Invited Review

Complications, morbidity and mortality of nonalcoholic fatty liver disease

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is an increasingly recognized public health problem, affecting up to a quarter of the world's adult population. The burden of NAFLD is influenced by the epidemics of obesity and type 2 diabetes mellitus (T2DM) and the prevalence of these conditions is not expected to decrease in the forthcoming decades. Consequently, the burden of NAFLD-related liver complications (non-alcoholic steatohepatitis [NASH], cirrhosis and hepatocellular carcinoma) and the need for life-saving liver transplantation are also expected to increase further in the near future. It is predicted that NAFLD will soon become the most important indication for liver transplantation. A large body of clinical evidence indicates that NAFLD is associated not only with increased liver-related morbidity and mortality, but also with an increased risk of developing other important extra-hepatic diseases, such as cardiovascular disease (that is the predominant cause of death in patients with NAFLD), extra-hepatic cancers (mainly colorectal cancers), T2DM and chronic kidney disease. Thus, NAFLD creates a considerable health and economic burden worldwide and often results in poor quality of life. This narrative review provides an overview of the current literature on main complications, morbidity and mortality of this common and burdensome liver disease.

Keywords: NAFLD, NASH, CVD, T2DM, complications, mortality, epidemiology

ABBREVIATION LIST

BMI, body mass index

CI, confidence interval

CKD, chronic kidney disease

CVD, cardiovascular disease

eGFR, estimated glomerular filtration rate

HCC, hepatocellular carcinoma

HCV, hepatitis C virus

LT, liver transplantation

MetS, metabolic syndrome

NAFLD, nonalcoholic fatty liver disease

NHANES-III, Third National Health and Nutrition Examination Survey

NASH, nonalcoholic steatohepatitis

OR, odds ratio

T2DM, type 2 diabetes mellitus

PNPLA3, patatin-like phospholipase domain-containing protein-3

TM6SF2, transmembrane 6 superfamily member 2

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a metabolic liver disease that is strongly associated with obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) [1-3]. The prevalence of NAFLD is increasing worldwide at approximately the same rate as the epidemics of obesity and T2DM [1-3]. Indeed, the global prevalence of NAFLD in adults is estimated to be ~25%, whereas the global prevalence of nonalcoholic steatohepatitis (NASH) has been estimated to be between 3% and 5% [1]. In Western countries, the prevalence of NAFLD in the pediatric population has been estimated to be between 3-10%, but its prevalence increases up to ~70% among children who are obese [1].

As NAFLD progresses, hepatic inflammation and hepatic fibrosis develop; in particular, hepatic fibrosis increases risk of liver-related complications, such as cirrhosis, liver failure, hepatocellular carcinoma (HCC) and death [1-3]. NAFLD-related liver complications are also predicted to become the most frequent indication for liver transplantation within the next decade. It is important to note that NAFLD is also closely associated with important extra-hepatic manifestations (e.g., cardiovascular disease [CVD], chronic kidney disease [CKD] and certain extra-hepatic cancers) that can further increase its disease burden, and CVD is the most common cause of mortality among patients with NAFLD [1,4]. **Fig. 1** illustrates the principal hepatic complications and extra-hepatic diseases associated with NAFLD.

Collectively, the ongoing global epidemic of NAFLD and the fact that a subgroup of individuals with this disease are at higher risk of developing advanced stages of liver disease, CVD and other cardiometabolic complications means that NAFLD is an important priority for health care and research.

In this narrative review we provide an overview of the current literature on complications, morbidity and mortality of NAFLD. In particular, we will discuss only NAFLD-related liver complications as well as the extra-hepatic diseases as listed in *panel B* of **Fig. 1**, for which there is stronger evidence of an association with NAFLD.

