

FROM NAFLD TO CARDIOVASCULAR DISEASE. IS IT (STILL) THE METABOLIC SYNDROME?

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in developed countries. The incidence of NAFLD in the general population is 30-38% depending on the geographical area and the diagnostic method used. NAFLD is considered to be the liver manifestation of the metabolic syndrome. A better understanding of the natural evolution would have practical consequences related mainly to the need of early and aggressive diagnosis, active monitoring and therapeutic solutions. Cardiovascular disease appears to be the main cause of death in these patients. The mechanisms linking NAFLD with cardiovascular disease are not fully understood yet, but attention was focused primarily on insulin resistance. The visceral adipose tissue, the epicardial adipose tissue, the systemic inflammatory response syndrome, the lipid profile, the procoagulants factors, the oxidative stress, and type 2 diabetes mellitus, they all might play a role in the link between NAFLD and cardiovascular disease. Currently, there isn't any medication specifically recommended for the treatment of NAFLD. Although the mechanisms underlying the association between NAFLD and cardiovascular disease are not fully known, attention must be paid to this association, given that these patients are more likely to die due to heart disease rather than liver disease.

Keywords: NAFLD, metabolic syndrome, cardiovascular disease, fatty liver.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disease in developed countries [1]. It represents the range of liver disease consisting of simple steatosis (fatty infiltration of more than 5% of hepatocytes), non-alcoholic steatohepatitis (hepatocyte injury consisting of steatosis, focal necrosis and inflammation) – NASH, which may lead to fibrosis and cirrhosis; these conditions occur in the absence of chronic consumption alcohol (under 20 grams of pure alcohol / day for women and under 30 grams of pure alcohol / day for men) [2]. Outside this etiopathogenetic framework, there are other factors and conditions that can lead to fatty liver, called non-NASH fatty liver (NNFL) such as: lipodystrophy, primary mitochondrial diseases, Wilson's disease, bariatric surgery, parenteral nutrition, medication (amiodarone, methotrexate, tamoxifen), toxins (carbon tetrachloride, ethyl bromide) [2].

The incidence of NAFLD varies greatly, being dependent, among other factors, on the geographic area and the diagnostic method used. In Europe, using ultrasonog-

raphy (US) as a method of diagnosis, the incidence in the general population is 20-30% [3,4], and in the USA, using histology as a diagnostic method, the incidence is 27-38% [5,6,7]. A Korean study of 589 patients, based on liver biopsy from potential donors for liver transplantation, reported a prevalence of NAFLD of 51% [8]. Regarding the subgroup of NAFLD patients with NASH, it is estimated to be 3-16% [5,9] in Europe and 6-15% in the USA [10,11,12]. These patients are considered as having a high risk, related to both, the progression of the hepatopathy and the liver disease associated comorbidities [13].

NAFLD is associated with components of the metabolic syndrome (MS), such as abdominal obesity, insulin resistance, dyslipidemia, glucose intolerance or type 2 diabetes mellitus (T2DM) [14]. This association, especially because insulin resistance was identified as a joint central pathogenetic mechanism, made NAFLD to be considered the liver manifestation of the metabolic syndrome [13]. Among the components of MS, the major associations of NAFLD are with obesity and with T2DM; the patients with these disorders have a prevalence of NAFLD of 70-90 % [1]. NAFLD represents an additional risk both for patients already diagnosed with T2DM and those with NAFLD

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without T2DM. Regarding patients with NAFLD and T2DM, they have an additional risk of developing NASH, advanced fibrosis and cirrhosis, and, less frequently, even hepatocellular carcinoma (HCC) [15,16], chronic kidney disease and retinopathy [13]. Patients with NAFLD have an increased risk of developing T2DM in time, with a more expressed risk among patients with NASH [17].

