2 Epidemiology and Etiologic Associations of Non-alcoholic Fatty Liver Disease and Associated HCC

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in the world and will soon become the number one cause of hepatocellular carcinoma (HCC), liver transplantation and liver-related mortality. The disease often occurs in the setting of metabolic conditions such as obesity and type II diabetes mellitus. These same metabolic drivers are also risk factors for NAFLD associated HCC which can occur even in the absence of cirrhosis or advanced fibrosis and appears to be phenotypically different to HCCs arising from other chronic liver diseases. The frequencies of liver-related events and HCC among NAFLD patients is low, especially when compared to cardiovascular disease and extrahepatic malignancies. However, the large denominator of total patients affected with NAFLD means that these events will impose an enormous clinical and economic burden on our society. Moreover, this burden is expected to rise further in the future. Therefore, the global NAFLD epidemic has arrived at our doorstep and demands our attention.

Keywords

Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Hepatocellular carcinoma · Epidemiology · Metabolic syndrome · Economic burden

2.1 Introduction

In the face of a global obesity epidemic, non-alcoholic fatty liver disease (NAFLD) has become the major cause of chronic liver disease worldwide [1, 2]. With continuing improvements in global hepatitis B virus (HBV) vaccination coverage and effective therapies to either control or eradiate chronic viral hepatitis, the proportional burden of NAFLD and its complications is set to rise dramatically. Accordingly, NAFLD is the fastest growing indication for liver transplantation (LT) in the United States (U.S.) over the past decade and is expected to surpass chronic hepatitis C virus (HCV) infection as the leading indication in next 5 years [3, 4]. In particular, the number of patients undergoing LT for hepatocellular carcinoma (HCC) secondary to NAFLD has increased by nearly fourfold to 13.5% of HCC-

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J. Yu (ed.), *Obesity, Fatty Liver and Liver Cancer*, Advances in Experimental Medicine and Biology 1061, https://doi.org/10.1007/978-981-10-8684-7_2

related LT. Although the absolute risk of HCC and liver-related mortality among NAFLD patients is low, the high (and rising) global prevalence of these patients translates into substantial numbers. Thus, on current trends, the future burden of NAFLD and associated HCC (NAFLD-HCC) will be staggering.

2.2 Epidemiology of NAFLD

2.2.1 Definitions

Non-alcoholic fatty liver disease is typically regarded as the hepatic manifestation of metabolic syndrome, a condition characterized by the presence of at least three of the following criteria: elevated body mass index (BMI) and waist circumference, dyslipidemia, insulin resistance and/or type II diabetes and hypertension [5]. NAFLD is defined as the presence of hepatic steatosis seen on imaging or histology (exceeding 5% of total liver weight) to the exclusion of secondary causes of hepatic fat accumulation [6]. It can be further classified into non-alcoholic fatty liver (also known as simple steatosis) or non-alcoholic steatohepatitis (NASH) based on the absence or presence of significant hepatic inflammation, respectively. The latter is considered a more aggressive form of disease which can progress to hepatic fibrosis, cirrhosis and NAFLD-HCC (Fig. 2.1).

2.2.2 Prevalence of NAFLD

The reported prevalence of NAFLD varies widely depending on the population studied and the diagnostic method used. In a landmark meta-analysis of 86 studies across 22 countries over 26 years, Younossi et al. estimated the global prevalence of NAFLD diagnosed on imaging to be 25.2% (range 22.1%–28.7%) [7]. Alternatively, when the prevalence of NAFLD was estimated using blood tests (elevated liver enzymes or other indices), only 9.3%–12.0% of individuals were diagnosed with the condition across the world. Indeed, the level of liver enzymes fluctuates throughout the course of NAFLD and may be normal in the vast majority of patients [8]. Hence blood tests, although simple and easily accessible, are thought

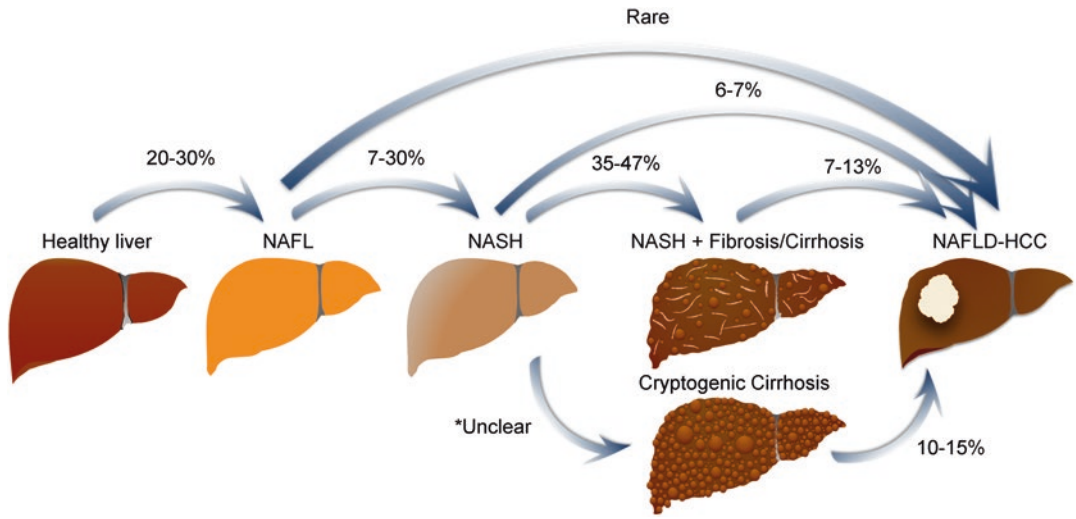

Fig. 2.1 The natural history of non-alcoholic fatty liver disease (NAFLD)
Although NASH accounts for up to half of cryptogenic cirrhosis cases, the proportion of NASH-cirrhosis patients misclassified as cryptogenic cirrhosis is not known