2. Risk of All-cause and Cause-specific Mortality

An impressive number of cohort studies have consistently documented that NAFLD (especially in its more severe forms) is associated with higher risk of all-cause and cause-specific mortality (**supplementary Table 1**) [5-42]. The leading causes of mortality among NAFLD patients are CVD, followed by extra-hepatic cancers and liver-related complications. In a meta-analysis of 45 studies including a total of almost 8.5 million individuals followed from 4 to 13 years, Younossi *et al.* estimated that the overall mortality rate per 1,000 person-years was ~15.4 for patients with NAFLD and ~25.6 for those with NASH; the pooled liver-specific mortality rate per 1,000 person-years was ~0.8 for patients with NAFLD and ~11.8 for those with NASH, whereas the pooled CVD-specific mortality rate among NAFLD patients was estimated to be ~4.8 per 1,000 person-years [41]. As specified in the aforementioned table, there are, however, some differences in the results of the cohort studies available so far. In a study of 229 Swedish individuals with NAFLD, who were followed for up to 33 years, Ekstedt *et al*. showed that compared to an age- and sex-matched population, NAFLD patients had increased risk of all-cause death, with a high risk of death from CVD and liver-related causes [23]. Moreover, the histologic NAFLD activity score was not able to predict all-cause mortality, whereas liver fibrosis stage predicted both all-cause and disease-specific mortality [23]. Similarly, in a multinational cohort study including 619 individuals with NAFLD, Angulo *et al.* confirmed that the histologic stage of liver fibrosis was the strongest predictor of all-cause mortality, liver-related morbidity and liver transplantation over a median of 12.6 years [22]. Cohort studies using data from the Third National Health and Nutrition Examination Survey (NHANES-III) have often reported conflicting results, with preliminary analyses showing no association between ultrasound-defined NAFLD and risk of all-cause and cause-specific mortality [15,16], and a subsequent analysis showing an independent association between advanced NAFLD (as determined by non-invasive fibrosis markers) and increased risk of mortality, mainly from CVD causes [19]. Another analysis of the NHANES-III database showed that severe steatosis on ultrasonography and liver enzyme elevation were associated with increased liver-related mortality, but were not independently associated with mortality from all causes (except for serum gamma-glutamyltransferase elevation), CVD or cancer, during a follow-up of up to 23 years [25]. Current studies on racial/ethnic disparities in mortality rates among NAFLD patients are discordant, with some studies from the USA reporting higher risk of all-cause mortality in Blacks than in non-Hispanic Whites and other studies showing higher risk of all-cause and liver-related mortality in non-Hispanic Whites than in other ethnic groups [43,44].

That said, in a recent analysis of the NHANES-III database involving almost 12,500 individuals followed for up to 23 years, Alvarez *et al.* estimated that in the Unites States approximately 8% of all-cause mortality and more than one third of liver disease-specific and diabetes-specific deaths were associated with NAFLD [40]. Furthermore, using a dynamic model of NAFLD to assess the health burden of this disease at a population level, Estes *et al*. estimated that all-cause and liver-related mortality will increase substantially by 2030 in NAFLD patients and that almost 40% of these deaths will occur in those with NASH [45].

3. Risk of Cirrhosis, HCC and Liver Transplantation

3.1 Cirrhosis

As with other chronic liver diseases, the severity of liver fibrosis is the strongest determinant of long-term clinical outcomes in NAFLD [22,23,46,47].

In a collaborative cohort study from four international centers, Bhala *et al*. showed that patients with biopsy-confirmed NAFLD with advanced fibrosis or cirrhosis (n=247) had lower incidence rates of liver-related complications and HCC than corresponding patients with hepatitis C virus (HCV) infection (n=264), but similar rates of CVD events and all-cause mortality over a mean follow-up of nearly 6.5 years [48]. Recently, in multi-national cohort study of 458 patients with biopsy-confirmed NAFLD with advanced fibrosis or compensated cirrhosis, Vilar-Gomez *et al.* reported that patients with NAFLD-cirrhosis had predominantly liver-related events, whereas those with advanced fibrosis had predominantly extra-hepatic cancers and CVD events during a mean follow-up of 5.5 years [49].

At present, there are a limited number of high-quality follow-up studies with a sufficiently long follow-up examining the risk of NAFLD progression, especially in the primary-care setting, as many of the available blood-based non-invasive surrogate scores for NAFLD assessment do not accurately reflect disease activity or progression [50,51]. Paired-biopsy studies from tertiary-care cohorts although open to ascertainment bias, offer some of the best available natural history data of NAFLD [52-62]. Pooled analysis of these tertiary-care cohorts suggests that more than one third of NASH patients have fibrosis progression and ~20% have some regression of liver disease during a mean follow-up of 5 years [58]. It has also been reported that approximately 20% of NASH

patients with advanced fibrosis or compensated cirrhosis progress to cirrhosis or develop liver failure, respectively, over a period of ~2 years [60]. In a cohort of 475 patients with biopsy-proven NASH and advanced fibrosis or compensated cirrhosis, Sanyal *et al*. showed that progression to cirrhosis occurred in 22% of NASH patients with advanced fibrosis, and liver-related outcomes occurred in 19% of patients with compensated cirrhosis over a follow-up of 96 weeks [61].

