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Nonalcoholic fatty liver disease and cardiovascular disease phenotypes

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Abstract

Nonalcoholic fatty liver disease is increasingly recognized as a major global health problem. Intertwined with diabetes, metabolic syndrome, and obesity, nonalcoholic fatty liver disease embraces a spectrum of liver conditions spanning from steatosis to inflammation, fibrosis, and liver failure. Compared with the general population, the prevalence of cardiovascular disease is higher among nonalcoholic fatty liver disease patients, in whom comprehensive cardiovascular risk assessment is highly desirable. Preclinical effects of nonalcoholic fatty liver disease on the heart include both metabolic and structural changes eventually preceding overt myocardial dysfunction. Particularly, nonalcoholic fatty liver disease is associated with enhanced atherosclerosis, heart muscle disease, valvular heart disease, and arrhythmias, with endothelial dysfunction, inflammation, metabolic dysregulation, and oxidative stress playing in the background. In this topical review, we aimed to summarize current evidence on the epidemiology of nonalcoholic fatty liver disease, discuss the pathophysiological links between nonalcoholic fatty liver disease and cardiovascular disease, illustrate nonalcoholic fatty liver disease–related cardiovascular phenotypes, and finally provide a glimpse on the relationship between nonalcoholic fatty liver disease and cardiac steatosis, mitochondrial (dys)function, and cardiovascular autonomic dysfunction.

Keywords

Cardiovascular, gastroenterology/hepatology, NAFLD, cardiovascular disease, cardiovascular risk

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Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic hepatopathy and a global health issue.[1–3] NAFLD is characterized by the presence of pathologic accumulation of fat in the liver with >5% of hepatocytes containing visible intracellular triglycerides (TGs), or steatosis affecting at least 5% of the liver volume or weight, in the absence of significant alcohol consumption and other specific causes of fatty liver disease, including hepatitis C, lipodystrophy, medications and inherited metabolic disorders.[4] The longitudinal risk of cirrhosis and hepatocellular carcinoma is rather low in NAFLD that is mostly an asymptomatic condition, progressing to nonalcoholic steatohepatitis (NASH) in about 15% of cases.[5]

Cardiovascular disease (CVD) is the leading contributory cause of death in subjects with NAFLD, and more severe forms of liver disease were associated with increased risk of CV morbidity and mortality.[6]

Nevertheless, current knowledge on the relationship between NAFLD and cardiac metabolism, structure, and function is still incomplete, and the most effective strategies to reduce the burden of CVD associated with NAFLD remain to be defined.

In this review, we aim to provide an updated overview of emerging CVD phenotypes associated with NAFLD and deliver a translational outlook spanning from the biological

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foundations of NAFLD down to CV implications and risk assessment.

Epidemiology

It is estimated that a billion people worldwide suffer from NAFLD,[2,7] with a global prevalence of approximately 25%.[8,9] The highest prevalence has been reported in the Middle East (32%) and South America (31%), followed by Asia (27%), the United States (24%), Europe (23%), and far less common in Africa (14%).[8] Ethnicity plays a significant role in the prevalence of the disease, which is significantly higher among Hispanic Americans than in other Americans of European descent, or in African Americans who display the lowest risk despite the relevant burden of essential comorbidities, such as obesity and hypertension.[10,11] Finally, genetics and environmental factors are likely to explain most of the residual disparities.

Genetics of NAFLD

Evidence of heritable components in NAFLD arises from studies of twins, familial aggregation, and interethnic differences in disease susceptibility.[12] According to genome-wide association studies (GWAS), susceptibility to NAFLD is linked to heritable components accounting for approximately 50% of the relative risk of disease.[13] Several genes were associated with NAFLD onset and outcomes, which will be presented below according to their effects on CV risk.

PNPLA3

A genetic variant of the PNPLA3 gene (encoding for *patatin-like phospholipase domain-containing protein 3*, or adiponutrin) was first linked to NAFLD in an analysis of data from the Dallas Heart Study in 2008;[14] this variant allele (I148M; rs738409) has been associated with increased liver fat content and inflammation, as well as to NAFLD severity[15] and NAFLD-related hepatocellular carcinoma.[16] A meta-analysis of data collected in the CARDIoGRAMplusC4D consortium showed a protective effect of PNPLA3 I148M with respect to coronary artery disease (CAD);[17] these results were replicated in a prospective study on patients undergoing coronary angiography, where PNPLA3 I148M was associated with lower levels of total serum cholesterol and low-density lipoproteins (LDL).[18] Conversely, in a Mendelian randomization study including 279,013 Danish individuals, the I148M variant was not significantly associated with higher risk of incident CAD. In a cross-sectional study of two different Italian cohorts, the I148M variant was associated with higher risk of subclinical atherosclerosis in young individuals. Finally, current evidence indicates that patients carrying the PNPLA3 I148M allele are at risk of developing NAFLD and its liver-related outcomes, but their CV risk may not be higher than in the general population.

