Cardiovascular and Systemic Risk in Nonalcoholic Fatty Liver Disease - Atherosclerosis as a Major Player in the Natural Course of NAFLD

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Abstract: Non-alcoholic fatty liver disease (NAFLD) encompasses pure steatosis through nonalcoholic steatohepatitis (NASH) and is the most common cause of chronic liver disease in Western countries. NASH is a progressive liver disease that increases the risk of cirrhosis and end-stage liver disease. Interestingly, the global health risk of NAFLD is not confined to the liver. Compared with those without NAFLD, patients with NAFLD exhibit not only increased liver-related complications and liver-related mortality but also increased risk of developing type 2 diabetes, cardiovascular disease (CVD) and chronic kidney disease, increased risk of post-operative complications after major liver surgery, and increased risk of developing certain malignancies, including primary liver cancer and colorectal cancer. In this review, we discuss the current evidence linking NAFLD with the risk of CVD in the setting of the more complex scenario of other hepatic and extra-hepatic complications that may occur during the natural course of NAFLD. Moreover, we provide a brief description of the putative biological mechanisms underlying such complications, particular emphasis being given to CVD. We conclude that NAFLD is a complex health problem with implications far beyond the liver. Hence, it may cause a significant global health burden and the assistance of patients with NAFLD impacts on the work of physicians from many different medical specialties.

Keywords: Atherosclerosis, colorectal cancer, cholangiocarcinoma, chronic kidney disease, cirrhosis, HCC, mortality, NAFLD, NASH, natural history, type 2 diabetes.

"LIFE IS A RISK. DRESS ACCORDINGLY"

INTRODUCTION

In its original 1980 description, Ludwig listed those co-morbidities that are associated with nonalcoholic fatty liver disease (NAFLD), i.e., type 2 diabetes (T2D), obesity and gallstones, and the risk of progressing to cirrhosis [1]. In the 1990s, some studies highlighted that NAFLD usually follows a benign course in most patients but that cirrhosis and hepatocellular carcinoma can develop in a minority of cases [2,3]. Collectively, these pioneer studies dating back to the early '80s and the '90s had already depicted the whole spectrum of the metabolic disorders related to NAFLD and the gamut of its hepatic complications.

More recently, however, it was also appreciated that, further to menacing the structural and functional integrity of the liver, NAFLD may pose even more substantial systemic health risks. Compared with those without steatosis, patients with NAFLD exhibit not only increased all-cause and liver-related mortality but also increased risk of cancer, increased risk of T2D, increased risk of post-operative complications after liver resection, and increased risk of cardiovascular disease (CVD) and chronic kidney disease (CKD).

This review article aims to focus on the increased risk of CVD in people with NAFLD in the setting of the more complex scenario of other hepatic and extra-hepatic complications that may occur during the natural course of NAFLD.

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I. RISK OF ALL-CAUSE AND LIVER-RELATED MORTALITY

A recent joint position paper by leading American hepatological societies states that patients with NAFLD have increased all-cause mortality compared to matched control populations [4]. The best evidence for such a conclusion results from a recent systemic review and meta-analysis by Musso *et al.* [5]. By pooling data from seven prospective studies with follow-up periods ranging from 7 to 28 years [6-13], the authors concluded that NAFLD as diagnosed by either imaging or histology was associated with a substantially increased risk of all-cause mortality (odds ratio [OR] 1.57, 95% confidence intervals [CI] 1.18-2.10, P<0.001) [5]. The main causes of mortality among patients with NAFLD were malignancy (28%) and CVD (25%). Interestingly, liver-related mortality not only ranked third among the causes of death in patients with NAFLD as compared with eleventh in the control population [5] but, more alarmingly, these deaths appeared to be concentrated in the more highly active 45-54-year age group [11], so posing a particularly heavy economic burden on the society. Although the conclusions of this meta-analysis appear to be methodologically correct, however, it should be pointed out that there was a high heterogeneity among the meta-analyzed studies (I^2=88%), and that the original data included in the meta-analysis come from either USA or northern Europe [5], implying that the natural history of NAFLD in countries other than those listed is, at best, characterized by an unproven inference.

Such a limitation in our understanding of the natural history of NAFLD needs to be emphasized since genetic factors, diet and lifestyle can affect the development and course of NAFLD [14,15]. The results of a recent Italian population-based cohort study, which was not included in the Musso's meta-analysis, is of interest [16]. In this study, the authors examined the 15-year mortality rates in

approximately 2,000 middle-aged individuals living in Cremona, a town in northern Italy. Interestingly, they found that the fatty liver index (FLI), a surrogate marker of NAFLD, was associated with increased all-cause and cause-specific mortality [16]. However, after adjustment for insulin resistance as estimated by homeostasis model assessment, the FLI lost its significant association with all-cause, CVD and cancer-related mortality while retaining a significant association with liver-related mortality alone [16]. This finding would suggest that the extra-hepatic complications of NAFLD might be likely due to concurrent insulin resistance rather than to NAFLD *per se*. However, the results of this observational study should be interpreted with some caution as the FLI is an index derived from serum liver enzymes, metabolic and anthropometric variables (i.e., body mass index, waist circumference, serum triglyceride level and serum gamma-glutamyltransferase level) that has been initially proposed to screen those individuals in need to undergo liver ultrasonography [17] and that has never been validated against liver histology.

It is worth to mention here that recent data from the National Health and Nutrition Examination Survey (NHANES-III) has reached unexpected results, conflicting with most of the literature data. Surprisingly, using data from the NHANES 1988-94 database, Lazo *et al.* failed to find a significant association between NAFLD on ultrasonography and the 14-year risk of all cause and cause specific (cardiovascular, cancer, liver) mortality in U.S. adults [18]. However, some important limitations of this study should be kept in mind in interpreting the results. Among these, we would like to underline the lack of a liver biopsy for diagnosing NAFLD, the relatively short-term follow-up (especially considering that NAFLD patients had a mean age of ~48 years at baseline) and the relatively low number of mortality outcomes [18].

Given that compared to the general population of same age and sex, NAFLD is associated with a significantly higher all-cause mortality [4], the ensuing question is whether the severity of NAFLD histology may add further prognostic value, especially for liver-related mortality. In order to answer such a question, Angulo [19] recently reviewed the published data from prospective studies on long-term mortality in NAFLD with an average follow-up period of 5 years or longer [3,9,13,20-25], and reported that liver-related mortality followed a steeper gradient from pure steatosis to NASH and to NASH-cirrhosis. Collectively, these studies showed that the liver-related mortality was significantly higher in patients with NASH as compared to pure steatosis (7.3% *vs.* 0.9%, respectively, P<0.001) [19]. In addition, it also appeared that the presence and severity of hepatic fibrosis dictated both all-cause and liver-related mortality in NAFLD as well as the development of the most important liver-related complications, including portal hypertension, end-stage liver disease and primary liver cancers [19,26]. Accordingly, to date, the identification of accurate non-invasive predictors of advanced liver fibrosis in patients with NAFLD represents a key clinical task. Several non-invasive clinical scoring systems have been developed to predict advanced liver fibrosis in routine clinical practice [4,5]. Most of such non-invasive clinical scoring systems include markers of insulin resistance and/or various components of the metabolic syndrome, suggesting that it is the dysmetabolic *milieu* along with older age that primarily drives the progression of NAFLD.