Noteworthy the progression of NASH to cirrhosis is generally slow, but it is *not* uniform in all patients and it is probably more dynamic than previously thought — NASH patients seem to have spontaneous progression or regression of their liver disease over a long period of time [46,47,59]. Although most of the available studies showed that NASH is the progressive form of NAFLD, recent paired-biopsy studies reported that a small subset of patients with simple steatosis can progress to NASH and even cirrhosis [62-64]. In a meta-analysis of eleven studies with paired biopsies, including 411 patients with NAFLD, Singh *et al*. found that ~34% of these patients had fibrosis progression, ~43% had stable fibrosis, and ~22% had improvement of fibrosis [64]. Of note, they also found that both patients with simple steatosis and NASH developed progressive hepatic fibrosis, progressing by one fibrosis stage (from baseline stage 0 fibrosis) over 14.3 and 7.1 years, respectively [64].

While there is strong evidence that MetS features (especially obesity and T2DM) promote a faster progression of NASH to cirrhosis [46-50,64-66], factors associated with spontaneous regression of NASH and NASH-related fibrosis are not fully understood. A meta-analysis of observational studies from 20 countries (involving a total of ~50,000 T2DM individuals) confirmed that the worldwide prevalence of imaging-defined NAFLD among T2DM patients was 55.5% [67]. In a smaller number of studies estimating the prevalence of biopsy-confirmed NASH and advanced fibrosis the authors also reported that the worldwide prevalence of these two conditions among T2DM patients was 37.3% and 17%, respectively [67]. Other important risk factors for progression of NAFLD to advanced fibrosis or cirrhosis are older age, aspartate aminotransferase-to-alanine aminotransferase ratio >1, hypertension, higher ferritin levels and ethnicity [46,47,50,68,69]. Evidence from familial aggregation and twin studies has also shown a heritable component to NAFLD [69-72]. Moreover, genome-wide association studies identified novel *loci* associated with NAFLD severity phenotypes. To date, non-synonymous single nucleotide polymorphisms in two genes in particular, *PNPLA3* (encoding for patatin-like phospholipase domain-containing protein-3) and *TM6SF2* (encoding for transmembrane 6 superfamily member 2) have most consistently been

validated as associated with greater susceptibility to NAFLD development and progression [46,69,73,74].

3.2 HCC

Although the evidence from high-quality, population-based studies investigating the association between NAFLD and HCC is lacking, NAFLD has been recognized as a major and increasing contributor to the global burden of HCC in developed countries [75]. Between 10% and 20% of HCC cases in the USA are now attributable to NAFLD [76,77]. Irrespective of underlying aetiology, cirrhosis is present in ~80% of HCC patients [77,78]. However, small series and case reports also reported the onset of HCC in NASH patients. In a population-based study of HCC patients in Olmsted County, Minnesota, showed that 27% of patients with NAFLD-associated HCC did not have cirrhosis [79]. Similarly, a Veterans Health Administration study of 1,500 HCC patients reported that approximately 13% of these HCC patients did not have cirrhosis and that patients with NAFLD-related HCC had a ~five-fold higher risk of HCC in the absence of cirrhosis, compared with those with HCV-related HCC [80]. In a recent meta-analysis, it has been estimated that the annual incidence of HCC in NASH patients was 5.29 cases per 1,000 person-years [41]. In a multicenter study of 756 patients with either NAFLD or HCV-related chronic liver disease, Piscaglia *et al*. showed that NAFLD-related HCC was more often detected at a later tumor stage and might arise also in the absence of cirrhosis (occurring in only ~50% of these patients), but after patient matching, it had a similar survival rate compared to HCV infection [81].

Currently, the extent to which HCC occurs in NAFLD, and the pathogenic mechanisms underlying the disease remain controversial. Nonetheless, in a cohort of NAFLD patients diagnosed at 130 facilities in the Veterans Administration, Kanwal *et al*. [82] confirmed that there was a stepwise increase in risk of incident HCC and cirrhosis with each additional MetS trait. T2DM conferred the highest risk of progression to HCC in this patient cohort, highlighting that T2DM is an important target for secondary prevention [82].