TM6SF2

A variant of the TM6SF2 gene (encoding for *transmembrane 6 superfamily member 2*), known as E167K or rs58542926, confers a significant risk of NAFLD onset[19] and progression to NASH.[20] An additive effect was found between PNPLA3 and TM6SF2 variants in NAFLD risk prediction in a recent cohort study from China.[21] Regarding CV implications, a meta-analysis investigating CV risk in NAFLD patients showed a protective effect of the E167K variant,[22] which accounts for lower levels of total cholesterol, LDL-cholesterol (LDL-C), and TGs; this result has been confirmed in other studies.[23–25]

Other genes

A risk locus located in the TMC4 gene (encoding for *transmembrane channel-like 4* protein; variant rs641738) was associated with a more severe NAFLD phenotype in patients of European ancestry;[26] however, subsequent investigations did not confirm such an association[27] and to date, this variant has not been demonstrated to modify CV risk.[17]

A meta-analysis found an increased risk of NAFLD in patients carrying the rs7046 A (V175M) allele of *PEMT* gene,[28] also associated with increased CV risk.[29]

Furthermore, GWAS and Mendelian randomization studies are needed to better clarify the role of genetics in the complex relationship between NAFLD and CVD.

Epigenetic factors

Discordance of NAFLD phenotypic expression and severity of disease in twins can be explained by microRNA epigenetic regulation.[30] Epigenetics might also explain how certain environmental factors may exert heritable effects on disease expression. Accordingly, DNA methylation remodeling has been associated with a lower fibrotic burden in mice models.[31] Furthermore, an epigenetic signature on circulating cell-free DNA is under investigation as a potential biomarker of disease severity.[32]

Environmental factors

Genetic predisposition and epigenetics cannot fully explain the disease onset or the rise in NAFLD prevalence observed in Western countries over the last decades. Environmental factors, such as dietary habits and physical activity, have been shown to play a significant pathophysiological role in NAFLD[1,2,8] and CVD (Figure 1).

The role of dietary composition in modifying the onset and severity of NAFLD has been shown in population-level studies, where NAFLD patients were commonly presenting with unhealthy eating habits (i.e. eating processed foods, frequently eating at restaurants), shallow levels of physical activity and higher sedentary behavior,[33] thus implying that risk factors are similar between NAFLD and CVD.[34]

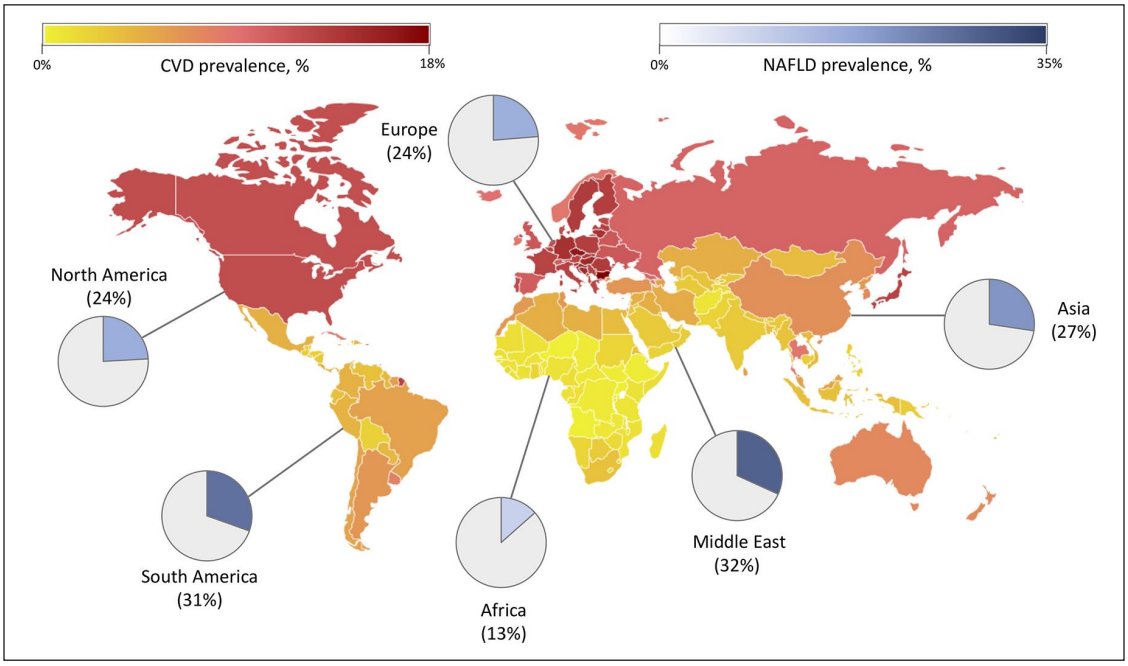

Figure 1. Geographical distribution of NAFLD and CVD prevalence. CVD prevalence is represented on each country's territory; NAFLD prevalence is represented as a pie chart for each world region. NAFLD and CVD prevalences were obtained from Younossi et al.[3] and the Global Burden of Disease Results tool (http://ghdx.healthdata.org/gbd-results-tool), respectively. Information from GBD Results Tool is made available under the ODC Attribution License (https://opendatacommons.org/licenses/by/1-0/index.html).