NAFL non-alcoholic fatty liver or simple steatosis, *NASH* non-alcoholic steatohepatitis, *NAFLD-HCC* non-alcoholic fatty liver disease-associated hepatocellular carcinoma
Figure courtesy of Dr. Weiqi Xu, Institute of Digestive Disease, The Chinese University of Hong Kong, Hong Kong

to underestimate the true prevalence of NAFLD. Liver histology is considered the most accurate yet least practical and most invasive method for diagnosing NAFLD. Autopsy series reveal a NAFLD prevalence of 13.0–15.8%, while liver biopsies obtained from potential living liver donors showed 20% of patients in the U.S. and 10.4% in South Korea had >30% steatosis [9].

Although the majority of literature arises from the North America and Europe where obesity and type II diabetes mellitus are epidemic, NAFLD has never been just a "Western disease" [1]. Indeed, it is highly prevalent in all continents. The highest prevalences of NAFLD are found in the Middle East (31.8%), South America (30.5%) and Asia (27.4%) where the prevalence rates of obesity are correspondingly high [7, 10]. The prevalence in U.S. and Europe are reported to be 24.1% and 23.7%, respectively while the lowest prevalence is reported in studies from Africa (13.5%). Hence the problem of NAFLD is just as common and important in other parts of the world as it is in the West [1].

2.2.3 Incidence

Compared to prevalence studies, NAFLD incidence studies are limited. The earliest study by Suzuki et al. showed the incidence of suspected NAFLD (as indicated by elevated serum transaminases) was 31 cases per 1000 person-years in a cohort of Japanese government employees without previous liver disease [11]. Most studies which used ultrasonography to diagnose NAFLD found an incidence rate of 18–27 cases per 1000 patient-years [12–15], although one Japanese study documented an incidence rate of 86 cases per 1000 patient-years [16]. A study of 565 community Chinese patients without NAFLD who underwent serial intrahepatic triglyceride content measurements with proton-magnetic resonance spectroscopy reported the incidence of NAFLD to be 34 per 1000 patient-years [17]. Finally, a population-based study of hepatology referrals in the United Kingdom showed a much lower incidence rate of 29 cases per 100,000 person-years [18], suggesting only a fraction of NAFLD patients are actually seen by hepatologists. Almost all incidence studies found that metabolic syndrome, or its components were strong predictors for NAFLD development. Regression of NAFLD is also known to occur, especially in the setting of weight loss. In the studies which also followed up patients with NAFLD at baseline, the regression rate was found to vary widely between 12 and 140 cases per 1000 patient-years [12–14, 16]. The average amount of weight loss in patients who demonstrated regression of NAFLD was small (2–3 kg) [12, 16].

2.2.4 Trends Over Time

In the past three decades, there has been a two to threefold increase in obesity across the Americas, Europe and Asia [2]. A parallel increase in the number of people with NAFLD has also been observed over this period of time. For instance, the prevalence of NAFLD has more than doubled in the U.S., Japan and some areas of China during the last two decades, while the prevalence of other chronic liver diseases has either remained stable or decreased [19–21]. However, recent pooled worldwide NAFLD prevalence estimates suggest a milder upward trend from 20.1% to 23.8% to 26.8% during 2000–2005, 2006–2010, and 2011–2015, respectively. This trend is also seen in patients at the severe end of the NAFLD spectrum. The percentage of NAFLD patients undergoing LT in the U.S. increased from 1.2% in 2001 to 9.7% in 2009 [22].

2.2.5 NASH

NASH is defined histologically by the presence of hepatic steatosis and two additional features: lobular inflammation and hepatocyte injury (ballooning) [1]. The global prevalence of NASH among biopsied NAFLD patients is estimated to be 59.1% (range 47.6%–69.7%) [7]. Since the condition can only be diagnosed by liver histology, NAFLD patients suspected of having it may undergo liver biopsy for the purpose of diagnosing NASH (with or without fibrosis), thus

creating a selection bias. Comparatively, NASH prevalence estimates among NAFLD patients without an indication for liver biopsy (e.g. elevated liver enzymes or clinical signs of liver disease) are much lower (6.7%–29.9%) [7]. The prevalence of NASH in the general population has been estimated to be between 3% and 5% [9]. However, in the obese population the median prevalence of NASH is 33% (range 10%–56%).

