOB) containing lipoproteins are the key events in atheroma outset [55].

Epidemiological studies, Mendelian randomization studies and Clinical trials have consistently established a log-linear relationship among LDL-C reduction and CV risk reduction; moreover, lowering LDL-C reduces the risk of ASCVD proportionally to the absolute achieved reduction in LDL-C [56-62].

Dyslipidemias are characterized by alteration of lipoprotein metabolism. Plasma lipids are transported through blood vessels as lipoproteins, that are a complex of lipids (mainly cholesterol and triglycerides) in a wrap composed by protein (apolipoprotein) and phospholipids. They are classified based on their density as shown in figure 3.

	Density (g/mL)	Diameter (nm)	TGs (%)	Cholesteryl esters (%)	PLs (%)	Cholesterol (%)	Apolipoproteins	
							Major	Others
Chylomicrons	<0.95	80–100	90–95	2–4	2–6	1	ApoB-48	ApoA-I, A-II, A-IV, A-V
VLDL	0.95–1.006	30–80	50–65	8–14	12–16	4–7	ApoB-100	ApoA-I, C-II, C-III, E, A-V
IDL	1.006–1.019	25–30	25–40	20–35	16–24	7–11	ApoB-100	ApoC-II, C-III, E
LDL	1.019–1.063	20–25	4–6	34–35	22–26	6–15	ApoB-100	
HDL	1.063–1.210	8–13	7	10–20	55	5	ApoA-I	ApoA-II, C-III, E, M
Lp(a)	1.006–1.125	25–30	4–8	35–46	17–24	6–9	Apo(a)	ApoB-100

Apo = apolipoprotein; HDL = high-density lipoprotein; IDL = intermediate-density lipoprotein; LDL = low-density lipoprotein; Lp(a) = lipoprotein(a); PLs = phospholipids; TGs = triglycerides; VLDL = very low-density lipoprotein.

FIGURE 3: LIPOPROTEINS CLASSIFICATIONS, ADAPTED FROM 2019 ESC/EAS GUIDELINES FOR THE MANAGEMENT OF DYSLIPIDAEMIAS: LIPID MODIFICATION TO REDUCE CARDIOVASCULAR RISK [53].

Dyslipidemias are classified as familial dyslipidemias and secondary forms. Hypothyroidism, nephrotic syndrome, nervous anorexia, T2DM, and Cushing syndrome are the main causes of secondary forms of dyslipidemias.

Familial hypercholesterolemia (FH) is the major genetic dyslipidemia. It is a co-dominant monogenic hypercholesterolemia, and the heterozygosis is associated with CVD already before the age of 55 years for men and 60 years for women. Homozygous familial hypercholesterolemia is a rare life-threatening disease with onset of CVD before the age of 20 years, characterized by extremely high levels of TC (more than 500 mg/dl). Other clinical manifestation are tendinous xanthomata and arcus cornealis before age 45 years [53, 63, 64]. Genetic dysfunction linked to FH are: the loss of function mutations in LDL receptor or in APO B gene [65], or the gain of function mutations in proprotein convertase subtilisin/kexin type 9 (PCSK9) gene, that are responsible of FH, familial defective APO B (FDB) and FH3, respectively. Mutations in LDL-R and in APO B cause a defective interaction between LDL and its receptor with accumulation of LDL in circulation. PCSK9 gene product is a protein able to degrade LDLR, and a gain of function mutation leads to LDL accumulation [53, 66].

However, familial forms of dysplipidemias are often polygenic: the pattern of inheritance in fact does not suggest a monogenic transmission. Many single nucleotide polymorphisms affecting lipoprotein metabolism were found: each one has a little effect on lipid profile, and patients with polygenic dyslipidemias have many of these SNPs. Thus, the clinical presentation of disease is the result of summation of all lipid alteration caused by each SNPs. [67].

Familial combined hyperlipidemia (FCH) is a phenotypic mixed form with high levels of LDL-C and/or TG, exposing patients to a high risk of CVD. Phenotype shows high intra-individual and interfamilial variability. Diagnosis is based on familial history of premature CVD and high level of TG or LDL-C, and high levels of APOB (≥120 mg/dl) [68, 69].