Conversely, active lifestyle and higher consumption of fruits and vegetables were linked to lower risk of NAFLD[35,36] and CVD.[34] Moreover, lifestyle-induced weight loss was found to improve liver histology and function, as well as cardiometabolic profile, among NAFLD patients.[37,38]

Smoking is an established CV risk factor, but its association with NAFLD is controversial. On pathophysiological basis, nicotine is known to trigger hepatic steatosis in the context of high-fat diet.[39] In 2018, a meta-analysis of 20,149 subjects reported a significant association between NAFLD and both active and passive smoking.[40] In two large cohort studies, current smoking was associated with NAFLD onset.[41,42]

The large overlap of risk factors between NAFLD and CVD depicts a complex framework of interactions between the two conditions, suggesting a redundant network of underlying biological mechanisms. Implementation of targeted prevention strategies is needed to reduce the growing burden of NAFLD and CVD.

NAFLD beyond the liver: a systemic threat

Patients with histologic NASH, and particularly those with overt fibrosis, show a higher risk of progression to cirrhosis and higher liver-related and all-cause mortality compared with less severe NAFLD phenotypes.[43–45] Importantly, evidence from longitudinal observational studies on NAFLD from different cohorts (Table 1) shows that CVD is one of the most important causes of death in the NAFLD/NASH population.

Metabolic comorbidities in NAFLD patients: chance or causality?

The majority of NAFLD patients have metabolic comorbidities, such as diabetes, obesity, and dyslipidemia.[1] NAFLD prevalence ranges from 50% to 75% in subjects with type 2 diabetes mellitus (T2DM),[54,55] from 80% to 90% in obese subjects,[56,57] and estimated around 50% in patients with metabolic syndrome,[58] while the prevalence of metabolic syndrome in NAFLD and NASH patients is reported at 43% and 71%, respectively.[3]

Most studies addressing the association between NAFLD and T2DM are observational in nature, and do not allow testing causality. However, a recent Mendelian randomization study[59] has shown evidence that genetically driven NAFLD phenotypes may be causally responsible for the onset of an atypical form of T2DM—late onset, type-1-like T2DM—characterized by deficient insulin secretion. In the same study, "genetic" NAFLD represented as well a causal factor for abdominal—but not central—obesity. Previous work had provided similar findings for genetically raised alanine transaminase (ALT) and aspartate aminotransferase (AST) levels.

Table 1. All-cause and CV mortality in NAFLD/NASH populations.

Study	Year	Country	Study group	Age (years)	Male sex (%)	Follow up (years)	Sample size (n)	All-cause mortality (1000 person-years)	CV mortality (1000 person-years)
Powell et al.[46]	1990	Australia	NASH	49	17	4	42	10.6	5.3
Adams et al.[47]	2005	USA	NAFLD		49	8	420	16.6	4.7
Ekstedt et al.[48]	2006	Sweden	NAFLD	46	87	14	58	8.8	6.3
			NASH	54			71	19.5	11.3
Rafiq et al.[49]	2009	USA	NAFLD	50	40	13	173	34.7	9.9
Lazo et al.[50]	2011	USA	NAFLD	47	53	14	2515	14.4	5.7
			Control	48	46		8856	10.2	4.0
Kim et al.[51]	2013	USA	NAFLD	45	50	14	4081	13.1	4.9
			Control	42	46		7012	10.0	3.8
Wild et al.[52]	2018	UK	T2DM–NAFLD	59	47	5	1452	31.2	5.8
Golabi et al.[53]	2019	USA	NAFLD	67	52	16	973	38.9	14.8
			Control		39		1122	34.7	13.2

NAFLD: non-alcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; CV: cardiovascular; T2DM: type-2 diabetes mellitus.

Lean NAFLD

Lean NAFLD is defined as NAFLD in the absence of obesity. This condition is common in areas where the risk of developing NAFLD is associated with ethnicity and genetic variation (PNPLA-3 gene), particularly in rural Asia, where prevalence reaches 25%.[3,61,62]

It has been suggested that the majority of lean NAFLD patients belong to the "metabolically obese–normal weight" phenotype,[63] described in about 5% of the Western population[8] and comprising non-obese, physically inactive individuals who have an increased CV risk, dyslipidemia, and impaired insulin sensitivity.

Lean NAFLD patients are generally young, usually insulin-resistant, presenting with increased plasma TG levels and by no means protected from liver fibrosis progression.[64]

Genetic predisposition and dietary composition are linked to the development of lean NAFLD. Current data suggest that a metabolic milieu like that of obese NAFLD patients is present in lean NAFLD patients, but the absence of obesity suggests they may hold for a distinct disease phenotype. Interestingly, lean NAFLD was associated with a greater visceral adiposity,[64] which corresponds to an ectopic fat distribution pattern characterized by higher values of neck and waist circumference. Visceral adipocytes, although smaller, are known to have a higher pro-inflammatory potential than subcutaneous adipocytes.[63]

Lean NAFLD diagnosis is challenging. CV risk assessment is crucial in patients with lean NAFLD,[53] as they are at increased risk of all-cause and CV mortality, and normal weight may be a relevant confounder. Management of lean NAFLD patients should follow the same principles used for obese NAFLD patients, requiring physical activity and good dietary habits.[61]

Sex differences and CV risk in NAFLD

NAFLD is a sexually dimorphic disease.[65] Epidemiological data confirm a higher prevalence in men than women; however, prevalence of NAFLD in menopausal women is comparable with that of age-matched men, and two-fold higher than in premenopause.[66]

