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Nonalcoholic Fatty Liver Disease 2020: The State of the Disease

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Nonalcoholic fatty liver disease (NAFLD) is the most common liver disease, with a worldwide prevalence of 25%. In the United States, NAFLD and its subtype, nonalcoholic steatohepatitis, affect 30% and 5% of the population, respectively. Considering the ongoing obesity epidemic beginning in childhood, the rise in diabetes, and other factors, the prevalence of NAFLD along with the proportion of those with advanced liver disease is projected to continue to increase. This will have an important impact on public health reflected in health care costs, including impact on the need for liver transplantation, for which nonalcoholic steatohepatitis is already close to becoming the most common indication. NAFLD patients with evidence of nonalcoholic steatohepatitis and advanced fibrosis are at markedly increased risk of adverse outcomes, including overall mortality, and liver-specific morbidity and mortality, respectively. Identification of this cohort of NAFLD patients is paramount, given the associated poorer outcomes, in order to target resources to those who need it most. Various noninvasive tools have been developed in this regard. This review provides an update on the epidemiology, clinical and prognostic features, and diagnostic approach to patients with NAFLD.

In 1849, the Austrian pathologist, Carl von Rokitansky, hypothesized that cirrhosis can result from fat accumulation[1]; however, since then, only sporadic descriptions of the relationship between fatty liver and both caloric intake and diabetes mellitus have been described in the literature more than a century later.[1–3] Hepatitis of the fatty liver was first described by Heribert Thaler in 1962,[4] before Jurgen Ludwig and his colleagues at Mayo Clinic added the phrase *nonalcoholic steatohepatitis* (NASH) to the lexicon in 1980, with their landmark case series.[5] At that point in time, it was generally believed that nonalcoholic fatty liver (NAFL) was a benign condition, and that patients with obesity-related comorbidities had more pressing medical issues to address. It was not until the 1990s that it became increasingly recognized that NASH was a serious medical condition in its own right, with associated morbidity and mortality.[6] The broader term of *nonalcoholic fatty liver disease* (NAFLD) started to be used in 2002, and encompassed the full spectrum of fatty liver disease from isolated hepatic steatosis or NAFL to NASH and NASH cirrhosis, in addition to the development of diagnostic criteria.[7,8] Over time, the incidence and prevalence of NAFLD has increased dramatically, in parallel with the global epidemic of obesity.[9] With more robust understanding of the natural history of NAFLD, it has become increasingly evident that even this nomenclature is inherently problematic, largely due to the heterogeneity of NAFLD and primary driving forces between individuals. For this reason, and in order to better characterize the disease, an alternate name that better reflects the underlying pathophysiology is needed. The rationale for this will be discussed in depth in another article from this Special Issue. Revised nomenclature in conjunction with the anticipated ability to sub-stratify the disease into different phenotypes will ultimately facilitate a more precise understanding of the natural history of NAFLD, disease variability, and promote a more individualized approach to treatment interventions. This review on NAFLD will explore its current epidemiologic patterns, established clinical and prognostic features, and diagnostic nuances, and should serve as a foundation ahead of the subsequent reviews of NAFLD in this issue of *Gastroenterology.*

Epidemiology

In 2016, the World Health Organization estimated that more than 1.9 billion adults (39% of the adult population) were overweight and 650 million (13% of the adult population) were obese.[10] In the United States, data from the

Abbreviations used in this paper: **AUROC, area under the receiver operating curve; BMI, body mass index; CAP, controlled attenuation parameter; CI, confidence interval; CPR, clinical prediction tool; FIB-4, Fibrosis-4; HCC, hepatocellular carcinoma; HSD17B13, hydroxysteroid 17β-dehydrogenase 13; LSM, liver stiffness measurement; MetS, metabolic syndrome; MRE, magnetic resonance elastography; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD Activity Score; NASH, nonalcoholic steatohepatitis; NASH CRN, Nonalcoholic Steatohepatitis Clinical Research Network; NFS, NAFLD Fibrosis Score; NPV, negative predictive value; PPV, positive predictive value; T2DM, type 2 diabetes mellitus; VCTE, vibration-controlled transient elastography.**

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National Health and Nutrition Examination Survey for 2015 to 2016 estimated that 39.8% of adults were obese.[11] In parallel with the obesity epidemic, there has been a rise in obesity-related complications, NAFLD notwithstanding. NAFLD is the most common liver disease worldwide,[12,13] and is now the second leading indication for liver transplantation in the United States, narrowly behind only alcohol-related liver disease.[14] Moreover, the proportion of NASH as an underlying etiology for hepatocellular carcinoma (HCC) in patients being listed for liver transplantation has increased 7.7-fold (from 2.1% to 16.2%) in the United States.[15] Data from the European Liver Transplant Registry database show similar trends, with 8.4% of all liver transplantations due to NASH, representing a 7-fold increase between 2002 and 2016.[16]

Currently, it is estimated that the global prevalence of NAFLD is approximately 25%, with more than 80 million individuals affected in the United States alone.[17] There are similar rates in Asia, with an estimated pooled prevalence rate of 27.4% (95% confidence interval [CI], 23.3%–31.9%) observed.[17] Both the prevalence of NAFLD and stage of liver disease appear to increase with age.[18,19] The overall prevalence of NAFLD appears to be higher in men, however, the prevalence of NASH with more advanced stages of fibrosis appears to be somewhat enriched in women.[20] Differential rates of disease progression resulting in an increased risk of severe fibrosis in postmenopausal women compared to men is possibly attributed to loss of the protective effects of estrogen against fibrogenesis.[21] Given the projected increasing rates of obesity and type 2 diabetes mellitus (T2DM), compounded by an aging population, NAFLD is projected to increase to 100 million people in the United States by 2030.[22] The data on incidence of NAFLD in the general population are less reliable. In this context, the incidence estimates of NAFLD vary worldwide, ranging from 28.01 per 1000 person-years (95% CI, 19.34–40.57) in Israel to 52.34 per 1000 person-years (95% CI, 28.31–96.77) in Asia.[12,17] The incidence of NAFLD has been increasing dramatically over time. A population-based study from Olmsted County, Minnesota, revealed a 5-fold increase in NAFLD incidence since 1997, as identified by diagnostic coding, most dramatically in younger adults aged 18–39 years old.[23]

Given that NASH requires histologic confirmation via biopsy, the prevalence of NASH in the general population can only be estimated from a small number of biopsy series. Based on these inherently incomplete data, the prevalence of NASH ranges between 1.5% and 6.45% in the general population.[12,17] Among those with NAFLD, the prevalence of NASH ranges from 7% to 30%, with an estimated 16.5 million individuals affected in the United States alone in 2015.[17,22] The pooled prevalence of NASH in Asia, ascertained from biopsy-proven NAFLD patients, was not surprisingly higher at 63.5% (95% CI, 47.7%–76.8%), given the selection bias of examining patients who warranted biopsy.[17] Cirrhosis due to NASH is quite common, with 3.3 million individuals estimated to have advanced fibrosis in the United States in 2015.[22] Rates of decompensated NASH cirrhosis are projected to increase by 168%, liver-related deaths by 178%, and incident HCC by 137% between 2015 and 2030.[22] Although these projected increases might appear dramatic, given the lack of approved therapies for NAFLD and the unabating obesity epidemic, these projected figures may in fact underestimate the future burden of liver disease related to NASH.

There is a noticeable variable prevalence of NAFLD among populations and phenotypic expression of severity. These differences are attributable to numerous factors, including metabolic comorbidities, microbiome, and environmental and genetic/epigenetic factors[24] (Figure 1). As an example, some of these variables likely explain the

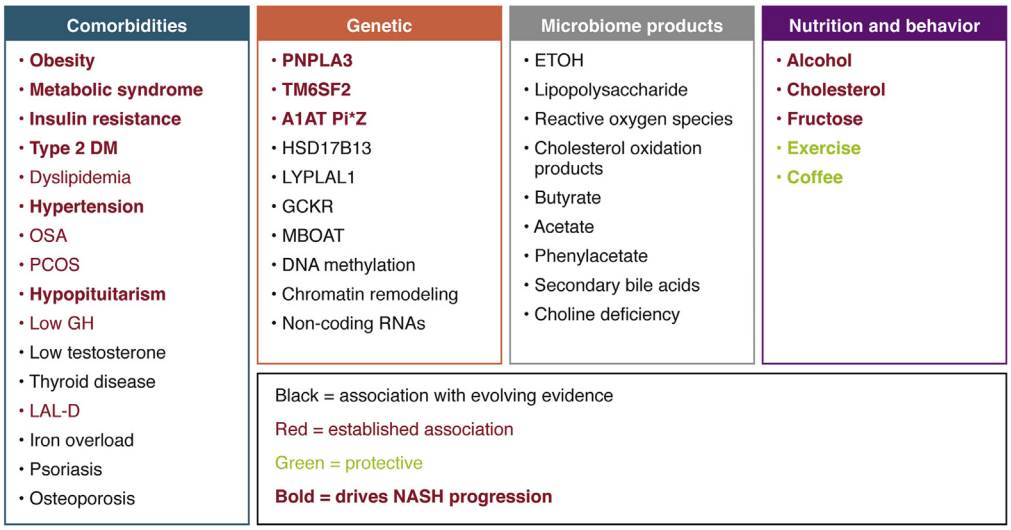


Figure 1. Modifiers of NAFLD. Factors influencing the course of NAFLD are outlined here and can be broadly divided into comorbid illness, genetic factors, microbial products, and nutritional/behavioral factors. Factors with an established association with NAFLD and NASH progression are *bolded* in *red*; factors clearly associated with NAFLD but for which no established link with disease progression has been established are noted in *red*; and disease factors with an appreciated association, but evolving evidence are noted in *black*. *Green* denotes a protective factor.