2.3 Risk Factors for NAFLD and Its Progression

2.3.1 Age and Gender

The prevalence of NAFLD increases with age. Pooled data show adult patients aged 30–39 years old have a prevalence of 22.4% which increments with each decade of life to 34.0% in those >70 years old [7]. In one population study by Wong et al., the prevalence of NAFLD in patients older than 60 years was >50% [23]. The same group also demonstrated older age was an independent predictor of incident NAFLD [17]. The prevalence of NAFLD in the pediatric general population (5–10%) is lower than adults, although still considerable especially in children with obesity (>30%) [24]. Unsurprisingly, the metabolic risk factors associated with NAFLD, including obesity, diabetes and hyperlipidemia and hypertension similarly, increase with age [9].

Data on the effect of gender on NAFLD are conflicting. Early reports published prior to 1990 suggested both NAFLD and NASH were more common in women [25]. However, most subsequent studies have consistently demonstrated a male predominance [9, 26]. The gender distribution also varies with age and race as NAFLD appears to be more common in Asian or black women than their male counterparts after the age of 50 [23, 26].

2.3.2 Race and Genetics

Considerable variation in NAFLD prevalence is observed around the world and in subjects of different ethnicities residing in the same country [7, 26]. Hispanics have the highest prevalence of NAFLD, while African Americans appear to be protected despite substantially higher rates of obesity and diabetes compared to other ethnicities in the U.S. [2, 9]. These disparities can be partially explained by variations in genetic polymorphisms associated with NAFLD. In the Dallas Heart Study cohort where 2287 subjects underwent proton-magnetic resonance spectroscopy, the frequency of hepatic steatosis was 45% in Hispanics, 33% in whites and 24% in blacks [8]. Using genome-wide association studies in 2008, Romeo et al. showed that two alleles of the patatin-like phospholipase domain containing protein 3 (*PNPLA3*) gene could account for 72% of ethnic differences in hepatic fat content seen in the Dallas Heart Study cohort [27]. The I148M allele which predisposes individuals to NAFLD is prevalent in Hispanics, while the S453I which is protective is commonly found in African Americans. The *PNPLA3* genotype can explain 10%–12% of the variance in the NAFLD trait overall. Since then, genetic variants in *APOC3, NCAN, GCKR, LYPLAL1, PPP1R3B, TM6SF2* and other genes have been discovered as significant NAFLD contributors [28–30] with both similarities and differences in frequency observed across ethnicities [1]. While genetic predisposition contributes to individual susceptibility to NAFLD and family clustering is known to occur [31], twin and family studies estimate the heritability of NAFLD to be roughly 39%–52% [32, 33]. Clearly environmental factors also play a big role.

2.3.3 Metabolic Factors

A strong relationship exists between the components of metabolic syndrome and prevalence of NAFLD. From a cohort of 12,454 adults, Lazo et al. calculated the age-, sex- and ethnicity-adjusted NAFLD prevalence ratios for patients with obesity, insulin resistance, diabetes, hypertension and hypercholesterolemia to be 3.93, 2.54, 2.40, 1.57 and 1.26, respectively compared to those without these conditions (26). The prevalence ratios for obesity, insulin resistance and diabetes remained significant even after further

adjustment for the other metabolic abnormalities and lack of physical activity. Furthermore, effect of these metabolic risk factors appears to be additive. Wong et al., demonstrated that each additional component of the metabolic syndrome increased the risk of NAFLD in a dose-dependent manner (prevalence of 4.5% in subjects without any component to 80.0% in those with all components) [23].

Indeed, the prevalence of NAFLD is exceedingly high in patients with features of metabolic syndrome. An Italian study of 187 young adult (age 18–50 years) non-diabetic obese patients detected hepatic steatosis on ultrasound in all but four patients, or 98% of patients [34]. The prevalence of histologically-proven NAFLD in those undergoing bariatric surgery similarly exceeds 90% [35]. In particular, central obesity as evidenced by increased waist circumference and/or waist-to-hip ratio has been shown to be a greater predictor of NAFLD than general obesity (BMI $\geq$30 kg/m^2) [36]. It should be noted that the distribution of visceral adipose tissue and percentage of fat for a given body mass differs between Asian and European subjects [1]. Previous studies conducted in Asian countries have reported non-obese individuals in 15–21% of NAFLD patients even after applying ethnic-specific anthropometric criteria [37]. These patients typically have a history of weight gain above their ideal body mass (but not reaching obese levels) and/or presence of other metabolic factors. It has been suggested that Asians may express the clinical phenotype associated with the metabolic syndrome at a lower BMI threshold than white populations [2]. However, pooled regional estimates of obesity prevalence among NAFLD patients (using a BMI cut-off of $\geq$25kg/m^2 for Asians and $\geq$30 kg/m^2 for others) are actually the highest in Asia (64.0%) followed by the U.S. (57.0%) and Europe (36.8%). Overall, obesity is present in 51.3% of NAFLD patients and 81.8% of NASH patients [7].

Insulin resistance is key in the pathogenesis of NAFLD and its progression, hence strong associations exist between NAFLD and diabetes. Up to 60–70% of individuals with type II diabetes exhibit ultrasonography evidence of NAFLD [38, 39]. In one study, 70.8% of diabetic patients with fatty infiltration seen on ultrasound underwent liver biopsy and NAFLD was confirmed in 86.7% of patients [39]. These ultrasound studies are supported by a prospective cohort study which screened diabetics for NAFLD using controlled attenuation parameter and found a prevalence of 72.8% [40]. Significant liver fibrosis was also detected by liver stiffness measurement in 17.7% of patients in this study. Furthermore, studies have shown that the risk of developing diabetes increases by three- to fourfold within 3 years of NAFLD diagnosis in patients without diabetes at baseline [1].