The main commonalities in sexual dimorphism of NAFLD and CVD concern hormonal regulation of metabolism, energy storage, immunity, and inflammation. Estrogens have been shown to confer protection from NAFLD in menopausal women receiving hormone replacement therapy;[66] conversely, a longer duration of estrogen deficiency has been associated with more severe liver fibrosis. Estrogens promote the gynoid phenotype of body fat distribution, limiting visceral fat accumulation (i.e. in the liver and the myocardium), and stimulating subcutaneous fat depots.[67] Moreover, estrogens trigger sex-specific immune responses and have a role in modulating inflammation and tumorigenesis in the liver. Accordingly, estrogens may represent a major contributor of NAFLD phenotype disparities between sexes. In a survey conducted on Australian adolescents with NAFLD,[68] males presented with worse cardiometabolic profile than females and larger visceral adipose tissue thickness, possibly indicating a higher degree of systemic inflammation and subsequent increased risk of CVD.

The overall higher prevalence of steatosis in men has been thoroughly investigated in a small study of 22 metabolically healthy men and women of comparable age, body mass index (BMI), and liver fat content recruited from the UKBiobank cohort.[69] In this subset, men presented with higher fasting and postprandial TG and very low-density lipoprotein (VLDL) levels than women. Moreover, after a test meal and subsequent metabolic tracing of ingested fatty acids, it was shown that women tended to favor oxidation pathways, whereas men favored synthetic pathways. This could partially explain the greater prevalence of NAFLD, and account for a possibly increased CV risk, in men. Interestingly, these results were confirmed in a study on 15,753 Chinese workers, which pointed out that in women, diabetes exerts a much greater effect on CV risk than NAFLD.[70] The authors

advocated sex disparities to be due to the greater amount of visceral adiposity typical of men.

NAFLD and CVD: biological foundations

The pathophysiology underlying the association of NAFLD and CVD is still not completely understood. NAFLD is now considered a systemic disease[71] sharing common pathways with other conditions, such as T2DM and atherosclerosis.

The development of a pro-inflammatory, pro-atherogenic, and pro-thrombotic milieu is essential for CV damage to take place in NAFLD patients.[72] The biological foundations of this milieu include endothelial dysfunction, altered lipid metabolism, systemic insulin resistance, oxidative stress, and systemic inflammation.[1,73]

Vascular alterations and endothelial dysfunction

NAFLD is associated with hepatic microvasculature alterations, with loss of the typical sinusoidal pattern and of fenestrae;[74] such changes occur in the liver early before inflammation and fibrosis.

Systemic endothelial dysfunction, an early step toward atherosclerosis, is present in NAFLD and NASH;[75] asymmetric dimethylarginine (ADMA) is an endogenous antagonist of nitric oxide synthase. The breakdown of ADMA is mainly driven by liver function, thus explaining the increasing ADMA levels observed in NAFLD patients, which may suffer from alterations in vasodilation.[76]

Vascular remodeling also happens in NAFLD patients. Indeed, histologic findings of active angiogenesis (such as centrizonal arteries and microvessels) are common in NAFLD, even in the absence of advanced fibrosis. These findings relate to other studies showing an increase in vascular endothelial growth factor (VEGF) serum levels in NAFLD and NASH[77] and to mouse models where anti-vascular endothelial growth factor receptor 2 (VEGFR2) treatment improved steatosis and inflammation.[78]

Members of the VEGF family, particularly VEGF-A, are established atherogenic factors and play a significant role in plaque instability.[79]

Altered lipid metabolism

The liver is the hub of lipid metabolic network, operating *de novo* lipogenesis and fat breakdown, as well as uptake and secretion of serum lipoproteins.[80] In NAFLD, serum lipid profile is significantly altered, leading to increased levels of TGs and LDL and decreased high-density lipoproteins (HDL). Resulting ratios (TG/HDL; cholesterol/HDL; and LDL/HDL) are considered pro-atherogenic, and were demonstrated to be altered along the severity spectrum of NAFLD.[81] The most detrimental lipid profile occurs during postprandial periods, when chylomicron remnants and LDL increase, and HDL decrease.[81,82]

Systemic insulin resistance

High blood levels of diacylglycerol determine activation of protein kinase C, which depresses hepatic insulin signaling,[1] inducing lipolysis, and alterations in glucose metabolism.[83] This also leads to a net effect of hepatic lipid accumulation (steatosis) and lipotoxicity, which further impairs insulin signaling, causes inflammation and oxidative damage, and promotes progression to NASH.