increased risk for NASH and related increased prevalence of fibrosis among Hispanic Americans. Although previously all Hispanics were thought to be at risk, more recent data suggest that origin also plays a role (ie, indigenous native Americans vs Africa), wherein Hispanics of Mexican origin have a prevalence rate of 33% and those of Puerto Rican and Dominican descent have prevalence rates of 18% and 16%, respectively.[25] These differences are attributable, in part, to the difference in carriage of a single polymorphism in the PNPLA3 gene, which has a higher relative frequency in Mexico. Other countries such as Japan and South Korea have a higher relative frequency of the PNPLA3 risk allele,[26] just as other genetic polymorphisms differ in prevalence across different regions.[26,27] Although African Americans have high rates of metabolic syndrome, NAFLD, particularly NASH, is less common in African Americans compared with Caucasian and Hispanic populations. This is attributable, in part, to lower carriage rates of PNPLA3, however, differences in adipose tissue distribution also play a role.[28,29] In African Americans, body type tends to be predominantly gynoid rather than android, as it typically is in Caucasians or Hispanics with NASH, and dyslipidemia in African Americans is less likely to be high triglycerides/low high-density lipoprotein, which are the typical features of the metabolic syndrome (MetS).[30] These observations are suggestive of a complex interplay between genetic and environmental factors that can dictate severity of NAFLD among different patient populations.

Until recently, our understanding of the natural history of NAFLD was based largely on retrospective data or fairly small case series unable to control for key confounders. The first glimpse of the short-term natural history began to emerge from phase 2b/3 clinical trials[31] and, over time, will continue to provide prospective insight into the natural history of NASH with established fibrosis, although these are a highly selected cohort, which can limit generalizability. Recently published data from the largest prospective cohort of NASH patients encompassing the full spectrum of disease confirmed the dynamic nature of NAFLD, with respect to both the development and progression of NASH, as well as fibrosis. Among other important findings, this study demonstrated that a larger number of those with NAFL are likely to progress to NASH (46.9%) than previously thought, and confirmed that fibrosis improves or progresses in approximately 30% during a mean period of 4.9 years.[32]

Drivers of Disease Progression

The diagnosis of NAFLD requires evidence of hepatic steatosis, either by imaging or histology, and absence of secondary etiologies of hepatic fat accumulation, such as significant alcohol consumption, long-term use of a steatogenic medications (eg, corticosteroids, methotrexate, amiodarone, and tamoxifen), hepatitis C virus infection (particularly genotype 3), monogenic hereditary disorders, severe malnutrition, and Wilson disease.[12] However, even after exclusion of other causes of steatosis, NASH, as it is currently defined, represents a very heterogeneous population. A number of factors have been proposed to accelerate disease progression and this is likely reflective of relative differences in the predominant mechanism of injury in a given individual (Figure 1). Alcohol use is pervasive and a confounding factor in many (possibly the majority) of patients with NAFLD. Furthermore, it is challenging to measure the extent to which alcohol intake impacts an individual due to inaccurate reporting and genetic differences in susceptibility to alcohol-induced liver injury. Increasing alcohol consumption patterns in the US population have been observed in recent years, with per-capita annual consumption of ethanol from all alcoholic beverages in the United States increasing to 2.35 gallons in 2016, from 1995's 33-year nadir of 2.17 gallons.[33] In 2015, the prevalence of binge drinking within 30 days of the study was 17% in the United States, with some states having a prevalence as high as 25%, and an average of more than 7 drinks was consumed per occasion, with males having higher alcohol consumption then females.[34] It is difficult to know what amount of alcohol is "safe" in a given patient, considering the absence of a consistent and precise definition of what constitutes significant amounts of alcohol consumption in the medical literature.[35] In the context of NASH clinical trials, significant alcohol consumption (defined as more than 21 standard drinks per week in men and more than 14 standard drinks per week in women) is exclusionary during a 2-year period preceding baseline liver histology,[36] with a standard alcoholic drink being any drink that contains about 14 g of pure alcohol.[37] These arbitrary thresholds are based on levels above which the risk of cirrhosis is higher (20 g of ethanol for women daily, 30 g daily for men),[38,39] however, these thresholds have not been studied in the context of NASH specifically. Alcohol significantly impacts disease progression in other forms of liver disease, including NASH.[40] Furthermore, patients with moderate alcohol consumption have decreased improvement in steatosis or resolution of NASH.[41] The role of alcohol in altering the disease course in NAFLD requires further elucidation and, as a field, we should be emphasizing that there is no specific innocuous amount of alcohol in the context of NASH rather than asserting that intake below the thresholds used in NASH clinical trials is safe or does not alter disease progression significantly.

Concomitant Liver Disease

Given its high prevalence, other causes of liver disease can coexist with NAFLD and these should be excluded, such as chronic viral hepatitis, autoimmune liver disease, and hemochromatosis.[12] Some indicators of other forms of liver disease can modify the course of disease (a serum ferritin level of >1.5 upper limit of normal, which generally reflects hepatic steatosis or secondary hemosiderosis rather than another form of liver disease, and α-1 antitrypsin heterozygosity are both associated with more advanced fibrosis),[42–44] while others, such as low titers of autoimmune antibodies (in the absence of concomitant autoimmune hepatitis) are common and of no apparent clinical consequence.[45] The presence of commonly associated comorbidities, such as obesity, hypertension, dyslipidemia,

diabetes or insulin resistance, hypothyroidism, polycystic ovary syndrome, and obstructive sleep apnea should also be documented, as they can increase the risk of advanced disease, worsen outcomes, and, in some, have a bidirectional association (Figure 1). The majority of what was previously considered cryptogenic cirrhosis is now recognized as "burned out" NASH, as such patients have a disproportionately high prevalence of metabolic risk factors despite the absence of NASH or even steatosis in the presence of cirrhosis.[46]

Nonalcoholic Steatohepatitis and Advanced Fibrosis

Importantly, those with histologic evidence of NASH and pronounced fibrosis (stage 2 or higher),[47–49] are at an even higher risk for adverse hepatic outcomes (hepatic decompensation, HCC, and liver-related mortality) and this risk increases in an exponential manner as fibrosis stage advances to cirrhosis.[12,47,49–57] Specifically, liver-specific and overall mortality rates among NAFLD and NASH patients are 0.77 (range, 0.33–1.77) per 1000 and 11.77 (range, 7.10–19.53) per 1000 person-years and 15.44 (range, 11.72–20.34) per 1000 and 25.56 (range, 6.29–103.80) per 1000 person-years, respectively.[17] Although acknowledging that fibrosis stage is the clearest histologic determinant of outcomes and NASH has a less clear independent association with poor outcomes, this is likely due to the high co-linearity between NASH and fibrosis severity.[58] Specifically, NASH is present in 94% of patients with stage 4 fibrosis compared with only 35% of those with stage 0 fibrosis.[58] Evidently, the identification of this subset of patients is of paramount importance to appropriate interventions, given their proximity to clinically relevant liver events.