MS is a set of metabolic and cardiovascular risk factors that have the capacity to predict a better cardiovascular and T2DM risk development than its individual components [18]. The last definition of MS is from 2009 and it requires the presence of at least three of the following five criteria: abdominal obesity (waist circumference increased, for the Europeans ≥ 94 cm in men and ≥ 80 cm in women, for the Americans ≥ 102 cm in men and ≥ 88 cm in women), elevated triglycerides (>150 mg/dl) or treatment for hypertriglyceridemia, low HDL cholesterol (< 40 mg/dl in men and < 50 mg/dl in women) or treatment for low HDL cholesterol, hypertension ($>130/85$ mmHg) or treated hypertension, high fasting plasma glucose (>100 mg/dl) or treatment for hyperglycemia [19]. Referring to association of NAFLD and MS, over 90% of patients with NAFLD have at least one component of the MS [20], and the complete diagnosis of MS is present at 55%-62.5% of the patients with liver disease, according to the definition of MS used [21].

Natural history

The natural evolution is not fully known, and there is still some controversy about the progression from NAFL to NASH and fibrosis. The complete elucidation of the mechanisms would be important, because a better understanding of the natural evolution would have practical consequences related mainly to the need of early and aggressive diagnosis, active monitoring and therapeutic solutions.

In 2006, Ekstedt et al. conducted a study which showed that in the presence of simple steatosis (NAFL), the mortality is not different from that recorded in the general population. Meanwhile, if steatosis with inflammation is present (NASH), there is increased mortality as compared to the general population both related to the liver disease (2.8% vs 0.2%, $p=0.04$), and especially due to cardiovascular events (15.5% vs. 7.5% $p=0.04$) [17].

Several studies have shown that NAFLD is a risk factor and a predictor for cardiovascular events, independent of the classical risk factors (MS, T2DM, obesity) [22].

Regarding the risk of progression to HCC, in a study conducted in the UK, Reeves et al. showed that at the end of 2010, of all patients with HCC, 35% had NAFLD, representing the most common etiology and constantly growing [23]. In a study from Japan, Yasui et al. have shown that among the 87 HCC cases from NAFLD, 49% were non-cirrhotic patients [24].

All these data support the hypothesis that NAFLD is not a trivial liver disease. It should be regarded as a

progressive disorder in which mortality from any cause is increased, and whatever the mechanisms involved in the progressive evolution of disease, they must be characterized in a short time, because NAFLD is the third cause of liver transplantation in the U.S., after the infection with HCV and the alcoholic liver disease (ALD). On the other hand, NASH is the only liver disease that is growing and is expected to surpass ALD over the next 5 years. Considering the introduction of new therapies for HCV infection, chances are that NAFLD will become the main etiology for liver transplantation in the not too distant [25].

Association between NAFLD and cardiovascular disease

Numerous epidemiological studies have reported an increased incidence of cardiovascular events in patients with NAFLD and cardiovascular disease appears to be the main cause of death in these patients, as mentioned above [17].

The association between NAFLD and cardiovascular disease (CVD) can be viewed from many angles, given the numerous studies that are assessing cardiovascular risk in terms of a wide range of risk factors.

There are a range of risk scores to assess the risk of developing CVD over time. These risk scores were applied among patients with NAFLD. As expected, patients with NAFLD have an increased risk of developing CVD, evaluated in terms of the risk scores. From this perspective, there appears to be an increased cardiovascular risk that is directly proportional to the severity of liver histological changes among patients with NAFLD [22]. However, there was some skepticism on the risk scores regarding patients with NAFLD. This is because some important determinants of NAFLD such as IR, obesity and increased levels of TGL, determinants which, in turn, each of them can increase the cardiac risk, are not taken into account when these risk scores are being calculated [22]. In addition, in patients with MS, the condition with which NAFLD shares most of its etiopathogenic links, the Framingham Risk Score underestimates the cardiovascular risk [26].

Studies that have addressed the relationship between coronary heart disease and NAFLD have identified in these patients an increased incidence of coronary atherosclerosis by using multislice CT [27-30]. Also, there appears to be an association between NAFLD and coronary artery disease, which is manifested by "unstable plaque" lesions [28]. The severity of coronary lesions in patients with NAFLD was also quantified by coronary angiography, reporting an increased association between patients with NAFLD and the severity of coronary lesions, even after adjusting the classic cardiovascular risk factors and MS [31-34]. These studies are, however, limited by the fact that they have not provided a functional assessment of these coronary lesions, knowing that the outcome is given by ischemia, not by the anatomical lesions [35-36].