More recently, major genetic modifiers of the natural course of NAFLD have also emerged. In fact, Sookoian *et al.* [14] gauged across different populations the effect of the rs738409 variant of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene, a known risk factor for NAFLD development [27], on the histological severity of NAFLD. In this meta-analysis, the authors reported that rs738409 is a major modifier of the natural history of NAFLD across different ethnicities on a worldwide basis [14].

I.a. Biological Basis of Hepatic Complications: From Intra-Hepatic Fat to Fibrosis and Beyond Via (Mild) Necro-inflammation

The intra-hepatic necro-inflammatory changes along with advancing age dictate the future development of hepatic fibrosis and are, therefore, critical to account for the variable clinical course of NAFLD [28].

A substantial evolution in the theories of NAFLD pathogenesis has occurred in the last years. The original *"two-hit"* theory of NAFLD proposed by Day and James 15 years ago suggested that, at the beginning, all patients with NAFLD develop pure steatosis (the so-called *"first hit"*), which progresses to NASH only in a minority of cases as a result of the so-called *"second hit"* [29]. Such a theory has been challenged by the results of some recent studies suggesting that pure steatosis and NASH are two different and probably not necessarily inter-related pathologic conditions (as reviewed in 30,31). Moreover, accumulating evidence suggests that pure steatosis is an adaptive phenomenon that may protect the liver against the chemical damage of free fatty acids (FFA), which are made less lipo-toxic when they are stored into the liver as inert triglycerides [30-32]. This view anticipates that those individuals in whom this adaptive phenomenon fails, will develop NASH. Unfortunately, to date, we cannot identify with certainty those individuals in whom NASH will develop. However, accumulating evidence supports the possibility that insulin resistance is a *"necessary but not sufficient"* morbid condition, which plays a role only in the early phases of the development of NASH [33]. Interestingly, specific genetic polymorphisms [14,27,30], chemical nature of the fat accumulated in the liver [e.g., saturated fatty acids [34] and free cholesterol [35]], increased serum uric acid level [36], increased serum ferritin level [37], and some endocrine disorders, e.g., hypothyroidism [38], play important roles in promoting the development of NASH. In addition, decreased antioxidant defenses, early mitochondrial dysfunction, altered sleep physiology, hypothalamic changes and gut microbiota may also contribute to the development of NASH but their pathogenetic role needs to be further elucidated [30,39].

At variance with other chronic hepatitides (such as viral hepatitis and autoimmune hepatitis), NASH is generally associated with far less prominent intra-hepatic necro-inflammation [40,41]. Such a milder degree of hepatic histologic changes might account for the more indolent course of NAFLD when compared with other chronic hepatitides. It is also possible, however, that a certain amount of chronic inflammation in patients with NAFLD also occurs in other organs. For instance, chronic inflammatory changes that occur in the adipose tissue ("adipositis") may result in abnormalities in the pattern of adipokine secretion [30,42], which together with endoplasmic reticulum stress and innate immunity, are central pathways in the pathogenesis of NASH [31], and may at least partly account for both hepatic and extra-hepatic complications of NAFLD. In particular, an abnormal cytokine secretion pattern may promote on the one hand a wound-healing pro-fibrogenic response into the liver; on the other hand, the development of systemic adverse metabolic and vascular outcomes [42-45].

Finally, insulin resistance and its related low-grade inflammatory state (which is typically associated with NASH) may also play a pathogenetic role in the development of primary liver cancers, principally hepatocellular carcinoma (HCC), in a subset of genetically prone NAFLD cases [46,47]. Major risk factors for the development of HCC in patients with NASH include older age, cirrhosis, T2D, obesity and iron overload [46]. However, iron overload is a controversial finding in NASH [48], and HCC has been also reported in non-cirrhotic NAFLD [49]. This finding suggests that NAFLD/NASH itself might promote HCC development in association with the mitogenic activity induced by chronic hyperinsulinemia and hyperglycemia [43,46].

II. RISK OF CANCER

In principle, it might be anticipated that the liver is the target organ for malignant transformation in patients with NAFLD. Indeed, NAFLD is associated with excess occurrence of primary liver cancer, principally HCC. However, based on the analogy with obesity and T2D [50,51], other organs might also be exposed to an increased cancer risk among patients with NAFLD. The putative underlying mechanisms by which NAFLD and its related metabolic disorders are linked to increased cancer risk have been extensively reviewed elsewhere [52].

II.a. Hepatocellular Carcinoma

The first circumstantial evidence that HCC could well be a part of the NAFLD spectrum may be dated back to the 1990s [2], approximately 10 years after the disease had been named by Ludwig. However, it was the seminal paper by Bugianesi *et al.* [53] that provided a systematic evaluation of the entity of the risk of HCC in patients with NAFLD. These authors by comparing 23 patients in whom HCC had developed on cryptogenic cirrhosis (likely representing burnt-out NASH) have identified the following independent risk factors for HCC: hypertriglyceridemia, T2D and lower aminotransferase levels. Because these three risk factors were previously reported as clues pointing to NASH in cryptogenic cirrhosis in the USA [54], the same authors also suggested that HCC may occur as a late complication of NASH-related cirrhosis [53]. Accordingly, in our recent review we also concluded that there is a strong inter-relationship between NASH, cryptogenic cirrhosis and HCC [49].

Notably, an Italian multicenter study has raised alarm about the fact that HCC is often diagnosed at a more advanced clinical stage among patients with cryptogenic cirrhosis, thus leading to more limited treatment options and hence a shorter survival of these patients compared with those with hepatitis C virus infection [55]. Similar findings have been also confirmed by other studies [56,57]. To further compromise the chances for an early diagnosis of HCC among patients with NASH is the observation that the dysmetabolic *milieu* related to NAFLD is an important risk factor for the development of HCC [58], and that HCC may also develop among patients with non-cirrhotic, low-fibrosis NASH [59-62]. All these findings strongly suggest the opportunity of more liberal surveillance schedules in patients with NASH [63]. However, the cost/effectiveness ratio of such a clinical approach needs to be better defined.

II.a.1. Intra-hepatic Cholangiocarcinoma

Some recent data have suggested that NAFLD is not only associated with an increased incidence of HCC but also of ICC.

Similarly to HCC, two large epidemiological surveys have shown that the dysmetabolic *milieu* related to NAFLD is an important risk factor for the development of ICC [64,65]. In USA, Welzel *et al.* [64], by comparing 743 consecutively diagnosed cases of ICC with a sample of inhabitants of the same geographic area, have reported that the metabolic syndrome was an independent predictor of ICC (adjusted OR 1.56; 95% CI 1.32-1.83, P<0.001). In a large prospective cohort study involving 578,700 European individuals, it has been also reported that components of the metabolic syndrome (especially high body mass index and increased fasting glucose levels) were associated with an increased risk of developing both HCC and ICC during an average follow-up of ~12 years. However, a subgroup analysis of these data revealed that only HCC retained a significant association with metabolic syndrome components [65].

II.b. Colorectal Neoplasms and Other Types of Cancer

Sørensen *et al.* who examined the incidence of cancer in 7,326 patients discharged with a diagnosis of fatty liver from a Danish hospital during 1977-1993 published the first evidence for a significant association between NAFLD and increased risk of colorectal cancer [66].