The Influence of Metabolic Syndrome and Type 2 Diabetes

There are a number of medical comorbidities that have varying degrees of association with disease progression among NAFLD patients (Figure 1). The clustering of disease comorbidities manifesting as MetS is by far the most characteristic feature of NAFLD, present in 36%–67% of patients,[17,59–65] and is a unifying contributor to disease progression.[17,59–65] In one study, 88% of patients with NASH met the criteria for MetS, compared with 53% of patients with NAFLD.[59] Similarly, in the Nonalcoholic Steatohepatitis Clinical Research Network (NASH CRN) data, the presence of the MetS increased the likelihood of histologically confirmed NASH by 40%,[66] and is independently associated with increased overall mortality among NAFLD patients in the National Health and Nutrition Examination Survey database.[67] Within the context of the MetS, strength of association varies and there is clear bidirectionality with respect to disease with some, particularly with T2DM and hypertension,[68] although definitive causation has not been proven. In a meta-analysis of 81,411, there was a significantly increased incidence of MetS during a 5-year follow-up period among NAFLD patients, with a relative risk of 3.22 for ultrasonography-diagnosed patients.[69] Obesity is nearly ubiquitous among patients with NAFLD, as up to 75% of patients who are overweight and 90%–95% of patients with morbid obesity have NAFLD.[70–72] The distribution of adiposity, specifically the presence of truncal obesity, is a more important determinant of NAFLD risk than body mass index (BMI).[73–75] Moreover, the extent of visceral adipose tissue area is associated with incident NAFLD in a dose-dependent manner (adjusted hazard ratio of 1.36 (95% CI, 1.16–1.59, per SD increase),[76] and is independently associated with increased risks of NASH and advanced fibrosis.[77] This contributes to the increased disease burden in Asians with a lower BMI who are more sensitive to increased visceral adipose tissue prompting adoption of different criteria for the diagnosis of the MetS in Asians.[78] While visceral adipose tissue only accounts for 7%–15% of the total body fat, it is much more metabolically active than subcutaneous fat and is a dominant source of adipocytokines and free fatty acids received by the liver via portal venous blood, which are instrumental in the development of insulin resistance and the activation of inflammatory pathways.[79]

The presence of insulin resistance is a nearly universal feature of NASH,[64,80] while the prevalence of T2DM in those with NAFLD is less consistent across studies with rates as low as 15% in the population-based Dallas Heart Study and as high as 33%–66% in others.[81–84] The presence of NAFLD increases the risk of incident T2DM, with a relative risk of 1.86 (95% CI, 1.76–1.95),[69] however, some studies have shown an almost 4-fold increased risk, especially among patients with more advanced disease.[85,86] Patients with NAFLD have demonstrably impaired ability of insulin to suppress glucose production, causing hyperglycemia and resulting in hyperinsulinemia, and inappropriate peripheral lipolysis that ensues.[87] NASH is also associated with metabolic dyslipidemia, characterized by high triglycerides and low high-density lipoprotein cholesterol. In a cross-sectional analysis of 9560 apparently healthy Chinese adults who underwent comprehensive health checkups including abdominal ultrasonography, 23% of patients with NAFLD had isolated hypertriglyceridemia, 10% had isolated low high-density lipoprotein cholesterol, and 18% had both abnormalities.[88]

NAFLD patients and/or patients with MetS have an increased rate of fatty acid and triglyceride-rich very low-density lipoprotein secretion into the plasma driven by increased fatty acid delivery to the liver, as well as increased rates of de novo lipogenesis, both mediated my insulin resistance.[89–92] These free fatty adds are central to the pathogenesis of NASH, resulting in the formation of lipotoxic species that lead to endoplasmic reticulum and oxidant stress, and inflammasome activation, which are thought to underpin the hepatocellular injury, inflammation, stellate cell activation, and progressive accumulation of excess extracellular matrix that characterizes the phenotype of NASH.[68] NAFLD at baseline or incident NAFLD is associated with an increased risk of incident hypertension,[93,94] thought to be related to increased renal sodium reabsorption due to hyperinsulinemia, enhanced stimulation of the sympathetic nervous system, and impaired vasodilation secondary to

insulin stimulation.[95,96] Some conditions also have emerging associations with NAFLD and include hypothyroidism, obstructive sleep apnea, hypogonadism, osteoporosis and psoriasis[63,97] (Figure 1). Hypopituitarism and growth hormone deficiency are frequently associated with more progressive NASH with advanced fibrosis.[98] Higher rates of chronic kidney disease are also increasingly recognized in patients with NAFLD.[99,100] Moreover, the frequency of simultaneous liver-kidney transplantation is disproportionally increasing in NASH cirrhosis.[101]

Genetic Influence

There has been vast research undertaken exploring the genetic drivers of disease in NAFLD, beyond MetS and insulin resistance. A classic example is the single rs738409 C>G polymorphism of PNPLA3, which encodes I148M regulating lipolysis of hepatocyte lipid droplets, and is strongly associated with NAFLD, steatosis extent, and histologic severity.[102,103] Furthermore, homozygous carriers of the p.148M mutation carry a 12-fold increased HCC risk, arguing for the potential for monogenic inheritance.[104,105] The risk-associated PNPLA3-I148M variant is resistant to normal proteasomal degradation and accumulates on lipid droplets, which interferes with lipolysis. The mutation occurs with the greatest frequency in Hispanics, followed by non-Hispanic whites, and the least in African Americans.[106] These associations are independent of the presence of T2DM and obesity.[107–109] The *PNPLA3* rs738409 GG allele is more common in Asians with lean NAFLD without MetS, which could account for the observation that Asian and Caucasian populations have a similar prevalence of NAFLD, but Asians have a lower metabolic burden.[110] Another example is the enzyme hydroxysteroid 17β-dehydrogenase 13 (HSD17B13), from a large family of enzymes primarily involved in sex hormone metabolism, which is a novel liver-specific lipid droplet–associated protein in mouse and humans. HSD17B13 expression is markedly up-regulated in patients and mice with NAFLD. Hepatic overexpression of HSD17B13 promotes lipid accumulation in the liver, supporting the pathogenic role of HSD17B13 in NAFLD.[111] A recent study showed a loss-of-function variant in HSD17B13 was associated with a reduced risk of chronic liver disease and of progression from steatosis to steatohepatitis, highlighting it as a potential therapeutic target.[112] While these genetic advancements have increased our understanding of the pathogenesis of NAFLD, and may account in some part for the so-called "lean" NAFLD patients, routine testing for these genetic variants is currently not advocated. A more comprehensive discussion on NAFLD genetics, including TM6SF2 and MBOAT7 gene variants,[113] can be found later in this Special Issue of *Gastroenterology*.

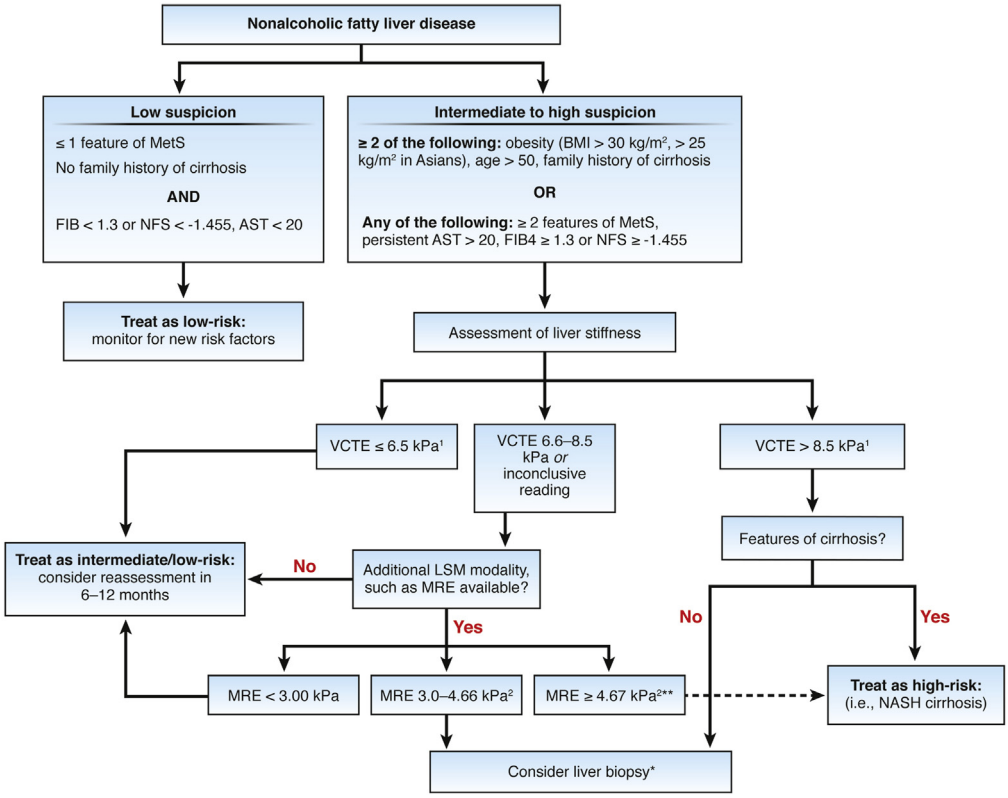
Cardiovascular and Malignancy Risk

Patients with NAFLD, largely driven by those with NASH,[57] have an overall higher rate of mortality compared with matched non-NAFLD controls.[114,115] The most common cause of death among NAFLD patients is cardiovascular disease,[116] with patients twice as likely to die of cardiovascular disease than liver disease. The increased risk of cardiovascular disease in patients with NASH appears to be beyond that conferred by the shared risk factors, likely related to systemic vascular endothelial dysfunction, proatherogenic dyslipidemia, myocardial remodeling, and heart failure.[90,117] A meta-analysis of 34,000 patients with NAFLD revealed a 65% increased risk of developing both fatal and nonfatal cardiovascular events.[118] Cancer-related mortality is the second leading cause of death in NAFLD patients, ahead of liver-specific mortality, which is far higher than the general population.[119] NAFLD is now the third most common cause of HCC in the United States, however, NAFLD is also associated with an increased risk of non-hepatic malignancies, particularly colon cancers.[120–125]