Hyperlipidemia or dyslipidemia is present in 69.2% of NAFLD patients [7] and diffuse fatty liver on ultrasound is seen in half of the individuals with hyperlipidemia [41]. In particular, hypertriglyceridemia may have a closer association with NAFLD than hypercholesterolemia. The above associations have led to changes to some international guidelines which now recommend screening for NAFLD in patients with obesity, insulin resistance or metabolic syndrome [42].

2.3.4 Progression to Fibrosis

As previously mentioned, approximately 7%–30% of NAFLD patients have NASH. Of these, up to 39.1%–40.8% will progress to develop fibrosis which occurs at a mean rate of 0.09–0.14 fibrosis stages per year [7, 43]. The incidence of advanced fibrosis in NASH patients is estimated to be 70.0 in 1000 person-years. Patients with simple steatosis have also been reported to develop fibrosis progression, although this is considered uncommon [9]. Factors associated with progressive or advanced fibrosis include older age, features of metabolic syndrome, elevated liver enzymes (especially aspartate aminotransferase [AST]) and low platelet count [44–47]. In terms of metabolic syndrome components, increased waist circumference, BMI, presence of diabetes (as well as insulin resistance or glucose intolerance), hyperlidemia and hypertension have all been associated with worsened fibrosis stage [1, 9, 43, 48, 49]. Multiple predic-

tive scoring systems using clinical and laboratory variables have been developed to identify NAFLD patients at risk of advanced liver fibrosis with area under the receiver operating characteristics curves (AUROCs) of 0.80–0.94 [50]. Almost all the risk factors mentioned above feature as a variable in one or more of these scoring systems. The *PNPLA3* I148M polymorphism has also been shown to favor NAFLD progression and liver fibrosis [51]. In terms of histological predictors, two studies observed that patients with higher steatosis grade were more likely to develop progressive fibrosis while no association was found between baseline severity of necroinflammation and risk of progressive fibrosis [43].

2.4 Epidemiology of NAFLD-HCC

Hepatocellular carcinoma is the fifth most common cancer in men and ninth most common in women globally [52]. The disease carries a high mortality rate and represents the second most frequent cause of cancer death worldwide accounting for 746,000 deaths in 2012. The median survival following diagnosis is poor, ranging from four to 20 months [53, 54]. Patients with NAFLD are at increased risk for developing HCC, however this risk is typically limited (but not exclusive) to those with advanced fibrosis or cirrhosis [6].

Since the first report of NAFLD-HCC in 1990 [55], the global incidence and prevalence of NAFLD-HCC has been steadily increasing [3, 56]. NAFLD is currently the third leading cause of HCC in the U.S. [56], however it is poised to become the leading cause of HCCs in the future [2, 57]. Indeed, a retrospective study of 162 HCC patients between 2007 and 2008 from one German center has already demonstrated that NAFLD was the most common underlying etiology of HCC [58]. A study of 632 consecutive HCC cases in the United Kingdom reported that between 2000 and 2010, there was a greater than tenfold increase in NAFLD-HCC compared to only a two to threefold increase in HCCs due to other liver diseases [59]. Changes are also occurring in non-Western countries, where the majority of the world's HCCs (>80%) currently arises mainly in the setting of chronic infection with HBV or HCV [60]. In particular, China contributes half of the world's HCC deaths, of which up to 80% are attributable to HBV [61]. However, since 1990 China has seen a 30% reduction in the rate of deaths due to HBV-related HCC [62]. A study from South Korea, another HBV endemic area, reported the proportion of patients with NAFLD-HCC increased from 3.8% in 2001–2005 to 12.2% in 2006–2010 while HBV-related HCC dropped from 86.6% to 67.4% during the same periods [63]. Similar trends have also been recorded in Japan [20].

The aforementioned rise in NAFLD-HCC burden is driven by the increase in proportion of NAFLD patients, since progression to HCC in NAFLD patients remains uncommon. The cumulative incidence of NAFLD-HCC has been reported across the world as 0.5%–2.3% after of 7.6–13.7 years of follow-up [44, 64–66]. Higher percentages of 6.7–7.6% after 5–10 years are seen in studies of NASH patients [67, 68]. In a large meta-analysis of 86 studies, Younossi et al. calculated that the HCC incidence rate is up to 12-fold higher in NASH patients as compared with NAFLD patients overall [7]. The rate in those with NASH-related cirrhosis is even higher still. The cumulative incidence of HCC in this group of patients is quoted at 6.7%–12.8% with follow-up times of between 3.2 and 10 years [64, 68–70]. One international cohort of 247 NAFLD patients across four Western countries found an HCC incidence of only 2.4% in patients with at least advanced fibrosis and 3.1% in patients with cirrhosis after a median follow-up of 7.2 years [71]. However, only patients with Child-Pugh class A liver disease were enrolled in this study.