3.3 Liver transplantation

NASH is now the most rapidly growing indication for liver transplantation (LT) both in the USA and other high-income countries [83-85]. Using the United Network for Organ Sharing and Organ Procurement and Transplantation 2003-2014 database that included 63,061 patients who

underwent LT from 2003 to 2014, Cholankeril *et al*. showed that NASH has become the second-leading indication for LT in the USA [82]. Another study of the same dataset that restricted the analysis to patients listed for LT for HCC also documented that NASH was the fastest growing cause of HCC in those listed [86].

4. Risk of Cardiovascular and Cardiac Diseases

4.1 Epidemiological data in adults

Strong evidence indicates that NAFLD is associated with increased prevalence and incidence of CVD after adjusting for traditional cardiovascular risk factors [87-91]. The magnitude of risk of CVD events parallels the underlying severity of NAFLD, such that NASH patients appear to be at higher risk of CVD events than those with simple steatosis. As previously discussed, it is well recognized that CVD is the predominant cause of mortality among NAFLD patients. Using mortality data from the National Vital Statistics System multiple-cause mortality data in the USA, Paik *et al.* recently confirmed that CVD was one of the most important causes of mortality among decedents with NAFLD [44]. A comprehensive meta-analysis of 16 observational studies (involving a total of ~34,000 individuals, 36% of whom had imaging-defined or biopsy-proven NAFLD) showed that patients with NAFLD had a higher risk of fatal and/or non-fatal CVD events than those without NAFLD (random-effects odds ratio [OR] 1.64, 95%CI 1.26-2.13) over a median period of 6.9 years [91]. Patients with more "severe" NAFLD were also more likely to develop fatal and non-fatal CVD events (random-effects OR 2.58; 95%CI 1.78-3.75) [91]. Other large cohort studies recently confirmed that NAFLD was independently associated with a higher incidence of myocardial infarction, *even* in primary care populations [92,93]. However, this latter finding has been recently questioned by the results of a large matched cohort study [94]. In fact, Alexander *et al*. did not find any significant association between recorded diagnoses of NAFLD and risk of incident acute myocardial infarction and stroke, after adjustment for established CVD risk factors, using electronic records from four European primary healthcare databases [94]. However, the lack of association of NAFLD with risk of acute myocardial infarction and stroke reported by Alexander *et al*. [94] is not likely because such an association does not exist, but is probably due to the important methodological problems within the study design. Indeed, the prevalence of a recorded diagnosis of NAFLD in this electronic primary healthcare database [94] was much lower than

expected (<2% that is ~15 fold lower than that usually reported in the European general population when the diagnosis of NAFLD is based on state-of-the art imaging methods), highlighting the potential for misclassification bias within the 'control' group.

In a large cohort study of 5,121 South Korean individuals without pre-existing CVD, Lee *et al*. examined the influence of NAFLD on subclinical coronary atherosclerosis as detected by coronary computed tomography angiography [95]. Notably, they showed that the presence of NAFLD (on ultrasonography) was independently associated with non-calcified, "vulnerable" coronary atherosclerotic plaques, suggesting an increased CVD risk in these asymptomatic individuals [95]. Recently, in a cohort of 304 German adults with biopsy-confirmed NAFLD, Labenz *et al.* also showed that the overall 10-year CVD event risk according to the Framingham risk score was high among these patients (about 60% were at intermediate or high CVD risk) and *even* greater among those with advanced liver fibrosis. However, only a minority of these patients received statin treatment [96]. Some observational studies also suggested that regression of NAFLD (on ultrasonography) over time was associated with a decreased risk of subclinical carotid atherosclerosis development [97].

Finally, convincing evidence also indicates that NAFLD adversely affects not only the coronary arteries (promoting accelerated atherosclerosis) but also other anatomical structures of the heart, conferring an increased risk of cardiomyopathy (mainly left ventricular dysfunction and hypertrophy, possibly leading to heart failure), cardiac valvular calcification (mainly aortic-valve sclerosis) and arrhythmias (mainly atrial fibrillation) [98,99].