Diagnosis of Nonalcoholic Steatohepatitis

As alluded to in the previous section, evidence of hepatic steatosis either by imaging or histology is required for the diagnosis of NAFLD. Liver biopsy is required for NASH but not for NAFLD, and remains the gold standard for characterizing liver histologic alterations in patients with NAFLD.[12] The current pathologic standard is to report grade (necroinflammatory "activity") separately from stage, which ascertains the location of abnormal collagen deposition and architectural remodeling (ie, "fibrosis"). NAFL is defined as the presence of $\geq$5% hepatic steatosis without evidence of hepatocellular injury in the form of hepatocyte ballooning.[126] NASH is distinguished from isolated hepatic steatosis or NAFL by the presence of hepatocellular injury characterized by the presence of lobular inflammation and hepatocellular ballooning independent of the presence of absence of fibrosis.[12]

There are 2 accepted scoring systems for the diagnosis of NASH—the NAFLD Activity Score (NAS) from the NASH CRN[126] and the Steatosis Activity Fibrosis from the European Fatty Liver Inhibition of Progression Consortium.[127] Both are used to standardize clinical trial outcomes and clinicians involved in managing NAFLD patients would benefit from familiarity in order to systematically interpret the efficacy of interventions. The basic histologic lesions focused on and evaluated by both are the same (steatosis, lobular inflammation, hepatocellular ballooning). However, they differ in that the NAS does not include fibrosis stage within it, while Steatosis Activity Fibrosis does.[128,129] The NAS (plus fibrosis stage) was created as a way to compare liver histology after therapeutic interventions but was not intended to replace the pathologist's separately reported diagnostic classification of the overall disease process (presence of NASH) reported as a single numeric value, the unweighted sum of steatosis, lobular inflammation, and ballooning (0–8). The Steatosis Activity Fibrosis numeric values were created to more holistically differentiate NAFL and NASH, and is reported with subscripts for each component, that is, S (0–3), A (0–4), and F (0–4). Both grade steatosis based on percentage of the parenchyma involved (<5%; 5%–33%; 34%–66%; >66%).[129] A major limitation common to both scoring systems is the assessment of response to treatment, with NASH resolution or loss of disease activity not completely validated.[129]



BMI, body mass index; T2DM, type 2 diabetes mellitus; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; FIB-4, fibrosis-4 index; ELF, Enhanced Liver Fibrosis panel; NFS, NAFLD fibrosis score; MRE, magnetic resonance elastography; VCTE, vibration-controlled transient elastography; LSM, liver stiffness measurement; PPV, positive predictive value.

*May also be required for clinical trial eligibility or if alternative/concomitant diagnosis is suspected.

**If low clinical suspicion for advanced fibrosis, consider liver biopsy.

[1]Siddiqui MS, et al. *Clin Gastroenterol Hepatol* 2019;17:156–163.e2.

[2]Loomba R, et al. *Hepatology* 2014;60:1920–1928.

NOTE: VCTE > 13.6 kPa is a reasonable cutoff for detecting advanced fibrosis, however, the PPV is 0.63. Thus, if clinicians utilize this cutoff, liver biopsy should be considered if the clinical picture remains unclear (Eddowes PJ, et al. *Gastroenterology* 2019;156:1717–1730).

Figure 2. Risk stratification of patients with NAFLD. A pragmatic risk stratification approach is important to identify patients at risk of advanced fibrosis in a cost-effective fashion. In this algorithm, patients are divided into low- or high-risk for advanced disease based on their clinical profile and clinical prediction rules. Patients who are identified as low risk of NASH or advanced fibrosis can be managed in primary care with periodic assessment of new risk factors. Patients who are intermediate or high risk should be considered for specialist evaluation, with further evaluation continuing with liver stiffness assessment.

There are a number of drawbacks to liver biopsy, not least the associated morbidity and very rare mortality, expense, and requirement for expert interpretation. Sampling "error" remains an issue, with studies showing that 35% of patients with bridging fibrosis on 1 sample may have only mild or no fibrosis on the other sample.[130] Furthermore, the diagnosis of NASH is not uniform among pathologists. This significant interobserver variability is illustrated by κ scores of 0.35–0.56 and 0.33–0.45 for ballooning and lobular inflammation, respectively. Another issue with the interpretation of liver pathology is fibrosis. Although κ scores for fibrosis are significantly better (0.61–0.84), the current scoring systems do not account for accumulating fibrosis burden as accurately or as optimally as they should, given the nonlinear increase in collagen burden, especially at later stages of fibrosis.[126,131,132] This is particularly relevant in the context of assessing response to therapy. It is also important to recognize that the interobserver agreements of the aggregate pathologic diagnostic criteria for NASH are also very weak.[48] In general, liver biopsy should be strongly considered in patients with NAFLD who are at increased risk of having NASH and/or advanced fibrosis on the basis of clinical evaluation, or in those patients with suspected NAFLD, in whom competing etiologies for hepatic steatosis and the presence and/or severity of coexisting chronic liver diseases cannot be excluded without a liver biopsy (Figure 2).

Noninvasive Assessment of Nonalcoholic Steatohepatitis and Liver Fibrosis

The ideal biomarker is a characteristic that can be measured in an objective manner that recapitulates normal physiologic or pathogenic processes and changes in parallel with disease improvement or worsening. In the context of NAFLD, biomarkers can be used for the diagnosis and quantification of steatosis, to determine the presence and extent of NASH-related liver injury, and to identify and quantify fibrosis. Ideally, a biomarker is also predictive of outcomes. The diagnosis of hepatic steatosis can be made using various imaging modalities—ultrasound, computed tomography, vibration-controlled transient elastography (VCTE) (Fibroscan) controlled attenuation parameter (CAP), and magnetic resonance imaging. Ultrasound and computed tomography are fairly reliable when there is >30% macrovesicular fat,[133,134] with CAP providing less favorable

reliability,[134] although a notable strength is its ability to detect significant steatosis when reading is >300 dB/m. CAP cutoffs from 281–310 dB/m have a sensitivity of 64%–100% and a specificity of 53%–92% for the detection of biopsy-proven S3 (≥66% hepatocytes with fat).[135] The assessment of dynamic change with either ultrasound, computed tomography, or CAP is not reliable. In contrast, magnetic resonance imaging–derived proton density fat fraction is currently the gold standard for the diagnosis and quantification of hepatic steatosis and has excellent accuracy in detecting dynamic change. Given its cost and lack of point-of-care access, its use is most relevant in the context of a clinical trial as a measure of treatment response.

However, it is the presence and extent of fibrosis, not steatosis, that determine liver-related outcomes. Clinical prediction rules (eg, NAFLD Fibrosis Score [NFS], Fibrosis-4 [FIB-4] Index, Aspartate Aminotransferase to Platelet Ratio Index) and others,[28,136] use readily available clinical variables, providing diagnostic and prognostic information in the practice setting at no cost. Their strength is in their ability to exclude advanced disease with a high degree of accuracy with their relatively robust negative predictive value (NPV).[136] Their limitations are mainly poor positive predictive value (PPV) (ranging from 27% to 79%) and inaccuracy at the extremes of age. Disease severity is overestimated in older patients and diabetics, which has prompted the development of age-adjusted lower cutoffs (NFS < 0.12 and FIB-4 < 2) that maintain the high NPV and thus help exclude advanced fibrosis in patients older than 65 years. In younger patients, the clinical prediction tools (CPRs) have very poor accuracy.[136,137] Furthermore, approximately one-third of patients will be classified as "intermediate" in the case of NFS and FIB-4, which has considerably lower diagnostic accuracy.[138,139] Recent data using a cohort from phase 3 NASH trials nicely illustrated the ability of NFS and FIB-4 in discriminating those patients with advanced fibrosis, with an area under the receiver operating curve (AUROC) ranging from 0.75 to 0.80.[140] However, in sum, CPRs lack sufficient PPV alone to diagnose NASH and fibrosis in isolation. Diagnostic performance can be enhanced by the incorporation of VCTE values as in the FAST Score (VCTE+CAP+aspartate aminotransferase)[141] or other fibrosis markers such PRO-C3 (FIB-C3 score).[142] Other proprietary predictive biomarkers, such as NIS4, that incorporate miR-34a, α2-macroglobulin, YKL-40, and hemoglobin A1c to predict the presence of NASH and F2 or higher fibrosis or pro-C hold some promise, but require larger-scale validation.[143] The most promising thus far to predict disease progression and outcomes is the Enhanced Liver Fibrosis panel.[31] The Enhanced Liver Fibrosis panel consists of plasma levels of 3 matrix turnover proteins (hyaluronic acid, tissue inhibitor of metalloproteinase 1, and N-terminal procollagen III-peptide) and performs marginally better than the NFS for diagnosing moderate fibrosis (AUROC, 0.90 vs 0.86) and severe fibrosis (AUROC, 0.93 vs 0.89), with the combination of the 2 tests yielding an AUROC of 0.93 for moderate fibrosis and 0.98 for severe fibrosis.[144] This promising biomarker panel has been approved for commercial use in Europe and was shown to be a useful adjunct in differentiating patients with indeterminate CPR scores,[122] but is not available for clinical use in the United States. In addition, Enhanced Liver Fibrosis will need to be validated in a real-world population with a lower prevalence of advanced fibrosis in order to determine its true performance characteristics.