No enough data are available on the prevalence and the clinical significance of NAFLD in patients with newly diagnosed dyslipidemias. Therefore, the aim of this study was to evaluate the prevalence of NAFLD and to assess CV risk in patients with newly diagnosed familial combined hyperlipidemia.

Materials and methods

We enrolled 80 patients (median age 53.5 years, range 18 -75 years) referred by general practitioners to the Department of Internal Medicine of Messina University Hospital from November 2018 to November 2020. All patients had a new diagnosis of dyslipidemia that was defined as follows: total cholesterol higher than 240 mg/dl, and/or LDL higher than 160 mg/dl, HDL lower than 40 mg/dl for man and 50 mg/dl for women, and APOB > 120 mg/dl.

Exclusion criteria were: diagnosis of T2DM, insulin-resistance assessed as HOMA> 2.5, BMI > 30 Kg/m², other chronic disease (hypothyroidism, renal dysfunction, chronic inflammatory disease) with exception for arterial hypertension, consumption of lipid lowering treatment in the last 24 months, alcohol intake higher than 2 UA/die, viral hepatitis (B, C, D), autoimmune and genetic causes of liver disease.

Steatosis was diagnosed by both abdomen ultrasound and HSI.

HSI was calculated as suggested by Lee [46] with the formula HSI = 8* ALT/AST + BMI (+2 if T2DM, +2 if female) (https://www.mdapp.co/hepatic-steatosis-index-hsi-calculator-357/). HSI higher than 36 was diagnostic for hepatic steatosis, whether lower than 30 ruled out steatosis [46]

US evaluated the following parameters to detect and quantify steatosis as absent, mild, moderate, or severe: parenchymal brightness, liver-to-kidney contrast, deep beam attenuation, bright vessel walls, and gallbladder wall definition [70]

Both FIB4 and TE using Fibroscan assessed liver fibrosis. FIB4 consisted of the following formula: age (years) * AST (U/L) / (platelet 10^9/L *√ALT (U/L). A cut off lower than 1.30 excluded significant fibrosis (F2), and a cut off higher than 2.67 predicted advanced fibrosis (F3) [47, 49, 50].

Liver TE was performed using the FibroScan® (Echosense, Paris), placing the transducer perpendicularly to the skin in the right intercostals spaces, with the patient lying in dorsal decubitus with the right arm in maximal abduction. The test was considered valid when at least 10 successful acquisitions with a success rate of at least 60% and an IQR (range of interquartile) ≤30% were obtained. [47]

We used a TE lower cut off of 7.9 KPa to exclude significant fibrosis (F2) and a TE upper cut off of 9.9 to confirm advanced fibrosis (F3) [38, 39].

We performed carotid ultrasound for the assessment of vascular damage with evaluation of c-IMT and presence of plaque. We consider plaque presence as a focal thickening of arterial wall higher than 1.5 mm or exceeding 50% the adjacent IMT, with lumen encumbrance and at least 0.5 mm of length [71, 72].

Written informed consent was obtained from all subjects in accordance with the Helsinki declaration. The study has been approved by the Ethics Committee of the University of Messina (protocol number 2018/71).

Statistical analysis

According to available data on dyslipidemia in NAFLD patients a power simple size of 100 patients ± 20% has been calculated.

The Kolmogorov-Smirnov test was used to verify variables distribution. Some variables had a non-normal distribution; thus a non-parametric statistical approach was applied. According to the presence/absence of steatosis we identified two groups, and the Mann-Whitney test was used to compare the variables between the groups. Spearman's test was used to verify the relationships between the variables. Chi-square test was used to test differences between categorical variables. Finally, we designed a conditional backward stepwise logistic analysis model testing FIB4, arterial hypertension, smoke habit and sex as dichotomized variables to evaluate the risk to develop atherosclerotic disease. Data are expressed as median and interquartile range (IQR), frequencies are expressed as number and percentage.