4.2 Epidemiological data in children

Children with NAFLD may also be at higher risk for subclinical atherosclerosis, early myocardial abnormalities and have a more adverse cardiometabolic risk profile than children without steatosis [100,101]. In a case-control study of 150 overweight children with biopsy-proven NAFLD and 150 overweight children without NAFLD, Schwimmer *et al*. firstly showed that children with NAFLD had higher blood pressure and a less favorable plasma lipid profile than control children [102]. In a cohort study of 3,170 children, Geurtsen *et al.* found that NAFLD (defined as ≥5.0% liver fat on magnetic resonance imaging) was associated with higher blood pressure, adverse lipid profile, greater insulin resistance and increased plasma C-reactive protein levels [103]. These

associations remained significant after adjustment for BMI and tended to be stronger in overweight and obese children. Notably, the authors also found that smaller percentages of liver fat content, such as 2% and 3% liver fat on magnetic resonance imaging, were already associated with an adverse cardiometabolic risk profile, suggesting that the threshold of liver fat content to detect CVD risk in children should be <5% [103].

4.3 Clinical implications and pathogenetic mechanisms

On this background of evidence, the European and American clinical practice guidelines for the management of NAFLD strongly recommended that all patients with NAFLD should undergo careful cardiovascular surveillance [104,105]. However, there is limited literature investigating the type of screening that should be used to assess CVD risk in NAFLD patients [106]. To this end, a possible strategy *at least* in adult individuals might be to rely on general CVD risk equations, such as the Framingham risk score or other risk scoring systems [104,106,107].

There are several pathophysiological mechanisms that are altered in NAFLD and may be associated with CVD development [87-90,108]. The liver plays a major role in regulating lipid metabolism by the combined action of hepatic *de novo* lipogenesis and lipid oxidation, as well as uptake and secretion of lipoproteins. NAFLD is associated with hepatic insulin resistance and induces very low-density lipoprotein (VLDL) production via changes in the rate of apolipoprotein B synthesis and stimulation of *de novo* lipogenesis [109,110]. Increased circulating levels of VLDL particles can lead to the generation of small, dense low-density lipoprotein (LDL) particles that are highly atherogenic [109,110]. In addition, NAFLD (especially in its more severe forms) releases a variety of proinflammatory and proatherogenic mediators that may promote the development and progression of CVD [87-90,98,108].

5. Risk of Diabetes

5.1 Epidemiological data in adults

Obesity is a major risk factor for NAFLD, which frequently occurs with CVD and T2DM [87]. Obesity, T2DM, NAFLD and CVD share many cardiometabolic risk factors, suggesting an overlap between many of the mechanistic pathways that contribute to each of the individual conditions. Although

liver fat can accumulate in obese individuals who are metabolically healthy [111], NAFLD often occurs in individuals with expanded/inflamed visceral adiposity, T2DM, insulin resistance and poor fitness (**Fig. 2**). As such, in individuals without a genetic predisposition to NAFLD, it is thought that NAFLD is the hepatic manifestation of the MetS [112]. However, the link between NAFLD and MetS traits, especially T2DM, is more complex than previously thought [113].

Development of MetS commonly occurs with ageing, polycystic ovary syndrome (PCOS) and after the menopause, and each of these conditions are also recognized risk factors for T2DM. The prevalence of NAFLD increases with ageing, in both sexes, until ~65 years and then its prevalence remains constant throughout the rest of life [45]. Menopause and PCOS are also risk factors for NAFLD [114,115], although it is uncertain whether early menopause increases risk of NAFLD. With ageing, menopause, PCOS or MetS, changes frequently occur in both insulin sensitivity and body fat depots. This is often manifest as a loss of subcutaneous fat (especially with ageing) and an increase in intra-abdominal visceral fat (especially with MetS and menopause). These changes in body fat distribution are often associated with insulin resistance and development of cardiometabolic risk factors. In fact, the menopause is often characterized by a loss of gluteo-femoral fat and an increase in intra-abdominal visceral fat accumulation. Post-menopausal women are also more susceptible to weight gain and atherogenic dyslipidemia (as well as body fat re-distribution), all of which are hallmarks of the MetS and are associated with higher risk of NAFLD [114].

We recently assessed the association between NAFLD and the development of incident T2DM in a meta-analysis of 19 observational studies involving a total of 296,439 adult individuals [116]. Around 30% of these individuals had imaging-defined NAFLD and nearly 16,000 cases of incident T2DM occurred over a median follow-up of 5 years. These data showed that NAFLD patients had a ~2.2-fold higher incidence of T2DM at follow-up, compared to subjects without NAFLD [116]. In a smaller subset of studies within this analysis, with non-invasive markers of fibrosis, there was a suggestion that patients with more "severe" NAFLD were also more likely to develop incident T2DM [116], but further research is needed to confirm this finding. Some evidence also suggested that improvement of NAFLD on ultrasonography was associated with T2DM incidence reduction [113,117].