The biggest unmet need in the NASH field is the development of a biomarker that can diagnose NASH and determine disease severity accurately. Multiple approaches have been explored or are under development that may hold promise, such as metabolomics or transcriptomic approaches.[145,146] However, the modest performance of any biomarker to diagnosis NASH so far is largely a function of the dynamic nature of the disease, wherein disease activity and even fibrosis stage can vary significantly over fairly short periods of time and limit its histopathologic interpretation. It is anticipated that the NIMBLE (Non-Invasive Biomarkers of Metabolic Liver Disease) and LITMUS (Liver Investigation: Testing Marker Utility in Steatohepatitis) consortia in the United States and Europe, respectively, will provide invaluable information on the performance of biomarkers in the context of NASH in the near future.

The most accurate noninvasive methods to identify advanced fibrosis and to dichotomize NAFLD patients into advanced vs nonadvanced fibrosis include VCTE (FibroScan) and magnetic resonance elastography (MRE).[140,147] Ultrasound-based acoustic force impulse elastography and supersonic shear-wave elastography are additional recognized modalities. VCTE, uses pulsed-echo ultrasonography to provide a liver stiffness measurement (LSM) as a surrogate marker of fibrosis,[148] and is approved by the US Food and Drug Administration for use in both adults and children with liver disease. VCTE has an AUROC of 0.83 with an NPV of 90%, and has a diagnostic accuracy of 80.8% in categorizing patients into subgroups with differing prognoses.[149] It is noteworthy that the cutoff values for different fibrosis stages can vary by both etiology of disease and population. The first study to evaluate the performance of VCTE in a US population (median BMI, 32.2 kg/m^2) found the optimal LSM cutoff to be 9.9 kPa, which yielded a 95% sensitivity and 77% specificity, with AUROC of 0.93 for detecting advanced fibrosis in NASH. An important limitation of this study was the fact that more than one-quarter of the 164 patients assessed had unreliable results, largely related to the lack of XL probe use when indicated.[150] The VCTE with M probe performed similarly in a Japanese cohort of 142 patients who had a noticeably lower median BMI (28.1 kg/m^2) and a lower failure rate of 10.5%.[151] Recently, the NASH CRN published its VCTE multicenter experience in 992 patients with biopsy-proven NAFLD (mean BMI, 33.6 kg/m^2) in the United States using a machine-guided protocol with either an M 1 or XL 1 probe.[152] The results were reassuring, with a low failure (3.2%) and high reliability (>95%) rates and reproducibility.[152] In this cohort, the XL+ probe was used in >60% of the "obese (BMI 30–34.9)" and in almost 90% of the "extremely obese (BMI ≥35)" populations, highlighting the improved diagnostic accuracy when the XL+ probe is used in patients with BMI ≥30 kg/m^2. A more recent study confirmed that the same LSM cutoffs can be used without further adjustment for steatosis for both M and XL probes, as

long these probes are used according to the appropriate BMI.[153] Further results from a subset of the NASH CRN cohort showed that at a fixed sensitivity, a cutoff LSM of 6.5 kPa excluded advanced fibrosis with an NPV of 0.91, and a cutoff LSM of 12.1 kPa excluded cirrhosis with an NPV of 0.99.[154] An important caveat to consider when interpreting VCTE results is that, while liver stiffness typically represents the extent of fibrosis, several other factors (eg, congestive hepatopathy, inflammation) can impact liver stiffness, as illustrated by its modest PPV.[155]

MRE has a sensitivity of 0.86 (95% CI, 0.65–0.97), specificity of 0.91 (95% CI, 0.83–0.96), PPV of 0.68, and NPV of 0.97, with an AUROC of 0.924 for diagnosing advanced fibrosis, using a stiffness cutoff of 3.63 kPa, in severely obese patients (mean BMI, 40.3 kg/m^2) with biopsy-proven advanced fibrosis.[156] Furthermore, the use of 3-dimensional MRE has shown at 40 Hz and a stiffness cutoff of 2.43 kPa to have an AUROC of 0.962 for diagnosing advanced fibrosis.[157] MRE is better for identifying varying degrees of fibrosis in patients with NAFLD,[158] and performs better than VCTE for identifying fibrosis stage 2 or higher, but they both perform equally well in identifying fibrosis stage 3 or higher with AUROCs for TE and MRE of 0.88 and 0.89, respectively.[151]

There are a number of factors to be considered when using these imaging modalities. While VCTE is more accessible and easier to use, its use is limited in patients with severe obesity, ascites, or acute inflammation.[159] MRE can overcome many of these barriers, except for iron overload and acute inflammation; however, MRE is limited by restricted availability at many centers, cost, required expertise for interpretation, presence of metal implants, patient size, and history of claustrophobia.[151] Importantly, neither of these modalities have shown significant promise in correlating with treatment response in clinical trials, which may be due to a number of factors, including the lack of granularity in the identification of individual stages, the limitations of the histologic reference standard, the absence of a highly effective drug, or possibly that liver stiffness may not change in real time with histologic fibrosis improvement.

Given the magnitude of the NAFLD epidemic, a logical algorithmic approach is essential in the initial evaluation of patients with NAFLD. The majority of patients have mild disease, however, patients with severe disease, either NASH or advanced fibrosis need to be identified in order to mitigate the associated complications. With the current lack of a simple, widely available, highly accurate biomarker for NASH, a pragmatic risk-stratification approach is important to disseminate "best practice" for the diagnosis of NASH, when patients are evaluated by both gastroenterology and hepatology providers. A reasonable approach is to divide patients into low-risk or high-risk for advanced disease based on their clinical profile (eg, metabolic profile, liver chemistries, and CPR), and use locally available noninvasive tools to triage patients, as outlined in Figure 2. Patients who are initially identified at low risk of NASH or advanced fibrosis can be managed in primary care with optimization of their metabolic risk profile, without necessarily the need for specialist referral. Patients who are intermediate or high risk should undergo further assessment with specialist referral starting with liver stiffness assessment, with further stepwise evaluation as outlined in Figure 2, which is intended to be used in the context of a Gastroenterology and Hepatology practice. It is challenging to propose a diagnostic algorithm that would apply to all clinical scenarios or practice settings including endocrinology and primary care. The proposed algorithm is deliberately conservative with a low threshold for LSM in order to not to miss any patients with advanced fibrosis given the implications of a missed diagnosis for patients. As LSM modalities continue to become widely available, we hope that this algorithm will be able to be applied more widely across all relevant specialties and primary care. AST has been incorporated given recent published evidence of its prognostication ability with more validating research anticipated shortly.[141,160] While ELF has been omitted as we await evidence of its performance at a general population level. It is important to consider that the incorporation of any biomarker into medical decision making, it is critical to consider the context of use when applying set cutoffs, as well as the population from which such cutoffs were derived, to most accurately apply on an individual patient level. Tailored interventions for patients with NAFLD are discussed in more detail in this Special Issue on NAFLD.

Conclusions

The NAFLD epidemic continues unabated in parallel with the ongoing rise in obesity. The impact of the rise in patients with NAFLD and an increasing proportion with advanced stage disease will be reflected in higher rates of HCC, the need for liver transplantation, and liver-related death, which will heavily burden the health care system. Impactful change in these trajectories will require a concerted effort to educate primary care providers on the prevention and treatment of obesity and the implementation of governmental initiatives to curb the obesity epidemic. Until then, we will be managing increasing numbers of patients diagnosed with NASH. The biggest unmet need in the field is an accurate biomarker that can diagnose and stage NASH to supplant the need for liver biopsy. Such a biomarker, when it is available, will expand the ability to identify patients at risk, monitor disease progression, and response to therapeutic intervention. Emerging data sets from phase 3 clinical trials will determine the performance characteristics of various biomarkers, as well as provide invaluable insight into how we may begin to phenotype patients with NASH. Once this is achieved, treatment can be individualized to better target the predominant driver of disease in a given patient and more robustly improve NASH-related liver injury.

References

1. Best CH, Hartroft WS. Liver damage produced by feeding alcohol or sugar and its prevention by choline. Br Med J 1949;2:1002–1006; pl.
2. Hanssen P. Enlargement of the liver in diabetes mellitus. JAMA 1936;106:914–916.
3. Zelman S. The liver in obesity. AMA Arch Intern Med 1952;90:141–156.