Notably, these findings have been further confirmed by other investigators [67-69]. Hwang *et al.* [67] reported that NAFLD was significantly associated with the number of colorectal adenomatous polyps among 2,917 Korean adults, who were consecutively submitted to colonoscopy. In a study involving two large cohorts of Chinese individuals, Wong *et al.* [68] found that NASH was associated with the presence of colon adenomas (adjusted OR 4.89, 95% CI 2.0-11.7) and advanced neoplasms (adjusted OR 5.34, 95% CI 1.9-14.8), independently of metabolic risk factors. In contrast, the presence of colon adenomas and advanced neoplasms was similar between patients with pure steatosis and control subjects [68]. Finally, in a study involving 1,211 Austrian men and women, who underwent screening colonoscopy, Stadlmayr *et al.* [69] reported that NAFLD was a independent risk factor for colorectal neoplasia (adjusted OR 1.47; 95% CI 1.1-2.0; P=0.01).

Collectively, these few available studies suggest that NAFLD is associated with the adenoma-carcinoma sequence in the large bowel; that this association follows a biological gradient and that it occurs irrespective of the underlying metabolic disorders. However, currently, it is still uncertain if screening colonscopy should be routinely performed in all patients with NAFLD; this decision should be better taken on an individual clinical basis [70].

Two other neoplasms, i.e., pancreatic [66] and breast [71] cancers, both belonging to the spectrum of diseases that are closely associated with obesity and T2D, have been also reported to occur more frequently among patients with NAFLD than among those without. However, these data are preliminary and the true entity of excess risk, if any, remains to be evaluated prospectively.

II.c. Biological Basis for the Development of Extrahepatic Cancers

In theory, at least three putative biological mechanisms might account for the increased risk of extrahepatic cancers observed among patients with NAFLD.

First, both NAFLD and extrahepatic cancers may result from the dysmetabolic *milieu* that is typically observed in T2D and obesity. Second, NAFLD may contribute to the development of extrahepatic cancers via dysregulation of the innate immunity and/or altered cytokine secretion patterns [72]. Third, in the case of colorectal cancer, an altered bile composition in patients with NAFLD may represent an additional risk factor that promotes the development of colorectal cancer.

The first putative biological mechanism may account for the association between NAFLD and the risk of pancreatic cancer: systemic insulin resistance promotes the development of fatty changes both in the liver and in the pancreas [43]. Experimental evidence supporting insulin resistance as a risk factor for the development of pancreatic cancer has been reviewed previously [73]. The second putative biological mechanism may account for the association between NAFLD and the risk of developing certain cancers in organs distant from the liver such as the breast [71]. Additionally, the last two biological mechanisms might also be involved in the pathogenesis of colorectal cancer. However, while a potentially pro-oncogenic cytokine profile has been reported in NAFLD [72,73], little is known about changes of the bile acid composition in NAFLD. However, the finding that gallstones in NAFLD patients go often in parallel with increasing insulin resistance further suggests that altered bile composition might also play a role in the development of colorectal cancer [74-76].

III. RISK OF TYPE 2 DIABETES MELLITUS

A systematic review and meta-analysis of 23 prospective, population-based studies confirmed that NAFLD diagnosed on serum liver enzymes or imaging is associated with a twofold to three-fold

Table 1. **Is nafld a risk for the development of type 2 diabetes? Data from prospective studies**

Author, year (Ref.)	Study Characteristics and Findings
Vozarova, 2002 (77)	Higher ALT level is associated with increased risk of incident T2D in nondiabetic Pima Indians followed-up for ~7 years
Lee, 2003 (78)	Higher GGT level is associated with increased risk of incident T2D in healthy workers followed-up for 4 years
Hanley, 2005 (79)	Higher ALT level is associated with incident metabolic syndrome in 1,625 individuals followed-up for ~5 years
Monami, 2008 (80)	Higher GGT level is associated with increased risk of incident T2D in 2,662 diabetes-free individuals followed-up for ~3 years
Goessling, 2008 (81)	Higher ALT level is associated with increased risk of incident T2D in 2,812 individuals followed-up for 20 years
Ford, 2008 (82)	Higher GGT level is associated with increased risk of incident T2D in 3,011 individuals followed-up for 7 years
Adams, 2009 (83)	Higher ALT level is associated with increased risk of incident T2D/metabolic syndrome in 358 individuals followed-up for 11 years
Fraser, 2009 (84)	Higher ALT and GGT levels are both associated with increased risk of incident T2D in a meta-analysis of 18 prospective, population-based studies; the fully adjusted hazard ratio for diabetes per increase in one unit of logged ALT was 1.83 (95% CI 1.57-2.14) and for GGT was 1.92 (1.66-2.21)
Balkau, 2010 (85)	Higher fatty liver index (FLI) is associated with increased risk of incident T2D in 7,711 individuals followed-up for 9 years
Okamoto, 2003 (86)	NAFLD on ultrasonography is not independently associated with incident T2D in 840 individuals followed for 10 years
Shibata, 2007 (87)	NAFLD on ultrasonography is associated the risk of incident T2D in 3,189 male middle-aged workers followed for 8 years
Kim, 2008 (88)	NAFLD on ultrasonography is associated the risk of incident T2D in 5,372 individuals followed for 5 years
Yamada, 2010 (89)	NAFLD on ultrasonography is associated the risk of incident T2D in 12,375 individuals followed for 5 years
Sung, 2011 (90)	NAFLD on ultrasonography is associated the risk of incident T2D in 11,091 individuals followed for 5 years
Bae, 2011 (91)	NAFLD on ultrasonography is associated the risk of incident T2D in 7,849 individuals followed for 5 years, especially among those with impaired fasting glycemia at baseline
Sung, 2012 (92)	NAFLD on ultrasonography is associated the risk of incident T2D in 12,853 individuals followed for 5 years, independently of insulin resistance and overweight/obesity
Ekstedt, 2006 (9)	Patients with histologically confirmed NASH are at higher risk of incident T2D than those with pure steatosis during a 13.7-year follow-up (n=129)

increase in the risk of developing T2D [5]. These data fit well with clinical experience suggesting that patients with NAFLD, who are not diabetic at baseline, frequently develop the disease during the follow-up.

In (Table **1**) are reported the principal prospective studies that have assessed the relationship between NAFLD (as detected by serum liver enzymes, imaging or histology) and the risk of developing T2D [9,77-92]. Many population-based studies that have used serum liver enzymes or radiological imaging have consistently shown that NAFLD is independently associated with an increased risk of incident T2D. However, the adjustment for potential confounders was often incomplete (for example, data on waist circumference, family history of diabetes, physical activity, fasting glucose level and insulin resistance were not always included in multivariate regression models). Only one study with a relatively small number of patients with biopsy-proven NAFLD (n=129) assessed the risk of T2D of different NAFLD histological subtypes, finding an OR of 2.98 (95% CI 1.23-7.22; P<0.01) in patients with NASH compared to those with pure steatosis over 13 years of follow-up (Table **1**).

A recent review of the literature data published by our group has confirmed that NAFLD is an optimal biological *milieu* in which T2D can develop [43]. The relationship between NAFLD and T2D,

however, is bidirectional: once developed, T2D has the potential for promoting the progression to NASH, cirrhosis and HCC [43]. Because of the "vicious circle" between NAFLD and T2D (as schematically shown in Fig. **1**), we believe that more careful surveillance of these patients will be needed.