5.2 Epidemiological data in children

In the pediatric population it is less certain whether NAFLD is a risk factor for T2DM (*not least* because T2DM occurs rarely in this age group) [118]. Although insulin resistance occurs almost universally among children and adolescents with NAFLD [119,120], the prevalence of abnormal glucose tolerance in children/adolescents with biopsy-proven NAFLD is also uncertain. In one of the best studies to date in a large multi-ethnic cohort study of 675 obese children/adolescents with biopsy-confirmed NAFLD enrolled in the NASH-Clinical Research Network (CRN) in the USA, the authors reported that the prevalence of prediabetes and T2DM was 23.4% and 6.5%, respectively [121]. Moreover, children/adolescents with prediabetes (adjusted-OR 1.9; 95%CI 1.21-2.9) or T2DM (adjusted-OR 3.1; 95%CI 1.5-6.2) had a higher risk of having NASH compared with those with normal glucose tolerance [121]. Recently, we have also undertaken a cross-sectional study of children/adolescents with and without NAFLD to compare the prevalence of prediabetes and T2DM [122]. We studied a cohort of 599 overweight Caucasian children/adolescents with biopsy-proven NAFLD, and 118 children/adolescents without ultrasound-defined NAFLD, who were recruited as they were similar for age, sex, BMI and waist circumference, to those with NAFLD [122]. Interestingly, children/adolescents with NAFLD had a remarkably greater prevalence of abnormal glucose tolerance (prediabetes or diabetes based on either hemoglobin A1c, fasting glucose or 2-hour post-load glucose levels) than those without NAFLD (20.6% *vs*. 11%). In particular, among NAFLD children/adolescents with abnormal glucose tolerance, the large majority of them satisfied the diagnostic criteria for prediabetes (19.8%) and less than 1% (0.8%, n=5 children) had diabetes [122]. In addition, the combined presence of prediabetes and diabetes was associated with a higher risk of NASH even after adjustment for age, sex, waist circumference and also the *PNPLA3* rs738409 polymorphism (adjusted-OR 1.69, 95%CI 1.06-2.69) [120]. Interestingly, in our cohort of children/adolescents with NAFLD [122], we found a prevalence of prediabetes (19.8% *vs*. 23.4%) and especially of T2DM (0.8% *vs*. 6.5%) that was much lower than that reported in the multi-ethnic cohort of children/adolescents with NAFLD enrolled in the NASH-CRN database [121]. It is reasonable to hypothesize that the marked differences in the prevalence of prediabetes and, especially, T2DM between these two pediatric cohorts reflect differences in race/ethnicity, adiposity measures and histologic stage of liver fibrosis. However, further studies are needed in this age group, in order to assess the relationship between NAFLD and incidence of prediabetes and T2DM.

5.3 Pathogenetic mechanisms

With the development of NAFLD, accumulation of hepatic fat results in lipid-induced mechanisms promoting hepatic insulin resistance and hepatic inflammation [123]. With increased hepatic lipid fluxes, increased VLDL secretion increases fasting levels of plasma triglycerides, which are one of the key features of the MetS [123]. The mechanisms contributing to hepatic insulin resistance, increased VLDL synthesis and increased hepatic glucose production are shown in **Fig. 3**. These mechanisms illustrate that there is a key role for the adipocyte-hepatocyte axis in the normal physiological insulin-mediated suppression of hepatic glucose production and it is likely that with hepatic insulin resistance this physiological axis is poorly regulated, leading to increased plasma fasting glucose concentrations and increased risk of T2DM in patients with NAFLD [123].