Measurement of carotid intima-media thickness (CINT) in B mode ultrasound is a noninvasive, sensitive and reproducible method, useful to identify and quantify subclinical vascular disease and to evaluate the cardiovascular risk [37]. Patients with NAFLD have an increased CINT compared to control population by 13% and a higher frequency of carotid plaques [38]. Also, it appears that the severity of liver histopathological lesions are proportionally associated with increased CINT, with NASH patients having lesions being at a greater risk than those with simple steatosis [39]. However, some studies have failed to show a strong association between NAFLD and CINT after statistical calculations were adjusted according to the presence of MS [39-44]. There are even two studies that reported no association between NAFLD and increased CINT [45-46]. Neither of these two studies evaluated the relationship between NAFLD and carotid atherosclerotic plaques. Carotid atherosclerotic plaques have at least the same meaning, if not stronger, in terms of developing future cardiovascular events as compared to CINT [47].

Regarding the assessment of the cardiac function in patients with NAFLD, it seems that in the presence of liver disease there may be thickening of cardiac walls, altering myocardial strain, concentric remodeling and evidence of diastolic dysfunction [48]. There is also evidence of a relationship between the severity of the diastolic dysfunction and the degree of fatty liver [49]. These findings are not accidental, even if they occur in apparently asymptomatic individuals with NAFLD, given that the LV dysfunction and LV mass are strongly correlated with IR, the main entity incriminated in the pathogenesis of NAFLD [50].

The impact of NAFLD on endothelial dysfunction and cardiac metabolism was also studied. It is known that endothelial dysfunction is the first pathological change that occurs in the development of atherosclerosis. Endothelial dysfunction has been proven in patients with NAFLD, both diabetic and non-diabetic patients [51-52]. Regarding the cardiac metabolism, the data are controversial. There are opinions that cardiac metabolic abnormalities may precede structural changes given by LV diastolic dysfunction and increased LV mass [22], and also new studies that do not support this hypothesis, given that they did not find any cardiac metabolic changes in the presence of NAFLD [48].

Considering the development of liver transplantation and NAFLD looming as one of the first indications for transplantation [25], we must also consider the potential cardiovascular events that could occur in these patients after transplantation.

Mechanisms linking NAFLD with CVD

The mechanisms linking NAFLD with CVD are not fully understood yet, but attention was focused primarily on insulin resistance (IR). IR seems to explain the accumulation of triglycerides in the liver, being the main pathogenetic link involved in the onset and progression of NAFLD.

IR and NAFLD act synergistically: on the one hand IR has consequences in the metabolism of fatty acids and carbohydrates, resulting in fatty liver, changes that, in turn, lead to the aggravation of IR [22]. Hyperinsulinemia induces disturbances in the transport of free fatty acids, leading to the accumulation of fat in key metabolic organs, such as the liver and the skeletal muscles. Due to deficiencies in the insulin sensitivity of these organs, it produces an exacerbation of IR, which causes metabolic cascade of unintended consequences [53]. In addition, there is also a selective hepatic IR, which probably due to different receptors leads to a paradox: on the one hand, insulin fails to validate its physiological action to reduce gluconeogenesis, and on the other hand, the same insulin continues to stimulate the production of free fatty acids and triglycerides [54]. Even though these mechanisms may explain the onset of steatosis, it remains unknown why some people develop only simple steatosis while others develop NASH.

There is a strong connection between the visceral adipose tissue (VAT) and liver fat [55]. This is not surprising, given that plasma free fatty acids are the main source of fat for the liver, because of the increased lipolysis due to the IR [56]. This situation partly explains the association between NAFLD and MS, which, according to recent recommendations include abdominal obesity between mandatory diagnostic criteria [19]. The question arose whether VAT could explain the increased risk for CVD in patients with NAFLD, rather than the fat content of the liver [22]. VAT is associated with glucose intolerance, IR, dyslipidemia, thus conferring increased risk for CVD, all of which are independent of the presence of diabetes [57]. VAT is strongly associated with IR in producing an increased cardiovascular risk, but IR, in turn, is also associated with increased risk for CVD and atherosclerosis [58]. It is thus unclear whether VAT confers a cardiovascular risk directly by secreted factors, or an indirect risk, by processes related to IR, or both [22]. An important finding comes from studies in patients with lipodystrophy, which demonstrated that, even in patients with lipodystrophy, IR may occur [59]. However, we must not underestimate the role of adipose tissue, which is a metabolically active tissue through the release of proinflammatory adipocytokines, which in turn increase the risk of CVD and atherosclerosis.