Results

The demographic, biochemical and clinical characteristics of the study group (80 patients, 37 males and 43 females) are described in table 1. The atherosclerotic plaque was present in 34/80 patients.

Liver steatosis was diagnosed in 56/80 patients (70%) of study cohort according to US; of these, 38 patients presented with mild steatosis (47.5%), 16 with moderate steatosis (20%), and 2 with severe steatosis (2.5%) (Table 2). When we tested liver steatosis by HSI, 34 out of 80 patients (42.5%) were diagnosed with steatosis, while 46 patients did not: 8 patients (10%) had no steatosis, and 38 patients (47.5%) had an inconclusive HSI score (Table 3). US examination diagnosed liver steatosis in 22 more patients compared to HSI; these patients presented an inconclusive HSI score, whereas all the 8 patients with an HSI score revealing absence of steatosis had also no US signs of liver steatosis. On the other hand, 4 patients had a diagnosis of steatosis according to HSI, but with no US signs of steatosis (Table 4).

We considered two groups of patients according to presence or absence of steatosis as identified by either US or HSI. Steatosis group (n=60, 75%) and a group without steatosis (both ultrasound and HSI ruled out steatosis) (n= 20, 25%) (Tab 5) We found that steatosis patients had a significantly higher BMI than those without steatosis ($p<0.05$).

Liver fibrosis, as evaluated by TE (> 7.9 KPa), was found in 7 patients, and one of these had a liver stiffness measurement (LSM) suggestive for advanced fibrosis (LSM >9.9 KPa); the remaining 73 patients had a LSM <7.9 KPa, showing either absent or not significant fibrosis.

According to FIB4, 59 patients (73.8%) had no fibrosis, and 21 (26.3%) patients had undetermined results (tab 6)

We found statistically significant correlations between steatosis and fasting insulin ($p <0.05$), LSM ($p <0.05$), BMI ($p <0.001$), and an inverse correlation with H-DLC ($p <0.05$).

We found statistically significant correlations between liver fibrosis as assessed by TE and BMI ($p <0.001$), c-IMT ($p <0.05$); when liver fibrosis was assessed by FIB4, we found a significant correlation with sex ($p <0.05$), c-IMT ($p < 0.05$), and atherosclerotic plaque ($p <0.05$).

We tested the potential association between fibrosis assessed by FIB4and atherosclerotic plaque (OR 4.760, IC 95%, [1.491 - 15.198] $p <0.05$), or with abnormal c-IMT (OR 1.179, IC 95%,

[0.364 - 3.823], p=ns). When liver fibrosis was identified by TE, we found a slight but not significant excess of risk of presenting with atherosclerotic plaque (OR 1.790, IC 95%, [0.281-11.406], p=ns), or with abnormal c-IMT (OR 1.667, IC 95%, [0.175 - 15.853], p=ns).

A multivariable logistic regression model was designed to evaluate the risk of developing atherosclerotic disease. Consistently with the pathophysiology, we tested the dependence of the variable "plaque presence" from FIB4, corrected for arterial hypertension, smoke habit, and sex. We found that FIB4 was able to predict independently the presence of plaque (OR 4.624, p=0.008), but arterial hypertension seems to have minor impact in this clinical setting (OR 3.088, p=0.048). Surprisingly, smoke habit did not reach a significant value (OR 1.873, p=0.15), as was sex (OR 0.923, p=0.884), and they were removed from the next step of the analysis (conditional backward stepwise logistic analysis).

Discussion

The link between NAFLD and dyslipidemia has been deeply investigated in the last 20 years [73-75]; it has been reported that 69% of NAFLD patients and 72% of those with NASH have dyslipidemia/hyperlipidemia [4, 76]. Moreover, the prevalence of NAFLD in high risk groups [2] has been investigated, ranging between 60-70% in Italian population to 42.6% in UK population with T2DM [77-80], 78.8% among patients with metabolic syndrome [78], and 50% in patients with dyslipidemia [81]. Moreover, in a large cross-sectional study on 44767 patients, the overall prevalence of NAFLD was 53%, whereas in patients with high TC/HDLC and TG/HDLC ratios the estimated prevalence was 78% [2, 82].