Studies have consistently shown a lower rate of HCC development in patients with NAFLD compared to other chronic liver diseases. In particular, NAFLD patients have a 15- to 35-fold lower HCC incidence than that of chronic HBV [7]. Differential susceptibility to HCC was also

seen in a retrospective study of 3200 Japanese elderly patients (>60 years old) with either NAFLD or HCV [66]. After a mean follow-up of 8.2 years, the cumulative incidence of HCC was 0.6% in the NAFLD group compared to 17% in the HCV group. Two separate prospective studies from the U.S. comparing patients with NASH-related cirrhosis and HCV-related cirrhosis both recorded a lower incidence of HCC in the NASH group: 12.8% vs. 20.3%, respectively after 3.2 years of follow-up [70] and 6.7% vs. 17.0%, respectively after 10 years of follow-up [68]. However, a Japanese study of 157 cirrhotic patients including 72 with NASH and 85 with alcoholic liver disease found similar rates of HCC development in the two groups after 5 years (10.5% vs. 12.3%, respectively) [69].

The estimation of NAFLD-HCC is further made difficult by HCC cases in patients with cryptogenic cirrhosis which accounts for 15–30% of cirrhosis and 30–40% of HCCs worldwide [3, 72]. Growing evidence suggests that "burned-out" NASH accounts for a large proportion (up to half) of cryptogenic cirrhosis [3, 73, 74]. Indeed, some cryptogenic cirrhosis patients demonstrate histological features of NASH, however these features may also be lost over time with the development of cirrhosis [6]. Patients with cryptogenic cirrhosis and associated HCC also share many characteristics with patients with NAFLD and NAFLD-HCC, respectively. In particular, those with cryptogenic liver disease and NAFLD are older with an increased occurrence of metabolic risk factors and less aggressive tumors when HCCs arise compared to patients with other chronic liver diseases [75–77]. In a prospective study of 105 consecutive HCC patients in the U.S., up to half of patients with cryptogenic cirrhosis and HCC had histologic or clinical features of NAFLD [78]. The authors concluded at least 13% of HCCs in the study were NAFLD-HCC. Hence, studies which do not account for the proportion of NAFLD patients in those with HCC arising from cryptogenic cirrhosis may be underestimating the true prevalence of NAFLD-HCC.

2.5 Risk Factors for NAFLD-HCC

While the classic risk factors associated with HCC, such as older age, male sex and cigarette smoking also apply in NAFLD-HCC, the following risk factors deserve mention.

2.5.1 Fibrosis

The majority of NAFLD-HCC, like other HCCs, occurs in the setting of cirrhosis [2]. The cumulative incidence of HCC in NASH-related cirrhosis is up to 25-fold higher than the overall NAFLD population. Advanced fibrosis is also an important risk factor for HCC. In a prospective cohort of 382 Japanese patients with biopsy-proven NASH, 34 patients were found to have HCC [67]. Of the NAFLD-HCC patients, 88% had advanced fibrosis, compared to only 31% in NASH patients without HCC. On multivariate analysis, the authors found that advanced fibrosis was the strongest independent risk factor for NAFLD-HCC with an odds ratio of 4.2 (95% confidence interval [95% CI] 1.8–9.7). In another Japanese study, 6508 patients with NAFLD diagnosed by ultrasonography were retrospectively studied for a median of 5.6 years. Since few patients in the study underwent a liver biopsy (<2%), the AST to platelet ratio index (APRI) was used to separate patients with significant fibrosis (F3-F4). The study reported a significantly higher cumulative rate of HCC in patients with significant fibrosis compared to those without (hazard ratio 25.0, 95% CI 9.0–69.5) [65]. However, NAFLD-HCC has also been well documented to occur without cirrhosis or advanced fibrosis in one third to one half of cases [79, 80]. HCC has even been demonstrated in patients with simple steatosis (without steatohepatitis or fibrosis) [81]. Despite the contribution by metabolic risk factors such as obesity and diabetes, hepatopcarcinogenesis in non-cirrhotic NAFLD patients remains complex and the precise molecular pathways are still not fully understood.

2.5.2 Obesity

Obesity is recognized as a significant risk for the development of several malignancies, including HCC [82]. A meta-analysis of 11 cohort studies from the U.S., Europe and Asia evaluated the association between being overweight (BMI ≥25 kg/m^2) or obese (BMI ≥30 kg/m^2) and HCC. The study found relative risks of 1.2 (95% CI 1.02–1.3) and 1.9 (95% CI 1.5–2.4) for HCC in overweight and obesity patients, respectively [83]. These findings were supported by a larger meta-analysis of 26 prospective studies which demonstrated similar relative risks of 1.5 (95% CI 1.3–1.7) and 1.8 (95% CI 1.6–2.1) for primary liver cancer in overweight and obese patients, respectively [84]. Of note on subgroup analyses, these associations were independent of geographic locations, alcohol consumption, history of diabetes or viral hepatitis status. Like in NAFLD, central obesity may be particularly important. A prospective multicenter European cohort study of over 350,000 subjects showed that among all anthropometric measures of obesity, waist-to-hip ratio had the strongest association with a relative risk of 3.5 (95% CI 2.1–5.9) when comparing first and third tertiles [85]. Obesity also increases the risk of HCC-related mortality. HCC is now the leading cause of obesity related cancer deaths in middle-aged males in the U.S. [3]. In a prospective study of more than 900,000 adults in the U.S. followed up for 16 years, Calle et al. reported that HCC mortality rates were 4.5-fold higher in men with BMI ≥35 kg/m^2 than men with normal BMI [86]. Among obese men, the relative risk of cancer death from HCC was the highest compared to all other cancers (4.5 vs. 1.3–2.6). Multiple obesity-mediated mechanisms are believed to play a role in development of HCC with and without NAFLD including low-grade chronic inflammatory response, increased lipid storage and lipotoxicity, and alteration of gut microbiota with increased levels of lipopolysaccharide [87]. In particular, there is accumulating evidence which links alterations in gut microbiota with obesity, NAFLD and HCC. A recent study of obese mice found the gut microbiome metabolite deoxycholic acid promoted obesity-associated HCC, while treatment of the mice with vancomycin inhibited deoxycholic acid production and HCC development [88].