The role of the epicardial adipose tissue level (EAT) was also studied, given its anatomical position, near the myocardium and coronary circulation, and the fact that they share the same microcirculation [60]. EAT thickness is associated with the severity of ischemic heart disease [61], with inflammatory markers and with oxidative stress [62], which might suggest potential similarities between the adipokine function secreted by the VAT with the ones secreted by EAT, which in turn is a visceral lipid layer [22].

The liver is a key organ in the systemic inflammatory response syndrome. In NAFLD, the liver is both a source of inflammatory factors and their target [1]. Hepatic steato-

sis increases the production of proinflammatory cytokines by both hepatocytes and by non-parenchymal cells, such as Kupffer cells and stellate cells [63]. Inflammatory markers like Hs-CRP, IL-6, TNF- α are elevated in patients with NASH and correlate with increased cardiovascular risk, with a progressive increased level of these markers with an increasing severity of the liver disease [64,65]. These inflammatory changes occur through activation of the nuclear factor kappa-B (NF- κ B), which, when it is activated, leads to increased hepatic production of IL-6, IL-1 β , TNF- α , and the activation of Kupffer cells and macrophages, which, in turn aggravate liver inflammation [69]. NAFLD should be considered a chronic inflammatory condition [63], and therefore, one should look at these factors secreted by the liver as having an important role in the pathogenesis of systemic inflammation and atherosclerosis [22].

The lipid profile in patients with NAFLD consist in increased levels of TGL, LDL-cholesterol, VLDL-cholesterol, apolipoprotein B100 and decreased levels of HDL-cholesterol, a lipid profile that is linked to cardiovascular events [66]. As a compensatory mechanism to reduce hepatic lipid content, it begins to produce excessive levels of TGL-rich VLDL, with consequent reduction in the quantity and quality of HDL-cholesterol, the lipid fraction that is considered protective against cardiovascular events [67].

PAI-1 and fibrinogen are procoagulants factors whose levels were observed to be elevated in the presence of NASH [64]. At the same time, both are closely related to the development of ischemic heart disease and T2DM [68].

Oxidative stress appears to play an important role in the progression of NAFLD from simple steatosis to NASH. The oxidative stress is characterized by increased reactive oxygen species and by increasing lipid peroxidation [70]. Also, the oxidative stress leads to mitochondrial dysfunction and to an inadequate response of the endoplasmic reticulum [71], and mitochondrial dysfunction and mitochondrial destruction are associated with both IR and atherosclerosis [58].

NAFLD contributes to the development of T2DM through increased gluconeogenesis, hepatic and systemic IR, and through synthesis of inflammatory factors and proteins with diabetogenic properties such as fetuin-A, fibroblast growth factor 21 (FGF-21), and 4 retinol binding protein (RBP-4) [13]. T2DM is a risk factor that contributes to the progression of NAFLD from simple steatosis to NASH and cirrhosis, in some cases even HCC, while being an important contributor to CVD [13].

Management of NAFLD

Currently, there isn't any medication specifically recommended for the treatment of NAFLD. Therapies for NAFLD can be divided into two categories: targeted therapies for the metabolic syndrome constituents through which we obtain an indirect liver benefit and therapies targeted directly to the liver.

Lifestyle changes in order to combat obesity consist of weight loss and regular exercise. Weight loss leads to improved cardiovascular risk profile and improve liver histology, both in terms of steatosis, and in terms of inflammatory activity [72]. Weight loss must be of minimum 5% in order to obtain an effect in terms of simple steatosis, respectively 7% in terms of steatohepatitis [73]. There are no data to objectify a benefit of these changes on liver fibrosis, but it is possible that they are missing because the studies were conducted over periods of time too short for validating any improvement of liver fibrosis. Since these patients compliance to the behavior modification decreases over time, we should take into account behavioral counseling to improve this compliance.