III.a. Biological Basis For A Diabetogenic Role of NAFLD

T2D only manifests as a result of defects in insulin secretion, insulin action, or both. [93]. Except for specific varieties of genetic hepatic steatosis which are not associated with insulin resistance [94], as a general rule, NAFLD and insulin resistance go always together [94,95], primarily as a result of protein kinase Cε activation which impairs insulin signaling in target cells [96]. The biological mechanisms leading from NAFLD to T2D and from the latter to progressive liver disease are summarized in schematic (Fig. **1**). Recently, our group has published comprehensive reviews on the basic mechanisms leading from NAFLD to both T2D and atherogenic dyslipidemia [43-45].

IV. SURGICAL RISK

Given the growing and epidemic prevalence of NAFLD in the general population, it is expected that these individuals may be prone to hepatic resection either as a therapeutic option for primary
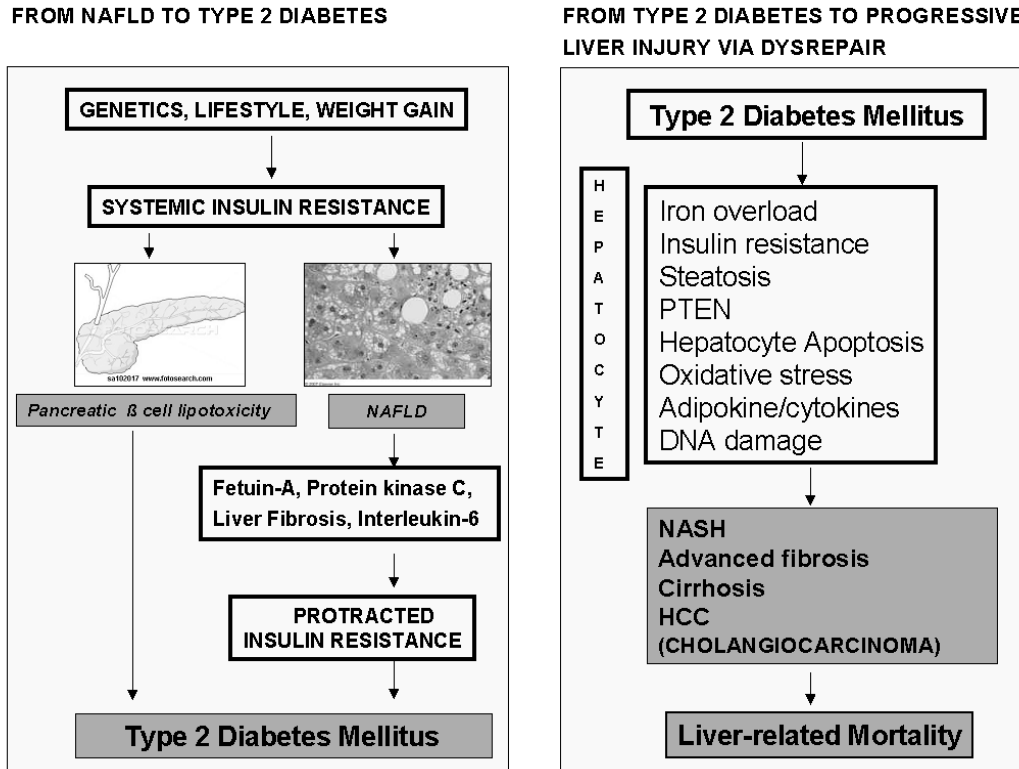
FROM NAFLD TO TYPE 2 DIABETES

FROM TYPE 2 DIABETES TO PROGRESSIVE LIVER INJURY VIA DYSREPAIR

Fig. (1). NAFLD and type 2 diabetes mellitus, "a vicious circle" (from Loria P *et al.* [43] with permission).
This figure depicts the closed loop leading from NAFLD to type 2 diabetes via long-standing insulin resistance (left section). Closing the circle, concurrent type 2 diabetes and NAFLD trigger ongoing liver injury and may result in end-stage liver failure as well as primary liver cancer (right section).

and metastatic hepatobiliary cancers or in the setting of living donation for liver transplantation [97]. Although advanced techniques in modern surgery, patients selection and postoperative medical management have resulted in negligible peri-operative mortality rates [98], however, recent studies have highlighted that hepatic steatosis is a risk factor for postoperative complications and death after major liver surgery [99]. For instance, McCormack *et al.* reported that compared with those without steatosis, patients with histologically confirmed hepatic steatosis have a significantly greater incidence of post-operative complications when considered either overall (50% *vs.* 25%) or major (27.5% *vs.* 6.9%) [99]. More recently, in a case-control study involving 102 patients with NASH, 72 patients with pure steatosis and 174 nonsteatotic control patients who underwent liver resection, Reddy *et al.* reported that 90-day post-operative overall morbidity, any liver-related morbidity and hepatic decompensation were significantly greater in patients with NASH, but not in those with pure steatosis, compared to corresponding controls [100].

Preliminary experimental evidence in animal models suggested that impaired liver regeneration (probably because of increased hepatocellular lipid peroxidation and damage in concert with Kupffer cell-mediated pro-inflammatory responses) may represent the biological basis accounting for increased rates of complications after major hepatic resection [101].

In order to estimate the potential adverse impact of NAFLD on patient outcomes after major surgery of the liver, de Meijr *et al.* recently performed a systematic review and meta-analysis of four prospective studies involving approximately 1,000 patients [97]. Notably, they reported that patients with NAFLD had a twofold to three-fold higher risk of major complications and death after hepatic resection compared with those without steatosis, and that this

risk increased in parallel to the histological severity of hepatic steatosis (Fig. **2**) [97].

Overall, these (preliminary) findings suggest the opportunity to implement specific changes in our routine clinical practice such as improved techniques of communication and, if possible, pre-operative interventions to improve NAFLD [97].

V. RISK OF CVD AND CKD

V.a. Risk of CVD

Originally deemed to be its hepatic manifestation [102-104], NAFLD has been more recently considered as one of the most important contributors to the development of metabolic syndrome [105]. On these grounds, and given that the first reported association between fatty liver and atherosclerosis dates back to the early 1950's [106], it is certainly not surprising that a lot of epidemiological studies have recently shown that NAFLD is linked with an increased risk of CVD, as also reviewed elsewhere [5,44,45, 107,108].