In our cohort of patients with newly diagnosed dyslipidemia (FCH) we found that a large number of these subjects had hepatic steatosis identified by either abdominal ultrasound or HSI (75%). Notably, this prevalence is similar to that detected in subjects with metabolic syndrome and in those with high TC/HDL-C or TG/HDL-C ratios [78, 82]

The prevalence of fibrosis in our population was 8.3% and 26.3%, according to TE and FIB4, respectively.

In our study group no patients had advanced fibrosis according to FIB4, and only one had advanced fibrosis according to TE. This finding can be partly explained by the relatively young age of population studied (median age 53 years). Indeed, we enrolled people at first diagnosis of dyslipidemia, thus excluding the possible confounding factor of lipid lowering drugs in evaluating either the prevalence of steatosis and its severity.

Nevertheless, a diagnosis of fibrosis through the FIB4 score (higher than 1.30 and lower than 2.67) predicts the risk of presenting with atherosclerotic plaque already at diagnosis (OR: 4.624).

After correction for arterial hypertension, sex and smoke habit, FIB4 maintained its statistical significance, thus being able to predict atherosclerotic disease in patients with dyslipidemia.

It has been demonstrated that features of dyslipidemia in NAFLD patients are characterized by high triglycerides, low HDL-C and high LDL-C, that are the same features of our cohort of patients. It has been hypothesized that dyslipidemia in NAFLD is caused by the same pathogenic mechanisms also driving hepatic steatosis including insulin-resistance [83].

Furthermore, it is well established the close association between NAFLD and atherosclerosis and NAFLD can be considered a risk factor of atherosclerosis [84].

Few studies investigated the association between FIB4 and atherosclerosis. In a previous study Xin and colleagues demonstrated an association between the progression of atherosclerosis detected by brachial ankle pulse wave velocity (ba-PWV) and higher incidence of NAFLD with high risk of liver fibrosis as assessed by NAFLD fibrosis score and FIB4, whereas they did not find association between c-IMT and fibrosis [85]. Histological advanced fibrosis (F2-F4) was found to be associated with higher ba-PWV; in this study authors concluded that older age, arterial hypertension and advanced liver fibrosis, even when assessed by FIB4 and NAFLD fibrosis score, were risk factors for atherosclerosis in NAFLD patients [86]. It is already demonstrated that FIB4 can predict risk of coronary artery calcification in NAFLD patients [87, 88]. None of these studies considered the variable presence/absence of plaque.

In our study FIB4 predicted the presence of atherosclerotic plaque already at the diagnosis. Insulin-resistance is a well acknowledged risk factor for both endothelial/arterial damage progression, and liver steatosis. In this study we excluded insulin-resistance patients; the association between FIB4 and atherosclerotic plaque could suggest further mechanisms linking fat ectopic accumulation both in liver and in arterial wall.

This study has some limitations. We found a significant association between steatosis and fasting insulin, as expected, but no association between fibrosis and fasting insulin or HOMA or HbA1c. It is likely due to selective exclusion criteria (BMI $\leq$30 Kg/m^2, HOMA $\leq$2.5, exclusion of diabetic patients) and relative sample size particularly according to those with fibrosis (8.3% of cohort according to TE, 26.3% according to FIB4).

Although robust statistically significance is lacking due to the relatively small simple size, particularly regarding patients with fibrosis, FIB4 can be suggested not only as an easy screening tool to assess liver fibrosis, but also as an adjunctive tool in clinical practice to better stratify dyslipidemic/NAFLD patients at risk of cardiovascular and liver disease progression.

With this study, we propose to screen for NAFLD all patients with "pure" primary dyslipidemia, namely all subjects with a lipid profile suspected for FCHL but no comorbidities nor other metabolic risk factors. Our data detect a 75% of prevalence of hepatic steatosis in these selected patients, with an increased risk of atherosclerotic disease among those with liver fibrosis.