2.5.3 Diabetes Mellitus

Type II diabetes mellitus and insulin resistance with associated hyperinsulinemia and increased insulin-like growth factor levels may also contribute to HCC development. Cohort and case-controlled studies report patients with diabetes have a two to fourfold increased risk of developing HCC, independent of viral hepatitis and alcohol use [89–91]. Similarly systematic reviews and meta-analyses estimate the increased risk of HCC in diabetic patients to be 1.9–2.2-fold [92, 93]. In addition, diabetes has also been shown to increase the incidence of HCC recurrence after curative therapy [94]. Indeed, in up to 70% of patients with diabetes there is associated NAFLD [40] which is itself a risk factor for HCC. However, a large prospective cohort study of 257,649 diabetes and 772,947 non-diabetics showed that the increased risk of HCC in diabetics persisted even after excluding patients diagnosed with NAFLD (adjusted hazard ratio 2.13, 95% CI 1.99–2.28) [89], thus suggesting an independent effect. Furthermore, the use of anti-diabetic medications, in particular metformin and possibly thiazolidinediones has been associated with a decreased risk of HCC among patients with diabetes [95, 96].

Since obesity, diabetes and NAFLD often coexist, the independent contributions of each factor to HCC risk are not known. Notably, it appears obesity and diabetes synergistically increase the risk of HCC development. A 14-year prospective follow up study of 23,820 Taiwanese residents showed that obesity was associated with a 3.3-fold increase in HCC among HCV-positive patients while diabetes increased HCC risk in both HBV-positive and HCV-positive patients, by 2.2-fold and 3.5-fold respectively [97]. However, in HBV or HCV chronic carriers

with both obesity and diabetes, the risk of HCC was increased by more than 100-fold compared with patients without these factors. A multicenter Italian case-control study also demonstrated a progressive increase in HCC with the number of components of metabolic syndrome [98]. In particular, the odds ratio for HCC in patients with obesity, diabetes and both were 2.0, 4.3 and 4.8, respectively. Hepatocarcinogenesis in NAFLD is multifactorial and clearly a complex interplay exists between the components of the metabolic syndrome.

2.5.4 Iron

Hepatic iron accumulation is thought to be involved in oxidative DNA damage, NASH, fibrosis and potentially HCC [99–101]. Increased iron absorption through up-regulation of divalent metal transporter 1 has been demonstrated in NASH patients compared to those with simple steatosis and control subjects [102]. Sorrentino et al. retrospectively studied the hepatic iron content in 153 patients with NASH-cirrhosis (51 patients with HCC and 102 age- and sex-matched patients without HCC) and showed that iron deposition was more frequent in the HCC group, thus implicating it as a cofactor in the pathogenesis of NAFLD-HCC [103]. Conversely, iron depletion has been shown to reduce oxidative damage in NASH patients and lower the risk of HCC in patients with chronic HCV [104, 105]. Further studies are needed to better understand the role of iron accumulation in NASH and HCC.

2.6 Characteristics of NAFLD-HCC

Until recently, inferences on the characteristics of NAFLD-HCC have largely been made based on summations of case reports or case series [80, 106, 107]. Typically, patients with NAFLD-HCC tend to be male, older, and have one or more features of metabolic syndrome (Table 2.1).

Table 2.1 Characteristics of patients with NAFLD-HCC and patients with HCC secondary to other chronic liver diseases

Characteristic	NAFLD-HCCs	Other HCCs
Dominant gender	Male	Male
Age at diagnosis (years)	65–70	60–65
Metabolic syndrome (%)	45–58	14–18
Type II diabetes mellitus	54–74	12–49
Obesity	48–66	12–37
Hypertension	47–60	18–52
Dyslipidemia	28–35	6–14
Cirrhosis at presentation (%)	51–62	78–97
Liver function	Largely preserved	Worse
Average tumor size (cm)	≥3	≤3
Tumor differentiation	Well-differentiated	Well- to moderately-differentiated