4. Thaler H. [The fatty liver and its pathogenetic relation to liver cirrhosis]. Virchows Arch Pathol Anat Physiol Klin Med 1962;335:180–210.
5. Ludwig J, Viggiano TR, McGill DB, et al. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–438.
6. Matteoni CA, Younossi ZM, Gramlich T, et al. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. Gastroenterology 1999;116:1413–1419.
7. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221–1231.
8. Clark JM, Brancati FL, Diehl AM. Nonalcoholic fatty liver disease. Gastroenterology 2002;122:1649–1657.
9. Swinburn BA, Sacks G, Hall KD, et al. The global obesity pandemic: shaped by global drivers and local environments. Lancet 2011;378:804–814.
10. Afshin A, Forouzanfar MH, Reitsma MB, et al. Health Effects of overweight and obesity in 195 countries over 25 years. N Engl J Med 2017;377:13–27.
11. Hales C, Carroll MD, Fryar CD, et al. Prevalence of obesity among adults and youth: United States, 2015–2016. NCHS Data Brief No. 288 October 2017.
12. Chalasani N, Younossi Z, Lavine JE, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018;67:328–357.
13. Younossi ZM, Stepanova M, Younossi Y, et al. Epidemiology of chronic liver diseases in the USA in the past three decades. Gut 2020;69:564–568.
14. Kim WR, Lake JR, Smith JM, et al. OPTN/SRTR 2016 annual data report: liver. Am J Transplant 2018;18(Suppl 1):172–253.
15. Younossi Z, Stepanova M, Ong JP, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. Clin Gastroenterol Hepatol 2019;17:748–755.e3.
16. Haldar D, Kern B, Hodson J, et al. Outcomes of liver transplantation for non-alcoholic steatohepatitis: a European Liver Transplant Registry study. J Hepatol 2019;71:313–322.
17. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease—meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology 2016;64:73–84.
18. Koehler EM, Schouten JN, Hansen BE, et al. Prevalence and risk factors of non-alcoholic fatty liver disease in the elderly: results from the Rotterdam study. J Hepatol 2012;57:1305–1311.
19. Kagansky N, Levy S, Keter D, et al. Non-alcoholic fatty liver disease—a common and benign finding in octogenarian patients. Liver Int 2004;24:588–594.
20. Zelber-Sagi S, Nitzan-Kaluski D, Halpern Z, et al. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. Liver Int 2006;26:856–863.
21. Yang JD, Abdelmalek MF, Pang H, et al. Gender and menopause impact severity of fibrosis among patients with nonalcoholic steatohepatitis. Hepatology 2014;59:1406–1414.
22. Estes C, Razavi H, Loomba R, et al. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an exponential increase in burden of disease. Hepatology 2018;67:123–133.
23. Allen AM, Therneau TM, Larson JJ, et al. Nonalcoholic fatty liver disease incidence and impact on metabolic burden and death: a 20 year-community study. Hepatology 2018;67:1726–1736.
24. Younossi Z, Tacke F, Arrese M, et al. Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2019;69:2672–2682.
25. Fleischman MW, Budoff M, Zeb I, et al. NAFLD prevalence differs among hispanic subgroups: the Multi-Ethnic Study of Atherosclerosis. World J Gastroenterol 2014;20:4987–4993.
26. Sayiner M, Koenig A, Henry L, et al. Epidemiology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in the United States and the rest of the world. Clin Liver Dis 2016;20:205–214.
27. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol 2018;15:11–20.
28. Younossi ZM, Loomba R, Anstee QM, et al. Diagnostic modalities for nonalcoholic fatty liver disease, nonalcoholic steatohepatitis, and associated fibrosis. Hepatology 2018;68:349–360.
29. Palmer ND, Musani SK, Yerges-Armstrong LM, et al. Characterization of European ancestry nonalcoholic fatty liver disease-associated variants in individuals of African and Hispanic descent. Hepatology 2013;58:966–975.
30. Després JP, Couillard C, Gagnon J, et al. Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: the Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) family study. Arterioscler Thromb Vasc Biol 2000;20:1932–1938.
31. Sanyal AJ, Harrison SA, Ratziu V, et al. The natural history of advanced fibrosis due to nonalcoholic steatohepatitis: data from the Simtuzumab trials. Hepatology 2019;70:1913–1927.
32. Kleiner DE, Brunt EM, Wilson LA, et al. Association of histologic disease activity with progression of nonalcoholic fatty liver disease. JAMA Netw Open 2019;2:e1912565.
33. US Department of Health and Human Services, Centers for Disease Control and Prevention. National Instiute on Alcohol Abuse and Alcoholism. Apparent per capita alcohol consumption: national, state, and regional trends, 1977–2016. Available at: https://pubs.niaaa.nih.gov/publications/surveillance110/CONS16.htm. Accessed November 12, 2019.
34. Centers for Disease Control and Prevention. Behavioral Risk Factor Surveillance System. Age-adjusted intensity of binge drinking among adults aged 18 years and older, by state, United States, 2015. Available at: https://www.cdc.gov/alcohol/data-stats.htm. Accessed November 12, 2019.
35. Liangpunsakul S, Chalasani N. What should we recommend to our patients with NAFLD regarding alcohol use? Am J Gastroenterol 2012;107:976–978.

36. Sanyal AJ, Brunt EM, Kleiner DE, et al. Endpoints and clinical trial design for nonalcoholic steatohepatitis. Hepatology 2011;54:344–353.
37. National Instiute on Alcohol Abuse and Alcoholism. What is a standard drink?. Available at: https://www.niaaa.nih.gov/what-standard-drink. Accessed November 12, 2019.
38. Rehm J, Taylor B, Mohapatra S, et al. Alcohol as a risk factor for liver cirrhosis: a systematic review and meta-analysis. Drug Alcohol Rev 2010;29:437–445.
39. Becker U, Deis A, Sørensen TI, et al. Prediction of risk of liver disease by alcohol intake, sex, and age: a prospective population study. Hepatology 1996;23:1025–1029.
40. Bedogni G, Miglioli L, Masutti F, et al. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. Hepatology 2005;42:44–52.
41. Ajmera V, Belt P, Wilson LA, et al. Among patients with nonalcoholic fatty liver disease, modest alcohol use is associated with less improvement in histologic steatosis and steatohepatitis. Clin Gastroenterol Hepatol 2018;16:1511–1520.e5.
42. Kowdley KV, Belt P, Wilson LA, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2012;55:77–85.
43. Regev A, Guaqueta C, Molina EG, et al. Does the heterozygous state of alpha-1 antitrypsin deficiency have a role in chronic liver diseases? Interim results of a large case-control study. J Pediatr Gastroenterol Nutr 2006;43(Suppl 1):S30–S35.
44. Strnad P, Buch S, Hamesch K, et al. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. Gut 2019;68:1099–1107.
45. Vuppalanchi R, Gould RJ, Wilson LA, et al. Clinical significance of serum autoantibodies in patients with NAFLD: results from the nonalcoholic steatohepatitis clinical research network. Hepatol Int 2012;6:379–385.
46. Caldwell SH, Lee VD, Kleiner DE, et al. NASH and cryptogenic cirrhosis: a histological analysis. Ann Hepatol 2009;8:346–352.
47. Angulo P, Kleiner DE, Dam-Larsen S, et al. Liver fibrosis, but no other histologic features, is associated with long-term outcomes of patients with nonalcoholic fatty liver disease. Gastroenterology 2015;149:389–397.e10.
48. Younossi ZM, Stepanova M, Rafiq N, et al. Pathologic criteria for nonalcoholic steatohepatitis: interprotocol agreement and ability to predict liver-related mortality. Hepatology 2011;53:1874–1882.
49. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: systematic review and meta-analysis. Hepatology 2017;65:1557–1565.
50. Angulo P, Keach JC, Batts KP, et al. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. Hepatology 1999;30:1356–1362.
51. Noureddin M, Yates KP, Vaughn IA, et al. Clinical and histological determinants of nonalcoholic steatohepatitis and advanced fibrosis in elderly patients. Hepatology 2013;58:1644–1654.
52. Bedogni G, Miglioli L, Masutti F, et al. Incidence and natural course of fatty liver in the general population: the Dionysos study. Hepatology 2007;46:1387–1391.
53. Musso G, Gambino R, Cassader M, et al. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. Ann Med 2011;43:617–649.
54. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274–285.
55. Dam-Larsen S, Franzmann M, Andersen IB, et al. Long term prognosis of fatty liver: risk of chronic liver disease and death. Gut 2004;53:750–755.
56. Rafiq N, Bai C, Fang Y, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol 2009;7:234–238.
57. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547–1554.
58. Hagström H, Nasr P, Ekstedt M, et al. Fibrosis stage but not NASH predicts mortality and time to development of severe liver disease in biopsy-proven NAFLD. J Hepatol 2017;67:1265–1273.
59. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–923.
60. Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. Crit Pathw Cardiol 2005;4:198–203.
61. Targher G, Bertolini L, Padovani R, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007;30:1212–1218.
62. Bellentani S, Scaglioni F, Marino M, et al. Epidemiology of non-alcoholic fatty liver disease. Dig Dis 2010;28:155–161.
63. VanWagner LB, Rinella ME. Extrahepatic manifestations of nonalcoholic fatty liver disease. Curr Hepatol Rep 2016;15:75–85.
64. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: Association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–1192.
65. Neuschwander-Tetri BA, Clark JM, Bass NM, et al. Clinical, laboratory and histological associations in adults with nonalcoholic fatty liver disease. Hepatology 2010;52:913–924.
66. Brunt EM, Kleiner DE, Wilson LA, et al. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. Hepatology 2011;53:810–820.
67. Stepanova M, Rafiq N, Younossi ZM. Components of metabolic syndrome are independent predictors of