High levels of saturated fatty acids also trigger insulin resistance by *de novo* ceramide synthesis and subsequent inhibition of Akt phosphorylation.[84]

Several liver-specific cytokines—*hepatokines*—have been showed to influence insulin sensitivity,[85,86] and some of them have been shown to exert CV effects. Among others, Fetuin-A causes insulin resistance by inhibiting insulin receptor tyrosine kinase in the liver and skeletal muscle. Serum levels of Fetuin-A are increased in NAFLD,[87] even higher in NASH,[88] and have been linked to a higher risk of myocardial infarction and stroke.[89]

Other hepatokines linked to insulin resistance in NAFLD are fibroblast growth factor 21 and selenoprotein P; both were associated with CV outcomes.[90–92]

Notably, CV risk in NAFLD patients with T2DM is greater than in T2DM non-NAFLD patients, and the association of NAFLD with CVD has been shown to be independent from T2DM and other cardiometabolic risk factors.[93]

Oxidative stress

Serum homocysteine, a marker of hepatic oxidative stress, is frequently reported to be elevated in NAFLD.[94–96] Oxidative stress is thought to contribute to disease progression to NASH. Intriguingly, NASH patients have lower homocysteine levels than NAFLD patients,[96,97] probably indicating a more severe liver dysfunction in NASH, where high oxidative stress is present,[98,99] but not correlated with serum homocysteine level.

Serum homocysteine is regarded as an independent CV risk factor.[100,101] It causes endothelial dysfunction, platelet activation, and redox status impairment, eventually leading to CVD.[101] Interestingly, the pioglitazone versus vitamin E versus placebo for the treatment of non-diabetic patients with nonalcoholic steatohepatitis (PIVENS) trial showed that levels of homocysteine in NASH patients lowered after treatment with vitamin E.[102]

Systemic inflammation

CV effects of NAFLD have been thought to be the result of inflammatory cytokines, released by the liver in the bloodstream, leading to systemic inflammation and CVD.[103] Inflammation triggers CVD by causing alterations in endothelial function, vascular tone, and coagulation, and by enhancing plaque formation.[104] Among serum markers of systemic inflammation, several are associated with

NAFLD; these include interleukin-6 (IL-6),[105,106] C-reactive protein (CRP), and tumor necrosis factor alpha (TNF-alpha).[107,108] In particular, high-sensitivity CRP was found to be higher in NASH patients compared with milder steatosis, possibly representing a marker of advanced disease.[109] Similar findings have been published about neutrophil-to-lymphocyte ratio[110] and the Th17/Treg lymphocyte ratio.[111] Of note, neutrophils, not just Th17 lymphocytes, were found to be themselves a source of IL-17, which is considered an essential initiator of liver disease. Moreover, in a mouse model, it has been demonstrated that spleen, bone marrow, and mesenteric lymph nodes were the primary source for liver-migrated lymphocytes.[112] This supports the idea that dysbiosis and gut-microbiota interactions may be responsible for low-grade systemic inflammation in NAFLD patients.[113]

Influence of NAFLD on cardiac function and metabolism

NAFLD has been linked to cardiac dysfunction. In particular, ultrasonographic findings of NAFLD have been associated with a three-fold increased risk of left ventricular diastolic dysfunction, independent of other cardiometabolic risk factors;[72] these findings were also confirmed in pediatric studies. Both liver stiffness and hepatic steatosis were independently associated with larger left atrial volume and left ventricular dysfunction in NAFLD patients.[114] Initial data showed impaired right ventricular function in NAFLD patients compared with age- and sex-matched healthy controls, and also in patients with hepatic fibrosis compared with those without.[115]

As for influences of NAFLD on cardiac metabolism, a study on NAFLD patients demonstrated that higher degrees of steatosis are related to lower myocardial insulin-stimulated glucose uptake and overall glucose extraction rate.[116]

Furthermore, cardiac magnetic resonance (MR) spectroscopy data[117] showed that phosphocreatine/adenosine triphosphate (PCr/ATP) ratio—a surrogate marker of cardiac energy metabolism[118]—was significantly reduced in NAFLD patients compared with controls. This may suggest that abnormalities in cardiac metabolism may precede the structural and functional changes induced by NAFLD. In another study on T2DM patients in which liver fat content was assessed using MR spectroscopy, the high liver fat group had slower cardiac metabolism compared with low liver fat group.[119] Patients with fatty liver were also found to have lower myocardial perfusion, even though values of cardiac mass and function were comparable between the two groups. Further research about the role of multimodality CV imaging, namely cardiac MR, could allow for early detection of subtle metabolic and tissue changes of the myocardium, even before the onset of overt structural and functional abnormalities.

Cholecardia

Bile acids dysregulation is currently recognized in NAFLD pathogenesis. Bile acids have been shown to act as gene regulators[120] and are thought to modulate glucose and lipid metabolism, enhance energy consumption in muscle tissue, and, most importantly, improve insulin resistance in healthy subjects.[121] In NAFLD, bile homeostasis is disrupted, and serum bile acid levels are higher with disease progression to NASH.[122] Elevated bile acid level is known to be associated with cirrhotic cardiomyopathy. Bile acids are well-known cardiotoxic agents, impairing ventricular function, and associated with increased risk of atrial fibrillation.[123] Accordingly, the term *cholecardia* was proposed to describe the cardiomyopathy phenotype associated with pathological levels of bile acids.[124]

Moreover, in a mouse model, it has been demonstrated that cardiac mitochondria do suffer from chemically induced cholestasis by exhibiting a reduction in calcium loading capacity—secondary to the activation of the mitochondrial permeability transition pore[125]—which is known to cause uncoupling of oxidative phosphorylation, accumulation of reactive oxygen species, and eventually cell death.[126]