2.6.1 Sex, Age and Initial Presentation

The male predominance seen with HCCs overall is also observed in NAFLD-HCC. Males make up 62.0%–88.9% of NAFLD-HCC patients [58, 65, 80, 106–108]. However, data are conflicting on whether differences in sex distribution exist between NAFLD-HCC and HCCs related to other diseases. In a large Italian multicenter prospective study by Piscaglia et al. comparing 145 NAFLD-HCC patients with 611 HCV-related HCC patients, significantly more males were seen in the NAFLD-HCC group (79.3% vs. 61.2%) [108]. On the contrary, Reddy et al. showed that in the subset of HCC patients undergoing curative treatments, females were more common in those with NASH relative to HCV or alcoholic liver disease (48.1% vs. 16.7%) [77]. Female gender was similarly more common in a Japanese nationwide cross-sectional study comparing NAFLD-HCC with alcoholic liver disease-related HCC (38% vs. 4%) [109]. Data are also conflicting on the age of HCC diagnosis in

NAFLD with respect to other chronic liver diseases. Published literature reports a mean and median age of NAFLD-HCC diagnosis at 66.7–74.7 years and 65–72 years, respectively [56, 58, 77, 80, 106–110]. While most studies demonstrate NAFLD-HCC patients are 5–8 years older at presentation compared to other HCC patients [56, 58, 75, 77, 110], the aforementioned Italian study found NAFLD-HCC patients were younger than patients with HCV-related HCC (67.8 vs. 71.1 years). However, the HCV-related HCC patients in this study were older than the typical age distribution for this disease [111]. Further prospective studies are needed to clarify these sex and age demographic associations observed in NAFLD-HCC.

One explanation for the older age of NAFLD-HCC patients is that fibrosis progression in NASH is slow (~0.1 fibrosis stages per year) and not universal (~40%) [7]. Although significant fibrosis is not essential for NAFLD-HCC development, it remains an important risk factor in 50% or more of patients. Furthermore, NAFLD-HCC patients tend to present late in the course of their disease. Up to half of patients who develop NAFLD-HCC have HCC diagnosed at time of initial referral [107]. Compared to HCV-related HCC, almost twice as many NAFLD-HCC patients at first presentation are symptomatic (7.4% vs. 13.8%), which is typically a late event in the course of HCC [108]. Correspondingly, patients with HCV-related HCC were more likely to receive surveillance prior to diagnosis (86.7% vs. 43.3%) and more likely to have their HCC picked up by surveillance programs (63.3% vs. 47.6%) than NAFLD-HCC patients [108, 110], hence facilitating earlier detection of HCC in non-NAFLD patients. Indeed, international guidelines for HCC surveillance in non-cirrhotic NAFLD patients are currently lacking.

2.6.2 Metabolic Syndrome

Metabolic syndrome and its constituents such as obesity and type II diabetes commonly co-exist with NAFLD and are independent risk factors for both NAFLD and NAFLD-HCC. It is therefore unsurprising that patients with NAFLD-HCC exhibit a higher prevalence of metabolic features compared to HCCs arising from other etiologies [58, 77, 106, 108]. Almost all patients (>98%) with NAFLD-HCC have at least one feature of metabolic syndrome while most (>75%) have two or more features [106]. Type II diabetes (54%–74%) and obesity (62%–66%) are most prevalent followed by hypertension (47%–60%) and dyslipidemia (28%–35%) [67, 77, 80, 109]. A retrospective study of 214 patients undergoing curative treatment for HCC found the presence of metabolic syndrome was three times more common in NAFLD-HCC compared to HCV- or alcoholic liver disease-related HCC (45.1% vs. 14.8%) [77]. Tokushige et al. found similar disparities in rates of metabolic syndrome with 58% seen in NAFLD-HCC patients and only 18% in patients with HCC due to alcoholic liver disease [109]. Clearly these metabolic features and their associated pathways play a key role in hepatocarcinogenesis.

2.6.3 Liver Function

Patients with NAFLD-HCC tend to have less severe liver dysfunction compared with other causes of HCC. Reddy et al. reported lower median model for end-stage liver disease (MELD) scores in NAFLD-HCC than those with HCC secondary to HCV or alcoholic liver disease (9 vs. 10) [77]. Using another measure of liver function, Piscaglia et al. showed proportionately more NAFLD-HCC patients with Child-Pugh class A (compensated) cirrhosis when compared to patients with HCV-related HCC (82.3% vs. 61.8%) [108]. Consistently, both studies found higher serum albumin levels, lower serum bilirubin and international normalized ratio values and lower rates of ascites in the NAFLD group. These differences are likely influenced by the substantial proportion (up to half) of NAFLD-HCC patients who do not have cirrhosis or advanced fibrosis.

Hepatic injury as reflected by elevation in transaminase levels appears to be less in NAFLD-HCC compared with other HCCs, especially AST

levels [77]. The predictive value of AST level for NAFLD-HCC was evaluated in two separate cohort studies of Japanese NAFLD patients. Interestingly, the studies reached opposing conclusions with one identifying elevated AST as risk factor for HCC [65] and the other showing it was protective [67]. The predictive value of AST level for NAFLD-HCC therefore remains uncertain.