Currently, there is a large body of evidence suggesting that NAFLD, especially in its necro-inflammatory form (NASH), is associated with a more athero-thrombotic risk profile (Table **2**), independently of overweight/obesity and other cardio-metabolic risk factors [109-132]. Therefore, NAFLD *per se* might represent an emerging risk factor for CVD. In line with this hypothesis, several cross-sectional studies have shown that NAFLD is associated with a greater prevalence of clinically manifest CVD as well as with markers of subclinical atherosclerosis such as increased arterial stiffness, reduced brachial artery flow-mediated vasodilation and increased carotid-artery intimal medial thickness or coronary-artery calcium [107,133-161], as summarized in (Table **3**). The
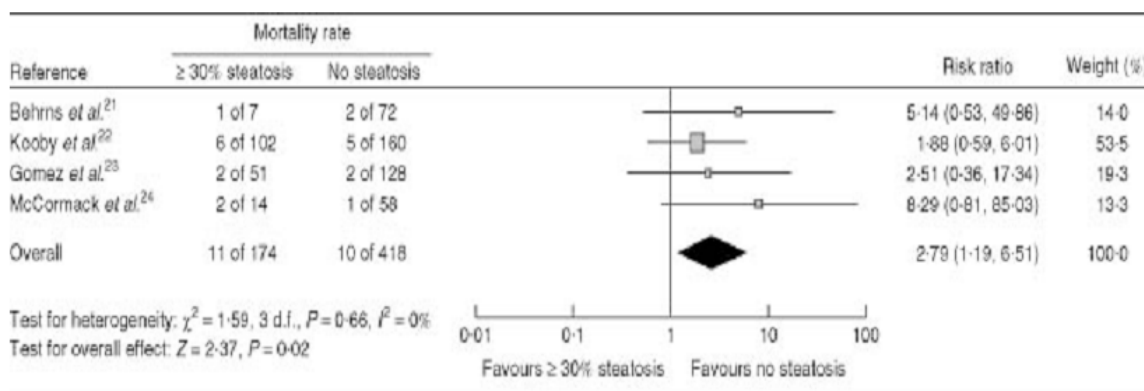
Fig. (2). Hepatic steatosis is associated with increased post-operative mortality rates (from de Meijer VE *et al.* [97] with permission).
Meta-analytic evidence that patients with histologically confirmed hepatic steatosis have a substantially higher risk of mortality following major surgical interventions on the liver than those without steatosis.

most worrying aspect of NAFLD as an emerging CVD risk factor is that the increased CVD risk is not only restricted to adults but it has also observed among children and adolescents. Additionally, the adverse impact of NAFLD on CVD risk is not only confined to vascular beds as several studies reported that NAFLD is also associated with early abnormalities in cardiac metabolism, geometry and function (as reported in Table **3**). Based on such findings, recent clinical recommendations to be applied to the individual patient with NAFLD have been put forward by national and international hepatological societies [4,70,162].

While the association between NAFLD and prevalent CVD seems to be robust and consistently replicated across different populations, the contribution of NAFLD *per se* to increased risk of incident CVD is more controversial. It remains debatable if NAFLD is simply a marker of CVD or if it actively contributes to the pathogenesis of CVD.

Several, but not all studies, reported that CVD morbidity and mortality are a serious menace to patients with NAFLD, and that CVD dictates the outcome (or outcomes) in patients with NAFLD more frequently and to a greater extent than does the progression of liver disease (Table **4**) [5,7-13,18,20,163-169]. On the other hand, in this review, we did not discuss the large number of prospective, population-based cohort studies that have used serum liver enzymes to diagnose NAFLD, and that have consistently shown that modestly elevated serum liver enzyme levels (i.e., a surrogate marker of NAFLD) are long-term, independent predictors of CVD morbidity and mortality both in adults and in adolescents [45,108,170].

Perhaps, the strongest evidence that supports a prognostic role of NAFLD on CVD risk is among people with T2D, as it is suspected that NAFLD *per se* is associated with an almost twofold increase in the risk of fatal and non-fatal CVD events even after controlling for several CVD risk factors, including metabolic syndrome features [166,167].

In addition, as reported in (Table **4**), some retrospective studies with a relatively long duration of follow-up that have examined the natural history of patients with histologically confirmed NAFLD have clearly shown that patients with NASH, but not those with pure steatosis, are at increased risk of CVD mortality compared with the reference population. Nevertheless, as also mentioned above, a recent systematic review and meta-analysis of retrospective/prospective studies by Musso *et al.* [5] concluded that NAFLD confirmed by ultrasound or histology is associated with a substantially increased risk of incident CVD (adjusted OR 2.05, 95% CI 1.81-2.31, P<0.0001; n=7 meta-analyzed studies) but that the severity of NAFLD histology does not appear to predict CVD mortality.

Indeed, CVD mortality did not significantly differ between patients with NASH and those with pure steatosis (adjusted OR 0.91, 95% CI 0.42-1.98, P=0.82; n=5 meta-analyzed studies). However, further larger and longer follow-up studies are needed to better elucidate this issue.

V.b. Risk of CKD

Epidemiological data supporting the view that NAFLD is associated with CKD, independently of overweight/obesity, T2D and hypertension, have been recently reviewed by our group [171]. Published data clearly demonstrate that NAFLD is associated with an increased prevalence of CKD, defined as presence of microalbuminuria, overt proteinuria or an estimated glomerular filtration rate (GFR) <60 ml/min/1.73m^2, in both nondiabetic and diabetic individuals [172-175]. Preliminary evidence also suggests that there is a positive, graded relationship between kidney function parameters (abnormal albuminuria and/or GFR reduction) and the severity of NAFLD histology [176,177]. The few available prospective studies seemingly also support a prognostic role for NAFLD in the development and progression of CKD both in patients without diabetes and in those with type 2 diabetes [178-181]. However, further research is urgently needed to ascertain whether NAFLD confers an excess risk over and above what would be expected from the shared cardio-renal risk factors.

Before such data become available, no systematic follow-up of patients with NAFLD for the early detection of CKD was recommended by practice guidelines endorsed by scientific societies [4,70,162]. Nevertheless, we believe that clinicians should be willing to implement such a follow-up evaluation in the individual patient with NAFLD, based either on personal/familial clues that suggest an increased risk for CKD or on the coexistence of multiple cardio-renal risk factors that may further amplify kidney damage.

V.c. Biological Basis for the Development of CVD and CKD

There are few but consistent pieces of biological evidence that may account for a role of NAFLD in the development and progression of CVD. For instance, particularly in patients with NASH, the liver disease is associated with structural and metabolic changes of all the resident cell populations of the liver (i.e., hepatocytes, Kupffer cells and stellate cells). Such phenotypic changes in liver cell populations may result in a systemic more athero-thrombotic risk profile possibly through the development of systemic/hepatic insulin resistance, atherogenic dyslipidemia and the up-regulation of synthesis and secretion of several pro-inflammatory and pro-coagulant factors (as schematically shown in Fig. **3** and Table **2**).

Table 2.   Nafld Increases The Risk Of Cvd: Summary Of The Biological Evidence From Human Studies.