6. Risk of Extra-hepatic Neoplasms

NAFLD is associated with an increased risk of extra-hepatic cancers (**supplementary Table 2**) [124-133]; the highest NAFLD-related cancer risk seems to be due to colon, stomach and some hormone-related cancers. In a recent large matched cohort study involving 4,722 US individuals with NAFLD and 14,441 age- and sex-matched individuals without NAFLD, who were followed for a median of 8 years, Allen *et al.* reported that NAFLD was significantly associated with 90% higher risk of malignancy, with the highest risk for gastrointestinal cancers (stomach, pancreas or colorectal cancers) [132]. In the absence of NAFLD, the association between obesity and cancer risk was small, suggesting that NAFLD may be an important mediator of the obesity-cancer association [132]. In a meta-analysis of eleven observational studies (8 cross-sectional and 3 longitudinal) with a total of ~91,000 asymptomatic adults (32% with NAFLD), we recently reported that NAFLD, diagnosed by either imaging or histology, was associated with a moderately increased prevalence and incidence of colorectal adenomas and cancer [134]. These risks were independent of age, sex, BMI, smoking or T2DM. Moreover, the severity of NAFLD seemed to be associated with a higher risk of colorectal cancer [133]. Notably, NAFLD patients with colorectal cancer seem also to have a worse prognosis when compared to their counterparts without steatosis [135,136].

As also shown in **supplementary Table 2**, few studies examined the association between NAFLD and hormone-related malignancies in women [129,132,133,137,138]. In a retrospective cohort

study, involving 25,947 South Korean individuals followed for median period of 7.5 years, Kim *et al.* reported that ultrasound-defined NAFLD was associated with a ~90% higher risk of developing breast cancer [129]. In the aforementioned study of Allen *et al.,* NAFLD was also associated with a nearly twofold increased incidence of uterus cancer [132]. In men, the association between NAFLD and prostate cancer appears to be more controversial [125,127,130]. Recently, in a nationwide study of almost 10.5 million South Korean men followed for a median period of 5 years, Choi *et al.* reported that NAFLD (as detected by fatty liver index or hepatic steatosis index) was associated with reduced incidence of prostate cancer [130].

Accumulating evidence suggests a role of NAFLD and expanded visceral adipose tissue as endocrine/paracrine organs for cancer development [139]. However, further research is certainly needed to better elucidate the link between NAFLD and increased carcinogenesis.

7. Risk of Chronic Kidney Disease/Dysfunction

7.1 Epidemiological data in adults

Over the last decade, an ever-increasing number of observational studies have shown that NAFLD is associated with an increased prevalence and incidence of CKD (defined as estimated glomerular filtration rate [eGFR] <60 ml/min/1.73 m^2, abnormal albuminuria or overt proteinuria) [140,141]. In these studies, the prevalence of CKD ranged from approximately 20% to 55% among adults with NAFLD compared to 5-30% among those without steatosis. Moreover, in most of these studies the association between NAFLD and increased CKD prevalence remained significant even after adjustment for traditional risk factors for CKD [140,141]. In a post-hoc analysis of a clinical trial that included 261 patients with biopsy-confirmed NASH, Vilar-Gomez *et al*. also reported that improvement in liver histology due to lifestyle modification (during 52 weeks) was independently associated with improved kidney function [142].

In a meta-analysis of 33 observational (20 cross-sectional and 13 longitudinal) studies, Musso *et al*. confirmed that NAFLD was significantly associated with an increased prevalence (random-effects OR 2.12, 95%CI 1.69-2.66) and incidence of CKD (random-effects hazard ratio 1.79, 95%CI 1.65-1.95) [143]. In a subgroup analysis of individual patient data from five small studies (including

nearly 430 adults with biopsy-proven NAFLD), the authors also suggested that advanced liver fibrosis was associated with a higher prevalence of CKD than non-advanced fibrosis [143]. Recently, Mantovani *et al.* performed a meta-analysis of 9 observational longitudinal studies (published up to August 2017) with aggregate data on ~96,000 individuals (34% with imaging-defined NAFLD) [144]. This updated meta-analysis showed that NAFLD was associated with a nearly 40% increase in the risk of incident CKD stage⩾3 (random-effects hazard ratio 1.37, 95%CI 1.20-1.53), over a median follow-up of 5.2 years. This risk appeared to increase further with the severity of NAFLD as assessed by non-invasive fibrosis biomarkers, and remained significant in those studies where analysis was adjusted for common risk factors for CKD [144].

7.2 Epidemiological data in children

Children with NAFLD may also be at higher risk for early kidney dysfunction (defined by an eGFR value <90 ml/min/1.73 m² or microalbuminuria) than children without steatosis. In a case-control study of 596 overweight or obese children and 130 nonobese children, Pacifico *et al*. found that NAFLD (on magnetic resonance imaging) was associated with decreased eGFR or microalbuminuria (adjusted-OR 2.54, 95%CI 1.16-5.57), irrespective of adiposity measures and other clinical risk factors [145]. Other smaller studies, though not all, have confirmed the presence of early kidney dysfunction among overweight or obese children with NAFLD [146-148].