2.6.4 Tumor Characteristics

Emerging data suggest NAFLD-HCCs may be phenotypically different to HCCs resulting from other liver diseases [3]. Most NAFLD-HCCs present as a well-differentiated, solitary lesion with an average size of 3–4 cm [79, 80, 107]. Up to 70%–78% of NAFLD-HCCs are solitary lesions [67, 75, 80, 106]. One study found that in patients eligible for curative treatments, those with NAFLD-HCC had a fewer tumor nodules compared to HCC secondary to HCV or alcoholic liver disease [77]. This finding was not supported by Piscaglia et al. however, the authors did document fewer small HCCs (Barcelona Clinic Liver Cancer Stage 0) and more advanced-stage HCCs (Barcelona Clinic Liver Cancer stage C) in NAFLD versus HCV groups [108]. NAFLD-HCCs were also more likely to be infiltrative and outside the Milan criteria for liver transplantation, while extrahepatic metastases were less likely. The same study demonstrated larger tumors from NAFLD-HCC compared to other HCCs (4.1 cm vs 3.3 cm) [108]. In another study, HCC patients with metabolic syndrome as their sole risk factor (a surrogate for NAFLD-HCC) also exhibited larger tumors compared to HCC patients with other chronic liver diseases (8.8 cm vs. 7.8 cm), although this fell just short of statistical significance [75]. These larger tumors seen with NAFLD-HCC may be a reflection of their aforementioned delayed presentation [107].

Tumor marker expression may also differ. Levels of α-fetoprotein (AFP) appear to be raised less often in NAFLD-HCC patients than in those with HCC due to other chronic liver diseases [3, 110]. In a Japanese prospective study of 34 patients with NAFLD-HCC, only 26.5% had an elevated AFP [67]. With regards to AFP levels, some studies have demonstrated lower levels in HCC patients with NAFLD versus non-NAFLD etiologies [75, 108], while others found no significant differences [77, 109]. Finally, a greater proportion of NAFLD-HCCs appear well-differentiated on histology compared to other HCCs [75, 77].

Similar to the clinical features of NAFLD-HCC, these tumor characteristics have been confirmed by most but not all studies. This highlights that HCC is still a heterogeneous disease even among the subset of NAFLD-HCC patients.

2.7 Cost and Economic Burden

The cost and economic burden of NAFLD and associated HCC deserves mention. In a study assessing economic burden of NAFLD, Younossi et al. estimated the annual cost to be US$103 billion in the U.S and €35 billion across four European countries – expenditures similar to that of diabetes and heart disease [112]. Based on recent trends, these costs associated with resource utilization are set to rise further in both inpatient and outpatient settings [113, 114]. Although a fraction of these costs may be mediated by comorbid diseases such as diabetes mellitus or angina pectoris [115], their economic impact is huge.

Furthermore, NAFLD patients consistently demonstrate lower health-related quality of life scores as measured by SF-36 or Chronic Liver Disease Questionnaire compared to the general population and patients with other chronic liver diseases [116]. NAFLD patients also experience higher levels of fatigue, physical inactivity and day-time somnolence than healthy controls [117]. These impairments result in loss of work productivity and reflect the unmeasured impact of psychological and psychiatric issues such as depression and anxiety associated with NAFLD. Therefore, NAFLD also imposes significant indirect costs on patients and society. The above study by Younossi et al. approximated that after adding the societal costs of quality-adjusted

life-years lost due to NAFLD, the annual burden of NAFLD in the U.S. and four European countries increases by two to sixfold to US$292.19 billion and €227.84 billion, respectively [112].

The economic cost associated with HCC are similarly considerable and higher than that of other cancers [118, 119]. In particular, the annual cost of NAFLD-HCC was quoted to be US$522.7 million in the United States and €90.2 million in Germany, France, Italy and United Kingdom combined [112]. Significant burdens have also been reported in other countries [118]. Therefore, NAFLD and associated HCC imposes a severe human and economic burden on patients, their families, and society. Of concern, the relative recency and ongoing rise of the obesity epidemic along with the lag period required for NAFLD to develop into cirrhosis and/or HCC has meant that the full impact of NAFLD-related advanced liver disease has not yet been felt [110].

While NAFLD is associated with increased liver-related mortality and HCCs [120], its clinical burden should be tempered by perspective. Indeed, non-liver-related death remains far more common than liver-related death or NAFLD-HCC combined [7]. Consistently, liver disease has been shown to be the third common cause of death in NAFLD patients behind cardiovascular disease and malignancies [64, 121–123]. For every 100 patients with NAFLD, only one to two will die from liver-related death [57]. Even in patients with NASH [44] including those with advanced fibrosis [121], cardiovascular disease remains the top cause of mortality. Therefore, the non-liver-related outcomes of NAFLD patients should not be neglected.

2.8 Conclusion

NAFLD is the most common chronic liver disease in the world and will soon become the number one cause of HCC, liver transplantation and liver-related mortality. The disease often occurs in the setting of metabolic conditions such as obesity and type II diabetes mellitus. These same metabolic drivers are also risk factors for NAFLD-HCC which can occur even in the absence of cirrhosis or advanced fibrosis and appears to be phenotypically different from HCCs arising from other chronic liver diseases. The frequencies of liver-related events and HCC among NAFLD patients is low, especially when compared to cardiovascular disease and extrahepatic malignancies. However, the large denominator of total patients affected with NAFLD means that these events will impose an enormous clinical and economic burden on our society. Moreover, this burden is expected to rise further in the future. Therefore, the global NAFLD epidemic has arrived at our doorstep and demands our attention.

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