These data are of particular interest because they further emphasize the strong link between altered lipid metabolism and liver disease. They also make clear that patients with suspected FCH should be investigated for atherosclerosis and liver disease already at diagnosis, in order to promptly recognize patients at higher risk of CV or liver disease progression.

TABLE 1 POPULATION CHARACTERISTICS

	Median	IQR
Age (years)	53	12
Sex (M/F)	37/43	
BMI Kg/m²	25.5	4.6
CT mg/dl	246.5	26
HDL-C mg/dl	50.5	20
TG mg/dl	118	86
LDL-C mg/dl	169	21
Fasting glucose mg/dl	89	12
HbA1c %	5,4	0.6
Fasting insulin mIU/l	7.25	3.4
HOMA	1.6	0.84
AST IU/l	19.5	9
ALT IU/l	19	14
GGT IU/l	24	23
PLT	245	75
FIB4	0.96	0.67
HSI	34.85	6.7
LSM KPa	5	2.05
c-IMT mm	0.98	0.29

TABLE 2: STEATOSIS ACCORDING TO US

Absent	24 (30%)
Mild	38 (47.5%)
Moderate	16 (20%)
Severe	2 (2.5%)

TABLE 3 STEATOSIS ACCORDING TO HSI

HSI < 30	8 (10%)
30 > HSI <36	38 (47.5%)
HSI > 36	34 (42.5%)

TABLE 4: US STEATOSIS IN 38 PATIENTS WITH INCONCLUSIVE HSI

Absent	16 (42.1%)
Mild	18 (47.4%)
Moderate	4 (10.5%)
Severe	0 (0%)

TABLE 5 COMPARISON BETWEEN STEATOSIS AND NO STEATOSIS

	STEATOSIS N = 60		NO STEATOSIS N =20		p
	MEDIAN	IQR	MEDIAN	IQR	
Age (years)	53.5	13	50	15	n.s.
BMI Kg/m²	26.13*	5	24.26*	4.3	$p < 0.05$
CT mg/dl	246	28	253	28	n.s.
HDL-C mg/dl	50	20	54	32	n.s.
TG mg/dl	116	91	123.5	78	n.s.
LDL-C mg/dl	169	21	170	23	n.s.
Fasting glucose mg/dl	89	61	91	12	n.s.
HbA1c %	5,4	0.6	5,65	0.8	n.s.

Fasting insulin mIU/l	7.25	3.6	7.25	2.5	n.s.
HOMA	1.52	0.91	1.67	0.56	n.s.
AST IU/l	20	9	19	14	n.s.
ALT IU/l	19.5	14	17.5	15	n.s.
GGT IU/l	26	23	22	25	n.s.
PLT	242.5	74	249	94	n.s.
FIB4	0.96	0.66	0.93	0.67	n.s.
HSI	36.85	5.7	32	3.8	n.s.
LSM KPa	5.15	2.32	4.5	2.8	n.s.
cIMT mm	1	0.32	0.97	0.22	n.s.

TABLE 6: LIVER FIBROSIS ACCORDING TO TE AND FIB4

LSM < 7.9 KPa	71 (88.75%)
7.9 KPa > LSM < 9.9 KPa	6 (7.5%)
LSM > KPa	1 (1.3%)
FIB4 < 1.30	59 (73.8%)
1.45 < FIB4 > 2.67	21 (26.3%)
FIB4 > 2.67	0 (0%)

TABLE 7: CONDITIONAL BACKWARD STEPWISE LOGISTIC ANALYSIS

	Step 1		**Step 2**		**Step 3**	
	OR (IC 95%)	p	OR (IC 95%)	p	OR (IC 95%)	p
FIB4	4,438	0,12	4.556	0.09	4.624	0.008
Arterial hypertension	2.824	0.076	2.883	0.061	3.008	0.043
Smoke habit	1.873	0.143	1.896	0.126	/	/
sex	0.923	0.884	/	/	/	/

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