mortality in patients with chronic liver disease: a population-based study. Gut 2010;59:1410–1415.
68. Friedman SL, Neuschwander-Tetri BA, Rinella M, et al. Mechanisms of NAFLD development and therapeutic strategies. Nat Med 2018;24:908–922.
69. Ballestri S, Zona S, Targher G, et al. Nonalcoholic fatty liver disease is associated with an almost twofold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol 2016;31:936–944.
70. Neuschwander-Tetri B. Fatty liver and nonalcoholic steatohepatitis. In: Friedman LS, Keeffe EB, eds. *Handbook of Liver Disease*. 3rd ed. Amsterdam, the Netherlands: Elsevier Health Sciences, 2012:106–114.
71. Sasaki A, Nitta H, Otsuka K, et al. Bariatric surgery and non-alcoholic fatty liver disease: current and potential future treatments. Front Endocrinol (Lausanne) 2014; 5:164.
72. Subichin M, Clanton J, Makuszewski M, et al. Liver disease in the morbidly obese: a review of 1000 consecutive patients undergoing weight loss surgery. Surg Obes Relat Dis 2015;11:137–141.
73. Park BJ, Kim YJ, Kim DH, et al. Visceral adipose tissue area is an independent risk factor for hepatic steatosis. J Gastroenterol Hepatol 2008;23:900–907.
74. Hamaguchi M, Kojima T, Itoh Y, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 2007;102:2708–2715.
75. van der Poorten D, Milner KL, Hui J, et al. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. Hepatology 2008;48:449–457.
76. Kim D, Chung GE, Kwak MS, et al. Body fat distribution and risk of incident and regressed nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2016;14:132–138. e4.
77. Yu SJ, Kim W, Kim D, et al. Visceral obesity predicts significant fibrosis in patients with nonalcoholic fatty liver disease. Medicine (Baltimore) 2015;94:e2159.
78. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. Lancet 2004;363:157–163.
79. McLaughlin T, Lamendola C, Liu A, et al. Preferential fat deposition in subcutaneous versus visceral depots is associated with insulin sensitivity. J Clin Endocrinol Metab 2011;96:E1756–E1760.
80. Chitturi S, Abeygunasekera S, Farrell GC, et al. NASH and insulin resistance: Insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–379.
81. Leite NC, Salles GF, Araujo AL, et al. Prevalence and associated factors of non-alcoholic fatty liver disease in patients with type-2 diabetes mellitus. Liver Int 2009; 29:113–119.
82. Prashanth M, Ganesh HK, Vima MV, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. J Assoc Physicians India 2009; 57:205–210.
83. Younossi ZM, Golabi P, de Avila L, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol 2019;71:793–801.
84. Browning JD. Statins and hepatic steatosis: perspectives from the Dallas Heart Study. Hepatology 2006; 44:466–471.
85. Chang Y, Jung HS, Yun KE, et al. Cohort study of nonalcoholic fatty liver disease, NAFLD fibrosis score, and the risk of incident diabetes in a Korean population. Am J Gastroenterol 2013;108:1861–1868.
86. Sung KC, Wild SH, Byrne CD. Resolution of fatty liver and risk of incident diabetes. J Clin Endocrinol Metab 2013;98:3637–3643.
87. Fujimoto WY, Abbate SL, Kahn SE, et al. The visceral adiposity syndrome in Japanese-American men. Obes Res 1994;2:364–371.
88. Du T, Sun X, Yuan G, et al. Lipid phenotypes in patients with nonalcoholic fatty liver disease. Metabolism 2016; 65:1391–1398.
89. Donnelly KL, Smith CI, Schwarzenberg SJ, et al. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. J Clin Invest 2005;115:1343–1351.
90. Siddiqui MS, Fuchs M, Idowu MO, et al. Severity of nonalcoholic fatty liver disease and progression to cirrhosis are associated with atherogenic lipoprotein profile. Clin Gastroenterol Hepatol 2015;13:1000–1008. e3.
91. Fabbrini E, Mohammed BS, Magkos F, et al. Alterations in adipose tissue and hepatic lipid kinetics in obese men and women with nonalcoholic fatty liver disease. Gastroenterology 2008;134:424–431.
92. Adiels M, Olofsson SO, Taskinen MR, et al. Overproduction of very low-density lipoproteins is the hallmark of the dyslipidemia in the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:1225–1236.
93. Ryoo JH, Suh YJ, Shin HC, et al. Clinical association between non-alcoholic fatty liver disease and the development of hypertension. J Gastroenterol Hepatol 2014;29:1926–1931.
94. Sung KC, Wild SH, Byrne CD. Development of new fatty liver, or resolution of existing fatty liver, over five years of follow-up, and risk of incident hypertension. J Hepatol 2014;60:1040–1045.
95. Yki-Järvinen H. Non-alcoholic fatty liver disease as a cause and a consequence of metabolic syndrome. Lancet Diabetes Endocrinol 2014;2:901–910.
96. Kotronen A, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. Arterioscler Thromb Vasc Biol 2008;28:27–38.
97. Li AA, Ahmed A, Kim D. Extrahepatic manifestations of nonalcoholic fatty liver disease. Gut Liver 2019 Jun 17 [Epub ahead of print].
98. Adams LA, Feldstein A, Lindor KD, et al. Nonalcoholic fatty liver disease among patients with hypothalamic and pituitary dysfunction. Hepatology 2004;39:909–914.