Cardiac steatosis

The idea that liver fat accumulation may trigger cardiac steatosis has made its way in the last years.[127] Hepatic fat content might be considered an indicator of systemic TG deposition, also accounting for fat accumulation within the myocardium. Subsequently, cardiac steatosis could trigger myocardial dysmetabolism and dysfunction. The presence of epicardial adipose tissue (EAT) is independently associated with NAFLD,[128,129] with a graded linear relationship between the severity of hepatic steatosis and EAT thickness. Importantly, thicker EAT is also a harbinger of coronary artery calcification.[130] Moreover, in a cohort of patients with metabolic syndrome, the severity of EAT and NAFLD was found to be highly correlated.[131,132] EAT is known as a source of pro-inflammatory cytokines (IL-1, IL-6, and TNF), which have an established role in the pathogenesis of atrial fibrillation and CV autonomic dysfunction (CVAD).[133–135] Unlike skeletal muscle where perimuscular fat is separated from myocytes through specific structures of connective tissue, within the heart, adipocytes are in close contact with both cardiomyocytes and nervous system and directly influencing their function.[136–138]

Clinical assessment of NAFLD

Diagnosis

Liver biopsy is considered the gold standard technique to diagnose NAFLD; however, current guidelines do not recommend to perform invasive tests for diagnostic purposes.[2,139–141] Liver biopsy should only be considered in

patients with suspected NASH or advanced fibrosis, basing on the presence of metabolic syndrome and/or potential competing liver failure etiologies.

NAFLD patients are often asymptomatic, and diagnosis is usually suspected in the presence of obesity, diabetes, and obstructive sleep apnea. Accurate alcohol history is necessary for diagnosis since histology does not accurately distinguish NAFLD from alcoholic FLD.[141]

NAFLD patients are usually identified by the presence of hepatic steatosis at abdomen ultrasonography or elevated transaminases in blood tests. Liver chemistry tests are found to be normal in more than two-thirds of cases, and usually do not predict histological severity of liver disease.[2,141] Nevertheless, an AST/ALT ratio of above 1.0 is highly suggestive of advanced disease.[142]

According to the latest guidelines,[2,140] asymptomatic or paucisymptomatic patients with non-harmful drinking habits (males $<$ 21 standard drinks/week; females $<$ 14 standard drinks/week), and known risk factors for metabolic syndrome, should undergo blood tests and first-level hepatic imaging (ultrasound) to confirm or rule-out the diagnosis of NAFLD.

Staging of liver disease

The purpose of staging liver steatosis is the distinction between low-risk NAFLD, in which lifestyle correction is sufficient for disease control, and high-risk NASH, where close follow-up and pharmacological therapy are required. NASH patients are indeed at a higher risk of extrahepatic morbidity and mortality, including CVD.[48]

Staging of liver disease is key for CV risk assessment and does include both invasive and noninvasive techniques, yet the gold standard is still represented by histological examination from liver biopsy.[1,2,141] Simple steatosis is characterized by a microvesicular accumulation of TGs in hepatocytes, whereas steatohepatitis includes signs of hepatocellular injury, mitochondrial changes, cell ballooning, and fibrosis.[141] Disease severity at histology can be evaluated through the NAFLD activity score,[143] based on the degree of steatosis, lobular inflammation, and hepatocyte ballooning, by which a score $>$ 5 is highly suggestive of NASH.

Although biopsy remains the gold standard for diagnosis and staging of disease, it is an invasive procedure not free of risks and sampling errors, also yielding high costs.[2] Noninvasive staging methods based on serum biomarkers, clinical scores, and imaging techniques are promising alternatives to invasive biopsy.[2,141]

Proposed serum markers include biomarkers of inflammation (CRP, IL-6), oxidative stress (vitamin E, thioredoxin), and apoptosis (cytokeratins 8–18),[5] although their prognostic yield is yet to be proven. Clinical scores—such as the NAFLD fibrosis score (NFS)—have successfully entered the clinical practice.[144] NFS is calculated based on the combination of the following parameters: age, BMI, altered glucose metabolism, AST/ALT ratio, platelet count, and albumin levels. Significant liver fibrosis (F3F4 fibrosis) is highly suspected when the NFS is $>$ 0.675.[141]

Ultrasound and MR are established noninvasive imaging modalities for the assessment of NAFLD. Transient elastography (FibroScan)[2,5] is an ultrasound-based test measuring liver stiffness as a surrogate of fibrosis. Beyond fibrosis quantification, FibroScan can also detect steatosis by measuring the controlled attenuation parameter (CAP).[145] MR elastography is an alternative technique to transient elastography for fibrosis assessment. However, although associated with higher diagnostic yield than FibroScan, MR elastography has not yet entered the clinical practice.[146] MR-based techniques are also highly accurate for the assessment of liver steatosis.[145]

CV risk assessment in NAFLD

Over the last decade, international scientific societies for the study of liver, diabetes, and obesity recommend routine CV risk assessment in NAFLD patients.[140] The American Association for the Study of Liver Diseases (AASLD) further recommends aggressive modification of CV risk factors in NAFLD patients.[147] Guidelines issued in 2018 by the Asia-Pacific Working Party on NAFLD state that all patients should receive advice and support for lifestyle interventions to reduce the risk of onset of CVD.[148] Similarly, Chinese guidelines confirm the importance of CV and cerebrovascular risk assessment in patients with NAFLD.[149] Importantly, the 2019 European Society of Cardiology/European Atherosclerosis Society (ESC/EAS) guidelines for the management of dyslipidemias recommend NAFLD assessment after systematic coronary risk evaluation (SCORE)[150] and consider NAFLD as a risk modifier in patients with low or moderate CV risk.