Author, year (Ref.)	Study Characteristics and Findings
	NAFLD or NASH is associated with increased circulating levels of proatherothrombotic mediators
Kugelmas, 2003 (109)	Increased serum levels of TNF-alpha, IL-6 and IL-8 (case-control study)
Hui, 2004 (110)	Increased serum levels of TNF-alpha, soluble TNF receptors and decreased levels of adiponectin (case-control study)
Chalasani, 2004 (111)	Increased serum levels of oxidized LDL-cholesterol and thiobarbituric acid reactive substances (case-control study)
Haukeland, 2006 (112)	Increased serum levels of interleukin IL-6, MCP-1, CCL2 and CCL19 (case-control study)
Targher, 2005 (113), Targher, 2008 (114)	Increased levels of hs-CRP, fibrinogen, von Willebrand factor, PAI-1 and decreased levels of adiponectin (case-control study)
Musso, 2007 (115)	Increased serum nitrotyrosine levels (case-control study)
Nobili, 2009 (116), Milner, 2009 (117)	Increased serum levels of retinol-binding protein 4 (case-control study)
Sookoian, 2010 (118)	Increased levels of soluble ICAM-1, PAI-1 and soluble CD40 ligand (case-control study)
Yilmaz, 2011 (119)	Increased levels of chemerin and vaspin (case-control study)
Kotronen, 2011 (120)	Increased levels of coagulation factor VIII, IX, XI and XII activities (case-control study)
Sookoian, 2012 (121)	Increased white blood cell and platelet counts (case-control study)
Alkhouri, 2012 (122)	Increased mean platelet volume, a marker of platelet activation (case-control study)
Ndumele, 2011 (123)	Increased serum levels of hs-CRP (community-based study)
Xu 2009 (124), Yilmaz 2012 (125)	Increased hemoglobin levels, especially in those with NASH without metabolic syndrome (community-based and cohort study)
	NAFLD/NASH is associated with abnormal hepatic expression of molecular mediators of atherogenesis, inflammation and coagulation
Sookoian, 2010 (118), Sookoian, 2011 (126)	Increased hepatic expression of hepatic expression of sICAM-1 and PAI-1; TGFB1, angiotensin I-converting enzyme, LAMA1, SERPINB2, CSF2, IL1A, IL3, IL4, LIF and MMP1 in NASH (case-control study)
Wieckowska, 2008 (127)	Increased hepatic expression of IL-6 (case-control study)
Yoneda, 2007 (128)	Increased hepatic expression of CRP (case-control study)
Thuy, 2008 (129)	Increased hepatic expression of PAI-1 (case-control study)
Westerbacka, 2007 (130), Greco, 2008 (131)	Hepatic up-regulation of several genes involved in monocyte/macrophage recruitment, inflammation and coagulation (case-control study)
	NAFLD is associated with impaired vascular repair capacity
Chiang, 2012 (132)	Decreased circulating levels of bone marrow derived-endothelial progenitor cells (case-control study)

Table 3.   Clinical evidence about the impact of nafld on cvd-related phenotypes

Author, year (Ref.)	NAFLD/NASH is associated with CVD-related phenotypes
	Carotid Atherosclerosis
Sookoian, 2008 (107)	NAFLD is associated with a 13% increase in carotid artery wall thickness (meta-analysis of 7 studies involving 3497 subjects)
Targher, 2006 (133), Colak, 2012 (134)	Severity of NAFLD histology is associated with carotid artery wall thickness
Kozakova, 2012 (135)	NAFLD as estimated by the fatty liver index is associated with early carotid plaques in middle-aged nondiabetic subjects
	Coronary Atherosclerosis
Gastaldelli, 2009 (136)	NAFLD as estimated by the fatty liver index is associated with coronary heart disease in middle-aged nondiabetic subjects

(Table 3) Contd….

Author, year (Ref.)	NAFLD/NASH is associated with CVD-related phenotypes
Lee, 2011 (137), Chen, 2010 (138), Kim, 2012 (139), Sung, 2012 (140)	NAFLD is associated with abnormal coronary artery calcium score
Akabame, 2008 (141)	NAFLD is associated with coronary vulnerable plaques on 64-detector multislice computed tomography
Assy, 2010 (142)	NAFLD is associated with greater severity of coronary artery disease on coronary CT angiography
Wong, 2011 (143)	NAFLD is associated with greater severity of coronary artery disease on coronary angiography
Yilmaz, 2010 (144)	NAFLD is associated with decreased coronary flow reserve
Targher, 2007 (145)	NAFLD is associated with greater prevalence of coronary, cerebrovascular and peripheral vascular disease in type 2 diabetic patients
Lin, 2005 (146)	NAFLD is associated with ischemic heart disease in a community-based cohort of male workers
	NAFLD and CVD Phenotypes in Children and Adolescents
Schwimmer, 2008 (147)	NAFLD on biopsy is associated with multiple cardiovascular risk factors
Pacifico, 2010 (148)	NAFLD is associated with reduced brachial artery flow-mediated vasodilation and increased carotid artery wall thickness
Alkhouri, 2011 (149)	NAFLD is associated with increased carotid artery wall thickness
	Increased Arterial Stiffness and Circulatory Endothelial Dysfunction
Vlachopoulos, 2010 (150), Salvi, 2010 (151), Lee, 2012 (152)	NAFLD is associated with increased arterial stiffness and reduced brachial artery flow-mediated vasodilation
Villanova, 2005 (153), Colak, 2012 (134)	NAFLD histology is associated with reduced brachial artery flow-mediated vasodilation
	Early Abnormalities in Cardiac Metabolism and Function
Perseghin, 2008 (154)	NAFLD is associated with impaired left ventricular energy metabolism in nondiabetic, normotensive, young subjects
Lautamaki, 2006 (155), Rijzewijk, 2010 (156)	NAFLD is associated with decreased myocardial perfusion in type 2 diabetic patients with/without ischemic heart disease
Goland, 2006 (157), Fotbolcu, 2010 (158)	NAFLD is associated with abnormalities in left ventricular morphology and function in nondiabetic, normotensive subjects
Fallo, 2009 (159)	NAFLD is associated with diastolic dysfunction in patients with never-treated essential hypertension
Bonapace, 2012 (160)	NAFLD is associated with diastolic dysfunction in type 2 diabetic patients without ischemic heart disease
Colak, 2012 (161)	NAFLD is associated with increased epicardial fat thickness

Table 4.   Does nafld/nash increase the risk of fatal and non-fatal cvd events?

Author, year (Ref.)	Study Characteristics and Findings
	NAFLD or NASH is not associated with increased risk of CVD events
Musso, 2011 (5)	NASH does not confer a significantly higher CVD mortality than pure steatosis (meta-analysis of 5 studies)
Lazo, 2011 (18), Stepanova, 2012 (163)	NAFLD on ultrasonography is associated with higher CVD prevalence but does not increase all-cause and cause-specific mortality in US adults (n=11,371, follow-up 14.5 years)
Dam-Larsen, 2004 (20)	No excess of all-cause and cause-specific mortality in patients with pure steatosis on biopsy compared with the general population (n=109, follow-up 16.7 years)
Domanski, 2012 (164)	No increased prevalence of CVD in patients with NASH compared with those with non-NASH fatty liver (a retrospective chart review of 377 patients with biopsy-proven NAFLD). Note of caution: cross-sectional study
	NAFLD or NASH is associated with increased risk of CVD events