Data from the literature support that bariatric surgery could be of benefit in patients with NAFLD, both in terms of steatosis and steatohepatitis, and a possible benefit on fibrosis [74]. However, bariatric surgery is not recommended as first-line treatment for NASH, but in obese patients requiring bariatric surgery, NASH is not a contraindication [86].

Antidiabetic medication seems promising because it is insulin-sensitizing medication. Studies on the benefit of metformin for NAFLD is controversial, although it turned out that this medication may be of benefit in lowering the risk of HCC in diabetic patients [75]. Glitazones appear to provide a direct benefit on liver disease by decreasing necro-inflammatory activity and steatosis, but with no effect on fibrosis [76]. Encouraging data come to support the effect of GLP-1 agonists, but further study is needed to prove the real benefit of this new class of drugs [77].

Lipid-lowering therapy mainly represented by statins is safe in patients with NAFLD, and they are not subjected to additional risk to the population without NAFLD in developing side effects such as hepatic cytotoxicity. Statin therapy, in addition to the beneficial effect on dyslipidemia, improves liver function [78] and reduce the risk of HCC [79]. Omega-3 fatty acids seem to decrease the liver fat load [80].

Antihypertensive medication seems to bring additional benefit to the hepatopathy, besides lowering hypertension, when there are used blockers of the renin-angiotensin-aldosterone system, and in particular, sartans [81].

In terms of therapy targeted on liver disease, it is reserved for patients with histologically proven NASH, according to current treatment guidelines [82]. Many studies support that antioxidants, especially vitamin E, appear to improve liver histology. Unfortunately, however, in terms of side effects, vitamin E predisposes to increased death risk from any cause, increased risk of hemorrhagic stroke and prostate cancer risk [83].

Pentoxifylline could provide a histological benefit, objectified by reduced steatosis, reduced lobular inflammation, but especially reduced fibrosis, through lowering TNF- α levels and lowering lipid oxidation, but we still

need further studies to confirm the usefulness of this substance in NAFLD therapy [84].

There are some data from uncontrolled studies on the benefits of ursodeoxycholic acid (UDCA) in the management of patients with NAFLD, consisting of a decrease in hepatic cytolysis, and improvement of steatosis [87]. Randomized studies have shown that 13-15 mg/kg/day doses or higher doses of 23-28 mg/kg/day of UDCA have no additional benefit over placebo [87,88], but UDCA combined with vitamin E appears to offer encouraging data [85].

There are also new therapies such as probiotics, IKK inhibitors, PPAR agonists, CB1 peripheral blockers, FXR/TGR5 agonists, autophagy activators, chaperons. All these resources require further additional studies to validate the usefulness in the treatment of NAFLD.

Conclusion

Although the mechanisms underlying the association between NAFLD and CVD are not fully known, attention must be paid to this association, given that these patients are more likely to die due to heart disease rather than liver disease. IR retains its key role in explaining the association between NAFLD and MS, which makes mandatory the inclusion of liver disease on the list of "official" MS constituents. Of course some questions remain looking for an answer. Is NAFLD associated with CVD as a result of the sharing of common risk factors or does NAFLD itself confer an increased cardiovascular risk independent of these factors? How often should these patients be monitored in terms of cardiovascular risk and how early should we intervene? Everyone agrees that IR has its place well established in the pathogenesis of NAFLD. But then, why doesn't insulin sensitivity therapy make its effect better and more readily felt? If this therapy fails to prove its usefulness, it can mean that we are looking at the problem under a totally wrong angle. Because most patients with NAFLD have normal liver function, the question arises whether and how screening should be conducted, considering the already high and increasing incidence of this disease. Given that there are tests that already identified a pattern of genetic changes for NAFLD, it is expected that the clinical utility of these genetic determinations be put to work and integrate them in daily clinical practice. There is need to conduct additional studies to elucidate both the mechanisms underlying the association of NAFLD with CVD, as well as the unknown aspects regarding the surveillance of these patients and their treatment.

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