Several CV risk scoring systems specific to NAFLD population have been considered over the years. The Framingham risk score (FRS) was proposed as an accurate predictor of coronary heart disease in NAFLD patients,[151] and has been shown to be significantly associated with severity of liver fibrosis[152] and to NAFLD Fibrosis Score as well.[153] However, the FRS has been shown to overestimate CV risk in European[154] and Asian[155] cohorts. The Italian National Institute of Health developed a CV risk assessment tool[156] which has been proposed for use in NAFLD patients from Southern Europe.[157]

A number of CV risk scores, including PROCAM, Qrisk2, and ASCVD, have been tested in NAFLD,[158–160] and in 2019, a risk score evaluating age, mean platelet volume, and diabetes has been proposed.[160] However, to date, no single model has demonstrated superior performance, clinical utility, or widespread global uptake.

NAFLD and CVD: from biological foundations to the evidence

Different long-term studies suggest that histologically defined NAFLD or NASH is associated with increased CV

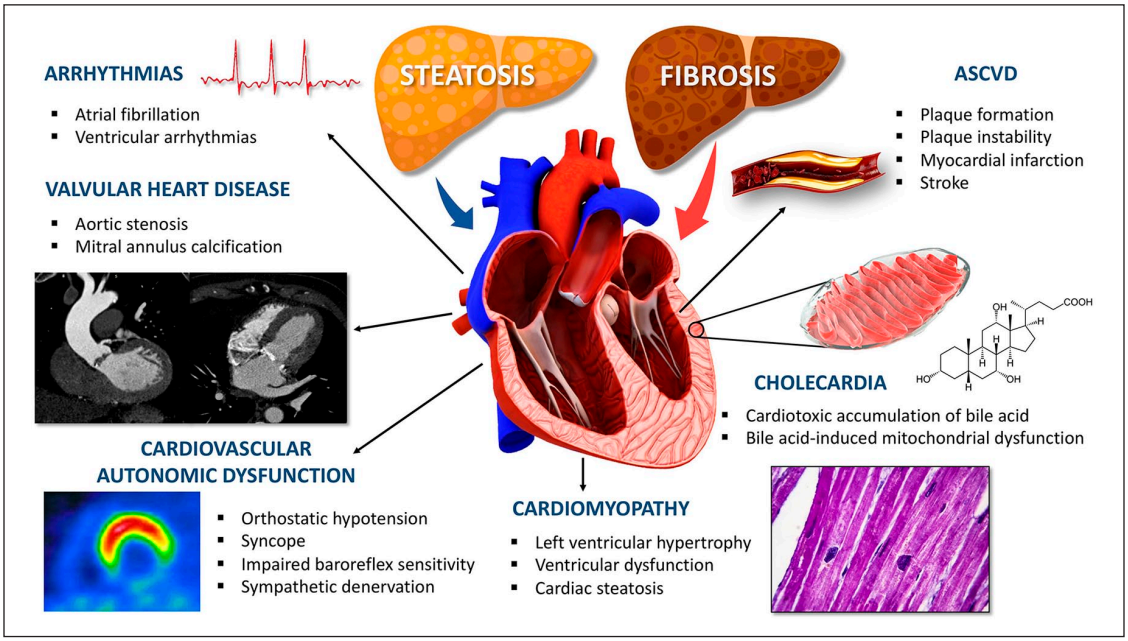

Figure 2. NAFLD and CVD phenotypes.

mortality.[43,48,161] A meta-analysis of 16 observational studies[162] found that NAFLD is indeed associated with increased risk of fatal and non-fatal CVD events in a graded fashion, even though the observational nature of the studies did not allow to establish causality. Interestingly, even after liver transplantation, CV complications were more frequent in NASH patients.[163]

Subclinical and clinical CVD phenotypes in NAFLD

CV morbidity and mortality

An association between NAFLD or NASH and CV events has been demonstrated in various observational studies and meta-analyses, regardless of different diagnostic modalities and statistical methods (Figure 2).[1,162] Ultrasound-diagnosed NAFLD was found to be associated with a nearly two-fold higher risk of symptomatic CVD events.[164] Such results were confirmed in a meta-analysis, also demonstrating that NAFLD severity was associated with an increase in CV events, even though no association between NAFLD and all-cause mortality was reported.[165]

NAFLD and atherosclerotic CVD

NAFLD increases both the risk of subclinical and clinically significant atherosclerosis.[166,167] Patients with NAFLD show impaired vasodilator response, increased carotid intima–media thickness (IMT), and carotid atherosclerotic disease.[168] Furthermore, NAFLD was associated with a 13% increase in IMT.[169] In a meta-analysis of 16 cross-sectional studies pooling 16,433 NAFLD patients and 41,717 control subjects,[170] NAFLD was associated with increased coronary artery calcification independent of traditional risk factors. The assessment of coronary artery calcium may be useful in identifying NAFLD patients at risk of future CV events.