99. Targher G, Byrne CD. Non-alcoholic fatty liver disease: an emerging driving force in chronic kidney disease. Nat Rev Nephrol 2017;13:297–310.
100. Park H, Dawwas GK, Liu X, et al. Nonalcoholic fatty liver disease increases risk of incident advanced chronic kidney disease: a propensity-matched cohort study. J Intern Med 2019;286:711–722.
101. Singal AK, Salameh H, Kuo YF, et al. Evolving frequency and outcomes of simultaneous liver kidney transplants based on liver disease etiology. Transplantation 2014; 98:216–221.
102. Rotman Y, Koh C, Zmuda JM, et al. The association of genetic variability in patatin-like phospholipase domain-containing protein 3 (PNPLA3) with histological severity of nonalcoholic fatty liver disease. Hepatology 2010; 52:894–903.
103. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011;53:1883–1894.
104. Liu YL, Patman GL, Leathart JB, et al. Carriage of the PNPLA3 rs738409 C>G polymorphism confers an increased risk of non-alcoholic fatty liver disease associated hepatocellular carcinoma. J Hepatol 2014;61:75–81.
105. Krawczyk M, Stokes CS, Romeo S, et al. HCC and liver disease risks in homozygous PNPLA3 p.I148M carriers approach monogenic inheritance. J Hepatol 2015; 62:980–981.
106. Younossi ZM, Stepanova M, Negro F, et al. Nonalcoholic fatty liver disease in lean individuals in the United States. Medicine (Baltimore) 2012;91:319–327.
107. Speliotes EK, Butler JL, Palmer CD, et al. PNPLA3 variants specifically confer increased risk for histologic nonalcoholic fatty liver disease but not metabolic disease. Hepatology 2010;52:904–912.
108. Valenti L, Al-Serri A, Daly AK, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2010;51:1209–1217.
109. Lallukka S, Sevastianova K, Perttilä J, et al. Adipose tissue is inflamed in NAFLD due to obesity but not in NAFLD due to genetic variation in PNPLA3. Diabetologia 2013;56:886–892.
110. Younossi ZM. Non-alcoholic fatty liver disease—a global public health perspective. J Hepatol 2019;70:531–544.
111. Su W, Mao Z, Liu Y, et al. Role of HSD17B13 in the liver physiology and pathophysiology. Mol Cell Endocrinol 2019;489:119–125.
112. Abul-Husn NS, Cheng X, Li AH, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med 2018;378:1096–1106.
113. Basyte-Bacevice V, Skieceviciene J, Valantiene I, et al. TM6SF2 and MBOAT7 gene variants in liver fibrosis and cirrhosis. Int J Mol Sci 2019;20(6).
114. Adams LA, Lymp JF, St Sauver J, et al. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. Gastroenterology 2005;129:113–121.
115. Liu Y, Zhong GC, Tan HY, et al. Nonalcoholic fatty liver disease and mortality from all causes, cardiovascular disease, and cancer: a meta-analysis. Sci Rep 2019; 9:11124.
116. Targher G, Day CP, Bonora E. Risk of cardiovascular disease in patients with nonalcoholic fatty liver disease. N Engl J Med 2010;363:1341–1350.
117. VanWagner LB, Wilcox JE, Colangelo LA, et al. Association of nonalcoholic fatty liver disease with subclinical myocardial remodeling and dysfunction: a population-based study. Hepatology 2015;62:773–783.
118. Targher G, Byrne CD, Lonardo A, et al. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. J Hepatol 2016;65:589–600.
119. Younossi Z, Henry L. Contribution of alcoholic and nonalcoholic fatty liver disease to the burden of liver-related morbidity and mortality. Gastroenterology 2016; 150:1778–1785.
120. Vilar-Gomez E, Calzadilla-Bertot L, Wai-Sun Wong V, et al. Fibrosis severity as a determinant of cause-specific mortality in patients with advanced nonalcoholic fatty liver disease. Gastroenterology 2018;155:443–457.e17.
121. Adams LA, Anstee QM, Tilg H, et al. Non-alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. Gut 2017; 66:1138–1153.
122. Yang YJ, Bang CS, Shin SP, et al. Clinical impact of non-alcoholic fatty liver disease on the occurrence of colorectal neoplasm: propensity score matching analysis. PLoS One 2017;12:e0182014.
123. Hamaguchi M, Hashimoto Y, Obora A, et al. Non-alcoholic fatty liver disease with obesity as an independent predictor for incident gastric and colorectal cancer: a population-based longitudinal study. BMJ Open Gastroenterol 2019;6:e000295.
124. Cho Y, Lim SK, Joo SK, et al. Nonalcoholic steatohepatitis is associated with a higher risk of advanced colorectal neoplasm. Liver Int 2019;39:1722–1731.
125. Mohamad B, Shah V, Onyshchenko M, et al. Characterization of hepatocellular carcinoma (HCC) in non-alcoholic fatty liver disease (NAFLD) patients without cirrhosis. Hepatol Int 2016;10:632–639.
126. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
127. Bedossa P; FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565–575.
128. Rinella ME, Tacke F, Sanyal AJ, et al. Report on the AASLD/EASL Joint Workshop on Clinical Trial Endpoints in NAFLD. Hepatology 2019;71:823–833.
129. Kleiner DE, Bedossa P. Liver histology and clinical trials for nonalcoholic steatohepatitis-perspectives from 2 pathologists. Gastroenterology 2015;149:1305–1308.
130. Ratziu V, Charlotte F, Heurtier A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128:1898–1906.

131. Chen SH, Peng CY, Chiang IP, et al. Comparison of collagen proportionate areas in liver fibrosis quantification between chronic hepatitis B and C. Medicine (Baltimore) 2016;95:e4736.
132. Younossi ZM, Gramlich T, Liu YC, et al. Nonalcoholic fatty liver disease: assessment of variability in pathologic interpretations. Mod Pathol 1998;11:560–565.
133. Bohte AE, van Werven JR, Bipat S, et al. The diagnostic accuracy of US, CT, MRI and 1H-MRS for the evaluation of hepatic steatosis compared with liver biopsy: a meta-analysis. Eur Radiol 2011;21:87–97.
134. Hannah WN, Harrison SA. Noninvasive imaging methods to determine severity of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Hepatology 2016; 64:2234–2243.
135. Mikolasevic I, Orlic L, Franjic N, et al. Transient elastography (FibroScan®) with controlled attenuation parameter in the assessment of liver steatosis and fibrosis in patients with nonalcoholic fatty liver disease—where do we stand? World J Gastroenterol 2016;22:7236–7251.
136. McPherson S, Stewart SF, Henderson E, et al. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;59:1265–1269.
137. McPherson S, Hardy T, Dufour JF, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol 2017; 112:740–751.
138. Angulo P, Hui JM, Marchesini G, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007;45:846–854.
139. Shah AG, Lydecker A, Murray K, et al. Comparison of noninvasive markers of fibrosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2009;7:1104–1112.
140. Anstee QM, Lawitz EJ, Alkhouri N, et al. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR Trials. Hepatology 2019;70:1521–1530.
141. Newsome PN, Sasso M, Deeks JJ, et al. FibroScan-AST (FAST) score for the non-invasive identification of patients with non-alcoholic steatohepatitis with significant activity and fibrosis: a prospective derivation and global validation study. Lancet Gastroenterol Hepatol 2020;5:362–373.
142. Boyle MP, Tiniakos DG, McPherson S, et al. Development and validation of the collagen neo-epitope biomarker pro-C3 “FIB-C3 Score” for detection and staging of advanced non- alcoholic fatty liver disease in a large international multi-centre patient cohort. Hepatology 2017;66(Suppl 1):54A.
143. Hanf R, Harrison SA, Bedossa P, et al. NIS4 for detection of active NASH (NAS≥4) and significant fibrosis (F≥2) in 714 patients at risk of NASH: diagnostic metrics are not affected by age, gender, type 2 diabetes or obesity. Hepatology 2018;68(Suppl 1):89A.
144. Guha IN, Parkes J, Roderick P, et al. Noninvasive markers of fibrosis in nonalcoholic fatty liver disease: validating the European Liver Fibrosis Panel and exploring simple markers. Hepatology 2008;47:455–460.
145. Mayo R, Crespo J, Martínez-Arranz I, et al. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: results from discovery and validation cohorts. Hepatol Commun 2018;2:807–820.
146. Hoang SA, Oseini A, Feaver RE, et al. Gene expression predicts histological severity and reveals distinct molecular profiles of nonalcoholic fatty liver disease. Sci Rep 2019;9:12541.
147. Castera L, Friedrich-Rust M, Loomba R. Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156:1264–1281.e4.
148. Sandrin L, Fourquet B, Hasquenoph JM, et al. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. Ultrasound Med Biol 2003;29:1705–1713.
149. Boursier J, Vergniol J, Guillet A, et al. Diagnostic accuracy and prognostic significance of blood fibrosis tests and liver stiffness measurement by FibroScan in non-alcoholic fatty liver disease. J Hepatol 2016;65:570–578.
150. Tapper EB, Challies T, Nasser I, et al. The performance of vibration controlled transient elastography in a US cohort of patients with nonalcoholic fatty liver disease. Am J Gastroenterol 2016;111:677–684.
151. Imajo K, Kessoku T, Honda Y, et al. Magnetic resonance imaging more accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. Gastroenterology 2016;150:626–637.e7.
152. Vuppalanchi R, Siddiqui MS, Van Natta ML, et al. Performance characteristics of vibration-controlled transient elastography for evaluation of nonalcoholic fatty liver disease. Hepatology 2018;67:134–144.
153. Wong VW, Irles M, Wong GL, et al. Unified interpretation of liver stiffness measurement by M and XL probes in non-alcoholic fatty liver disease. Gut 2019;68:2057–2064.
154. Siddiqui MS, Vuppalanchi R, Van Natta ML, et al. Vibration-controlled transient elastography to assess fibrosis and steatosis in patients with nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol 2019;17:156–163.e2.
155. Eddowes PJ, Sasso M, Allison M, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2019;156:1717–1730.
156. Chen J, Yin M, Talwalkar JA, et al. Diagnostic performance of MR elastography and vibration-controlled transient elastography in the detection of hepatic fibrosis in patients with severe to morbid obesity. Radiology 2017;283:418–428.
157. Loomba R, Cui J, Wolfson T, et al. Novel 3D magnetic resonance elastography for the noninvasive diagnosis of advanced fibrosis in NAFLD: a prospective study. Am J Gastroenterol 2016;111:986–994.
158. Loomba R, Wolfson T, Ang B, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with

nonalcoholic fatty liver disease: a prospective study. Hepatology 2014;60:1920–1928.
159. Srinivasa Babu A, Wells ML, Teytelboym OM, et al. Elastography in chronic liver disease: modalities, techniques, limitations, and future directions. Radiographics 2016;36:1987–2006.
160. Harrison SA, Alkhouri N, Davison BA, et al. Insulin sensitizer MSDC-0602K in non-alcoholic steatohepatitis: A randomized, double-blind, placebo-controlled phase IIb study. J Hepatol 2020;72:613–626.

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Conflicts of interest
This author discloses the following: M. Rinella consults for Intercept, Gilead, Genfit, Enanta, Bristol-Myers Squibb, Novartis, NGM, Immuron, CymaBay, Merck, Viking, Gelesis, Metacrine, Allergan, Thetis, 3vBio, Madrigal, Boehringer Ingelheim, Terns, Genentech, and Fractyl, and has independent research funding from Novartis. The remaining author discloses no conflicts.