| Author, year (Ref.)	Study Characteristics and Findings
Musso, 2011 (5)	NAFLD, as assessed by either liver enzymes or ultrasonographic/histological methods, is associated with increased risk of all-cause, CVD and liver-related mortality compared with the reference population (meta-analysis of 7 studies)
Jepsen, 2003 (7)	NAFLD (especially NASH and cirrhosis) is associated with increased CVD mortality in 1,800 patients discharged with a hospital diagnosis of NAFLD followed for 6.2 years
Adams, 2005 (8)	NAFLD (especially NASH and cirrhosis as confirmed by imaging or biopsy) is associated with increased CVD mortality in a community-based cohort of 420 patients with NAFLD followed for 7.6 years
Ekstedt, 2006 (9)	Patients with histologically confirmed NASH but not those with pure steatosis are at higher risk of all-cause, CVD and liver-related mortality compared with the reference population (n=129, follow-up 13.7 years)
Dunn, 2008 (11)	NAFLD diagnosed by liver enzymes is associated with CVD mortality especially in the 45-54 yr-age group (NHANES-III, n=7,574, follow-up 8.7 years)
Haring, 2009 (12)	NAFLD on ultrasonography increases prediction of CVD mortality from elevated serum GGT levels in a population-based cohort of individuals (n=4,160, follow-up 7.3 years)
Soderberg, 2010 (13)	Patients with histologically confirmed NASH but not those with pure steatosis are at higher risk of all-cause, CVD and liver-related mortality compared with the reference population (n=118, follow-up 24 years)
Sung, 2009 (165)	Young, nonobese subjects with ultrasound-diagnosed NAFLD have a higher CVD risk (by Framingham risk score), especially those with suspected NASH (n=30,172 subjects). Note of caution: cross-sectional study
Targher, 2005 (166), Targher, 2007 (167)	NAFLD on ultrasonography is associated with an increased risk of fatal and non-fatal CVD events in type 2 diabetic patients free from CVD and viral hepatitis at baseline (n=2,103, follow-up periods ranging from 5 to 6.5 years)
Hamaguchi, 2007 (168)	NAFLD on ultrasonography is associated with an increased risk of non-fatal CVD events in a community-based study (n=1,637, follow-up 5 years)
Zhou, 2012 (169)	NAFLD on ultrasonography is associated with an increased risk of all-cause and CVD mortality in a community-based study (n=3,543, follow-up 4 years)
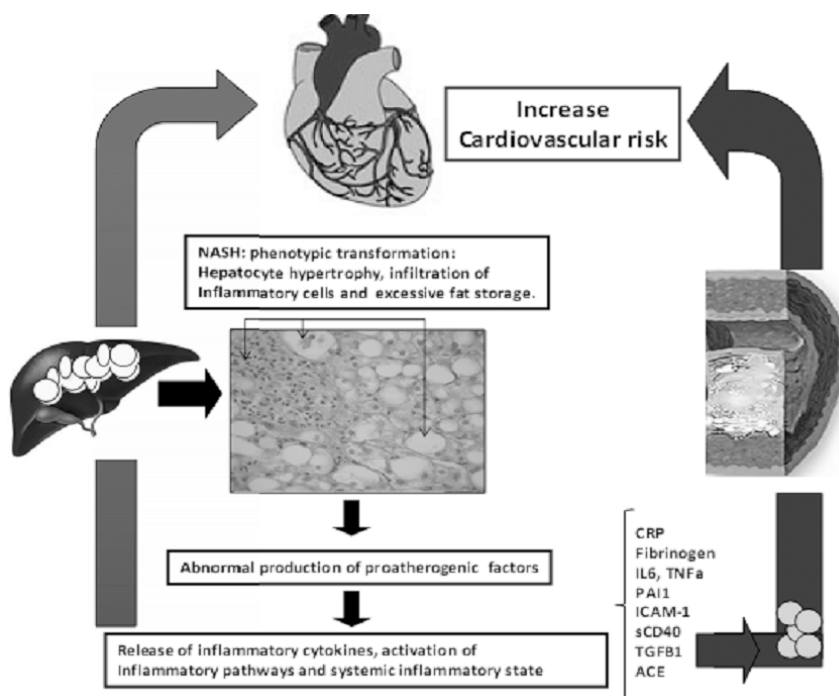
Fig. (3). Putative underlying mechanisms linking NAFLD to accelerated atherogenesis.
NAFLD, especially in its necro-inflammatory form (NASH), is associated with phenotypic changes in liver cell populations that may directly contribute to a more atherothrombotic risk profile via atherogenic dyslipidemia, hepatic/systemic insulin resistance, dysglycemia and increased secretion of several pro-inflammatory and pro-coagulant mediators.
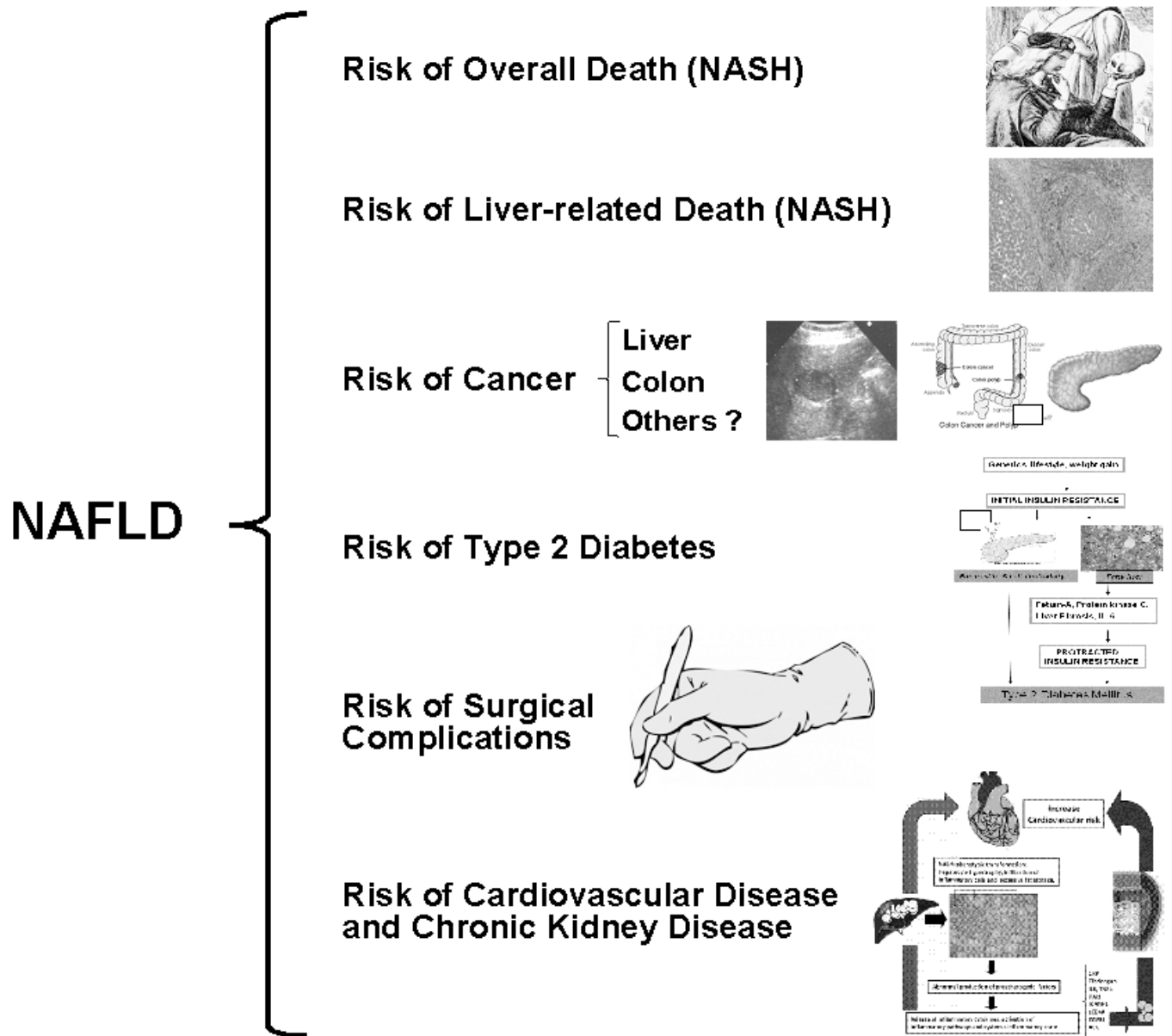
Fig. (4). The prominent role of CVD in the natural history of NAFLD
This figure summarizes the wide spectrum of hepatic and extra-hepatic complications of NAFLD. CVD represents one of the most worrying adverse outcomes of NAFLD. The biological forces driving the individual patient with NAFLD towards hepatic and extra-hepatic complications are increasingly being unveiled.