Moreover, in a recent cohort of 455 patients without known CVD, heightened hepatic metabolism was associated with coronary artery calcium and arterial inflammation[171] and was also found to be an independent predictor of CV events.

NAFLD has also been shown to be associated with an increased risk of adverse outcomes in the setting of primary percutaneous coronary intervention.[72] Notably, high-risk plaque features were shown to be more common at CT angiography in NAFLD patients,[172] and higher severity patients had a higher risk of death.[173]

Cardiomyopathies

NAFLD has been associated with morphological and structural changes in the myocardium. This was reported in the Coronary Artery Risk Development in Young Adults (CARDIA) study,[174,175] where NAFLD patients showed significant subclinical myocardial remodeling and dysfunction, possibly linking NAFLD to the onset of heart failure. Left atrium enlargement was also highly frequent in patients with an ultrasonographic diagnosis of NAFLD.[175] In small studies where NAFLD was diagnosed using liver biopsy, the existence of a significant relationship between the severity of

liver histology and abnormality in left ventricular morphology and function was demonstrated, suggesting the importance and of the heart–liver axis in this pathology.[176]

According to MR spectroscopy data, overt structural and functional abnormalities of the myocardium in NAFLD patients are most likely to be preceded by depression of cardiac metabolism due to cardiotoxic effect exerted by high levels of serum bile acids.[117]

Valvular heart disease

Beyond myocardial disease, NAFLD was also linked to valvular heart disease, particularly aortic stenosis (AS)[177,178] and mitral annulus calcification (MAC).[178] AS has become the most common valvular heart disease in developed countries.[179] Compared with the general population, the prevalence of AS and MAC was three-fold higher in a cohort of NAFLD patients, regardless of traditional risk factors.[177] Steatosis was responsible for an additional 33% increased risk of AS and associated with an odds ratio of 2.70 for incident AS or MAC.[178] Pooled AS prevalence has been reported to be 41.3% (95% CI: 32.0%, 51.4%) among NAFLD patients versus 24.6% (95% CI: 18.4%, 32.0%) in non-NAFLD patients.[180]

CV autonomic dysfunction

NAFLD has been linked to CVAD. The first recognition of CVAD in NAFLD derives from a cohort of NAFLD patients with overrepresented nocturnal hypotension, orthostatic hypotension, and susceptibility to vasovagal syncope.[181] The same authors later confirmed a broader connection of NAFLD to CAVD symptoms, such as syncope and falls.[182] NAFLD has also been associated with deterioration in heart rate recovery (HRR), a marker of decreased parasympathetic activity,[183] and higher mortality.[184,185] Furthermore, a reduced standard deviation of beat-to-beat intervals (SDNN) was found to be associated with NAFLD-related risk of falls.[186] A graded relationship between HRR reduction and NAFLD severity has also been confirmed in the diabetic population.[187] Finally, a recent study demonstrated an association between NAFLD severity and reduced diastolic and systolic variability, increased baroreceptor sensitivity, and impaired cardiac function,[188] promoting the hypothesis that NAFLD patients might be exposed to pathologically sustained sympathetic activity and resistance to parasympathetic stimuli.

Atrial fibrillation and ventricular arrhythmias

The literature on the association between NAFLD and the risk of cardiac arrhythmias is still scarce. Data from the Framingham Heart Study showed that high serum transaminase levels and NAFLD are both independently associated with an increased incidence of atrial fibrillation.[189,190] A pilot case-control study found a significant association between NAFLD and impaired atrial conduction properties, particularly P-wave dispersion and electromechanical delay, as assessed by 12-lead electrocardiogram (ECG) and echocardiography.[191]

A number of studies focused on QTc prolongation also suggest a potential link between NAFLD and ventricular arrhythmias.[72,192,193] In both community-dwelling individuals and diabetic patients, NAFLD was associated with a significant increase in QTc duration.[194,195]

Further research on the impact of NAFLD on cardiac electrical properties and other biological phenomena may provide novel insights about NAFLD and risk of arrhythmias and sudden cardiac death.[1]

Review methodology and limitations

Authors performed a narrative review and searched Medline, the Clinical Trials Registry, the Cochrane Library, Web of Science, ResearchGate, as well as reference lists of all identified articles and previous reviews and meta-analyses, from January 1966 through March 2020 for potentially relevant articles; ultimately, a selection of most relevant papers was finally included in the current review according to authors' opinion.

We acknowledge the lack of dedicated sections covering the fundamentals and state-of-the-art of imaging techniques in NAFLD, and the therapeutical aspects of NAFLD and related CV risk; however, this was beyond the scope of the current review.

Conclusion

NAFLD plays a major role in the pathogenesis and progression of CVD. NAFLD management should be focused on both specific lifestyle modifications and aggressive risk factors modification, which would not only reduce the risk of liver disease progression but may also provide benefit by reducing the risk of developing CV complications. Future prospective multimodality CV imaging studies aiming at the early detection of metabolic, structural, and functional alterations of the CV system may help refine current strategies of CV risk assessment in NAFLD and determine the impact of the full histologic spectrum of NAFLD on subsequent risk for clinical heart failure. Randomized controlled trials are also needed to test whether effective NAFLD treatment will translate into better CV outcomes.

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