Emerging evidence is now also suggesting that there is an association between *PNPLA3* rs738409 polymorphism and decreasing eGFR levels or raising albuminuria, irrespective of known renal risk factors and presence and severity of NAFLD, in both adults and children [147-151].

Collectively, despite the growing evidence linking NAFLD with a greater risk of CKD, it remains to be definitely established whether a causal association also exists [140]. Further research is needed to better understand whether NAFLD is a risk factor for the development and progression of CKD.

8. Conclusions

This review describes the morbidity and mortality from hepatic and extra-hepatic complications associated with NAFLD. NAFLD is accepted as a major cause of advanced fibrosis, cirrhosis, HCC

and liver failure (necessitating liver transplantation) but in addition, in the last decade, convincing evidence has been produced to show that NAFLD also increases risk of T2DM, CVD (i.e. the major cause of death in NAFLD), CKD and certain extra-hepatic cancers, such as colorectal cancers. Thus, with a wide range of NAFLD-associated hepatic and extra-hepatic complications/manifestations, NAFLD is a cause of a poor quality of life for many patients, and results in a considerable global health and economic burden for healthcare providers.

Although a large body of research has taken place over two decades, and has resulted in improvements in patient care, many knowledge gaps related to NAFLD still remain. Specifically, and relevant to this review, the natural history of NAFLD needs better clarification. Understanding why there is such substantial inter-individual variation in NAFLD progression and outcomes is crucial to target diagnostic strategies at "at risk" individuals. Additionally, global awareness programs are needed for NAFLD and its related long-term complications. Only with better awareness, is it likely that clinicians will then seek to diagnose the different stages of liver disease and be suspicious about potential NAFLD-related extra-hepatic complications.

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FIGURE LEGENDS

FIGURE 1 – Principal hepatic complications and extra-hepatic diseases associated with NAFLD (Panel A and Panel B). Only those shown in Panel B are discussed in the text.

FIGURE 2 – Characteristics of the metabolically unhealthy state (A) associated with normal weight, overweight or obesity, occurring with non-alcoholic steatohepatitis (NASH) and leading to increased risk of both type 2 diabetes mellitus (T2DM) and cardiovascular disease (CVD). Characteristics of the metabolically healthy state (B) are shown for comparison.

FIGURE 3 – Development of NAFLD and putative mechanisms contributing to hepatic insulin resistance, increased very low-density lipoprotein (VLDL) synthesis and increased hepatic glucose output. With increased fluxes to the liver of long-chain fatty acids (LCFAs), proinflammatory cytokines and decreased adiponectin from expanded and inflamed (dysfunctional) visceral adipose tissue undergoing lipolysis, there is increased hepatic synthesis of various lipid species. These lipid species include ceramide, di-palmitoyl phosphatidic acid (di-P PA), di-acylglycerol (DAG) and tri-acylglycerol (TAG), which act to promote hepatic insulin resistance, hepatic inflammation and accumulation of a lipid globule. With remodeling of the lipid globule, and synthesis of phospholipids, cholesterol esters and apolipoproteins, VLDL particles are assembled for export from the liver. With NAFLD, particularly in the absence of genetic polymorphisms known to be associated with increased accumulation of the lipid globule (such as the rs738409 polymorphism of the *PNPLA3*/adiponutrin gene), there is increased VLDL output, which is often manifest in the patient as increased plasma triglyceride concentrations. Increased plasma triglyceride concentrations are a characteristic of the MetS and, with other MetS features, contribute to increased risk of CVD with NAFLD. With increased hepatic insulin resistance and increased flux of glycerol and LCFAs to the liver from adipose tissue lipolysis, there is also increased acetyl Co-A mediated activation of pyruvate carboxylase (catalyzing the irreversible conversion of pyruvate to oxaloacetate), and with decreased insulin action relative to glucagon action, there is increased activity of phosphoenol pyruvate carboxykinase (PEPCK), catalyzing the key rate limiting step in hepatic gluconeogenesis, i.e., the conversion of oxaloacetate to phosphoenolpyruvate leading to increased hepatic glucose output in the fasting state. Thus, increased gluconeogenesis in the fasting state increases hepatic glucose output, which increases plasma fasting glucose concentrations, which is another one of MetS features occurring in NAFLD.