Indeed, several case-control studies have confirmed that patients with NAFLD, especially those with NASH, have increased circulating levels of several pro-inflammatory and pro-coagulant biomarkers compared with subjects who do not have steatosis (Table **2**). Additionally, the finding that NASH is associated with an abnormal intrahepatic expression of these pro-inflammatory and pro-coagulant biomarkers further supports the conclusion that the increased circulating levels of these biomarkers result from the up-regulation of their own synthesis in the steatotic and inflamed liver (Table **2**). These latter observations fit well also with our recent data demonstrating that the hepatic transcriptional profile of several candidate genes responsible for accelerated atherogenesis is selectively dysregulated among patients with NASH. The list includes genes associated with atherosclerotic risk (*SERPINB2*, *alias PAI2*, *TGFB1*, *ACE*), genes involved in inflammation and cytokine signaling (*CSF2*, *IL1A*, *IL3*, and *IL4*), and genes involved in cell prolif-

eration/growth (*LIF*), extracellular matrix remodeling (*MMP1*) and cell adhesion-cell-matrix glycoconjugate (*LAMA1*) [126].

Although all the experimental and biological data would tend to suggest that it is NASH rather than pure steatosis that leads to increased risk of CVD, published data from prospective studies to date do not entirely support such a conclusion (as summarized in Table **4**). More research is needed to draw a firm conclusion on the role of the severity of NAFLD histology on the development and progression of CVD.

Is there any biological explanation for these observations? We might speculate that the liver tissue of NASH patients suffers from a phenotypic transformation similar to that observed in other tissues in presence of the metabolic syndrome, such as the adipose tissue [182]. Hence, the metabolic consequences of the "transformed" liver might be crucial to the pathogenesis of CVD as the liver is

tightly connected to the circulatory system not only by releasing very low density lipoproteins (VLDL) but also by secreting several systemic coagulation/fibrinolytic factors (e.g., fibrinogen, factor VII, von Willebrand factor, PAI-1), pro-inflammatory mediators (e.g., C-reactive protein, TNF-alpha, IL-6 and other acute-phase proteins) and key factors of the renin-angiotensin-aldosterone system (RAAS). For example, angiotensinogen is constitutively produced and released from the liver into circulation where it is converted by angiotensin converting enzyme (ACE) to angiotensin II, i.e., a powerful vasoconstrictor factor that is strictly involved in systemic inflammation by inducing the up-regulation of endothelial adhesion molecules and pro-inflammatory cytokines. Accumulative evidence has confirmed the strong link between RAAS, human atherosclerosis and thrombotic risk. Surprisingly, patients with NASH have an over-expression of ACE as recently shown by our group [183]. Why should we consider that the hepatic over-expression and even the release of RAAS proteins from the liver to the circulation is clinically relevant? As previously shown, all these molecular mediators may act in concert to produce both cardiovascular and endothelial effects. For instance, angiotensin II stimulates the release of PAI-1, and increases the expression of endothelial adhesion molecules such E-selectin and ICAM-1 [184-186]. Interestingly, we showed that patients with NAFLD have an increased hepatic expression of ICAM-1, particularly in focus of lobular inflammation [118]. Thus, treatment with RAAS inhibitors might be useful not only to improve blood pressure, but also to reduce the release of some proatherothrombotic mediators from the inflamed/steatotic liver [183,187]. Accordingly, we also showed that treatment with enalapril may improve hepatic fibrosis scores in patients with NASH [183]. Other pharmacological approaches such as pioglitazone have been proposed to be effective for the treatment of liver disease, glucose metabolism and CVD risk in NASH [188]. Finally, studies demonstrating that further to NAFLD, other chronic liver diseases that are typically characterized by the presence of steatosis, such as chronic hepatitis C, are linked to an increased risk of CVD [189,190], may provide further evidence to the notion that hepatic steatosis itself may play a role in accelerated atherogenesis.

With regard to the biological basis for the development of CKD among patients with NAFLD, it is important to note that there is a strong, mutual relationship linking CKD and atherosclerosis [191,192]. Interestingly, the putative biological mechanisms linking NAFLD to CVD that we briefly discussed above might also be largely implicated in the pathogenesis of kidney damage. In fact, it has been reported that NAFLD might play a part in the pathogenesis of CKD, possibly through the systemic release of several pro-inflammatory/pro-coagulant mediators from the steatotic/inflamed liver or through the contribution of NAFLD itself to hepatic/systemic insulin resistance and atherogenic dyslipidemia [170,193].

CONCLUSIONS

This review has provided an unbiased view of the whole spectrum of the hepatic and extra-hepatic complications that may occur in the natural course of NAFLD. In particular, the increased risk of CVD has been discussed against the more complex scenario of other hepatic and extra-hepatic complications of NAFLD (as summarized in schematic (Fig. **4**)).

As previously discussed, the currently available evidence from published studies suggests that the long-term prognosis of patients with NAFLD particularly the development of liver-related complications and liver-related death greatly depend on the severity of liver injury on liver biopsy with a life expectancy similar to the general population in those with pure steatosis, but a significantly worse prognosis in those with histologically more severe liver disease. Accumulating evidence also suggests that CVD is the leading cause of morbidity and mortality in patients with NAFLD. The association between NAFLD and CVD seems to have strength, consistency, temporality and biological plausibility. However, further research is required to ascertain whether the contribution of the liver to the increased risk of CVD observed in patients with NAFLD occurs independent of the shared cardio-metabolic risk factors; whether the increased CVD risk is associated with the severity of NAFLD histology and to what extent it may be reversible when NAFLD is treated effectively. The adverse impact of NAFLD on the well established diabetes- and obesity-related risk of CVD deserves particular attention in view of the possible implications for screening and surveillance strategies in the growing number of patients with NAFLD.

There is an urgent need to conduct larger and longer follow-up studies in patients with biopsy-confirmed NAFLD, ideally comprising of both serial biopsies and clinical observations to include a detailed examination of the actual risk of disease progression and adverse extra-hepatic (fatal and non-fatal) outcomes. Only with this sort of information, will we be able to provide patients with accurate prognostic information, initiate randomized treatment trials on a more rational basis and predict the likely burden of NAFLD-related complications on health care systems.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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ABBREVIATIONS

ACE	=	Angiotensin converting enzyme
ALT	=	Alanine aminotransferase
BMI	=	Body mass index
CKD	=	Chronic kidney disease
CVD	=	Cardiovascular disease
FLI	=	Fatty liver index
GGT	=	Gamma-glutamyltransferase
HCC	=	Hepatocellular carcinoma
ICC	=	Intrahepatic cholangiocarcinoma
NAFLD	=	Nonalcoholic fatty liver disease
NASH	=	Nonalcoholic steatohepatitis
PAI 1	=	Plasminogen activator inhibitor 1
PNPLA3	=	Phospholipase domain-containing protein 3 gene
RAAS	=	Renin-Angiotensin-Aldosterone System
T2D	=	Type 2 diabetes
VLDL	=	Very low density lipoproteins

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