MAYO CLINIC

REVIEW

Recommendations for Diagnosis, Referral for Liver Biopsy, and Treatment of Nonalcoholic Fatty Liver Disease and Nonalcoholic Steatohepatitis

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Abstract

Nonalcoholic fatty liver disease (NAFLD) is the primary cause of chronic liver disease in the United States, afflicting an estimated 80 to 100 million Americans. Nonalcoholic fatty liver disease is a spectrum of liver diseases composed of nonalcoholic fatty liver and nonalcoholic steatohepatitis (NASH). Although nonalcoholic fatty liver has a negligible risk of progression, patients with NASH often develop cirrhosis or hepatocellular carcinoma. Although liver biopsy is required to diagnose NASH, only patients with a high risk of NASH or advanced fibrosis require this evaluation. Despite the high prevalence of NAFLD, well-defined screening recommendations are currently lacking. In this review, suggestions for screening, diagnosis, and initial work-up of NAFLD are given on the basis of established guidelines and recent publications. Proposed drug treatments of NASH are also discussed, highlighting the study outcomes, as well as proposed uses and limitations of these drugs. The literature was searched in PubMed using search terms *nonalcoholic fatty liver disease* and *nonalcoholic steatohepatitis*, with filters of "English language." A date range of January 1, 2000, to May 1, 2015, was used for the search. The bibliographies of key references were also searched manually, and seminal publications before the year 2000 were included.

 ■ Mayo Clin Proc. 2015;90(9):1233-1246

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the United States, and its prevalence and clinical importance is increasing worldwide.[1,2] Recent studies estimate that between 30% and 40% of the population in the United States (80 to 100 million Americans) is affected by NAFLD.[3-6] The number of people at risk for NAFLD is even greater, given the increasing prevalence of obesity, diabetes, and metabolic syndrome.[6] Nonalcoholic steatohepatitis (NASH) is a frequently progressive subset of NAFLD that can be complicated by cardiovascular disease, cirrhosis, and hepatocellular carcinoma (HCC).[7,8] Although there are no Food and Drug Administration–approved medications for NASH, there are several medications that have shown benefits in clinical trials. The uses and limitations of these medications will be discussed in detail (Table 1). Prompt diagnosis, timely referrals, and effective treatments are necessary to improve the long-term outcomes of patients with NAFLD and NASH in the setting of primary care and general gastroenterology practices. This review will focus on these important aspects of patient care.

The content of this review is based on a search of the literature performed in PubMed using the following search terms: *nonalcoholic fatty liver disease* and *nonalcoholic steatohepatitis*. Studies published in the non-English scientific literature were excluded. A date range of January 1, 2000, to May 1, 2015, was used for the search. The bibliographies of key references were also searched manually, and seminal publications before the year 2000 were included.

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HISTOLOGY, EPIDEMIOLOGY, AND DISEASE COURSE

Nonalcoholic fatty liver disease is characterized by hepatic steatosis, without a history of excessive alcohol use, in the absence of other known liver diseases.[1] Nonalcoholic fatty liver disease is categorized into 2 subtypes: nonalcoholic fatty liver (NAFL), which

ARTICLE HIGHLIGHTS

- Nonalcoholic fatty liver disease (NAFLD) is a spectrum of liver diseases composed of nonalcoholic fatty liver, which has a negligible risk of progression, and nonalcoholic steatohepatitis (NASH), which has a higher risk of liver disease progression. Nonalcoholic steatohepatitis is histologic diagnosis based on liver biopsy findings of steatosis, ballooning, and lobular inflammation; this disease is associated with an increased risk of cardiovascular death, cirrhosis, end-stage liver disease, and hepatocellular carcinoma.
- Patients with suspected or known NAFLD and a high risk of NASH or advanced fibrosis should be referred for consideration of liver biopsy.
- Lifestyle modifications, including weight loss and exercise, form the cornerstone of NAFLD treatment and should be strongly encouraged. Vitamin E and pioglitazone have been shown to benefit select patients with biopsy-proven NASH.
- Statins and metformin therapy are not indicated for the treatment of NASH, but are safe and effective in patients with NASH with other clinical indications for their use, such as dyslipidemia and diabetes.

is usually nonprogressive, and NASH, which is often progressive and can lead to cirrhosis and HCC.[3] Nonalcoholic fatty liver and NASH have traditionally been considered 2 separate clinical entities, rather than 2 points on a disease continuum.[9] Recent studies evaluating sequential liver biopsies are challenging this notion.[10-12] A systematic review and meta-analysis of paired biopsy studies found that both patients with NAFL and NASH have the potential to develop progressive liver disease.[13] The fibrosis progression rate from stage 0 to stage 1 for NAFL vs NASH is 14 years vs 7 years, providing suggestive evidence that NAFL, NASH, and fibrosis progression are a continuum rather than separate diagnoses.[13] Patients with NAFL and mild lobular inflammation, without ballooning, had an increased risk of disease progression as compared with those without inflammation.[13] Another retrospective study evaluated serial liver biopsies in 108 patients and found no significant difference in the proportion of fibrosis progression between patients with NAFL and those with NASH at index biopsy (37% vs 43%; P=.65).[12] Similarly, a recent study analyzing paired liver biopsies over time found that even patients with bland steatosis can progress to NASH, especially in the setting of metabolic risk factors.[14]

Establishing an accurate diagnosis of NASH is of major clinical importance. A histologic diagnosis of NASH is associated with cardiovascular disease[15] and more rapid progression of liver disease.[13] To accurately distinguish NASH from NAFL requires liver biopsy. Nonalcoholic fatty liver is defined as bland steatosis with minimal or no inflammation, whereas NASH is characterized by macrovesicular steatosis, ballooning, and mixed lobular inflammation with or without zone 3 perisinusoidal fibrosis[16] (Figure 1). Steatosis and ballooning in adults with NASH are most commonly zone 3 predominant or panacinar.[17,18] When advanced fibrosis develops, the zonal distribution of steatosis and ballooning is often lost.[17,18] Acidophil bodies (compact eosinophilic cells representing apoptotic hepatocytes) and Mallory-Denk bodies (ropey intracytoplasmic inclusions composed of damaged intermediate filaments) are also frequently seen on biopsies from patients with NASH but are not required for the diagnosis.[16]

In the Western world, NAFLD is most commonly associated with obesity, metabolic syndrome, and diabetes.[19] As with other metabolic conditions, NAFLD appears to have a strong genetic component. Both family history of diabetes and Hispanic ethnicity have been identified as risk factors.[19] The first genome-wide association study of NAFLD identified that the variant I148M (rs738409) located in human patatin-like phospholipase domain–containing protein 3 gene (*PNPLA3*) was associated with increased hepatic fat content and hepatic inflammation.[20] This allele was found at a higher frequency in Hispanic patients, providing one possible reason for increased susceptibility in this population. Metabolic syndrome, diabetes, and advanced age have all been shown to increase the risk of liver disease progression in patients with NAFLD.[19,21,22]

It is estimated that NASH occurs in 20% of patients with NAFLD (3%-12% of the US population).[5,6] Approximately 30% to 40% of patients with NASH will develop

Mayo Clin Proc. ■ September 2015;90(9):1233-1246 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.013
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TABLE 1. Medications for Use in Patients With NAFLD

Medication	Indications	Contraindications	Limitations	Adverse effects
Pioglitazone	Primary treatment of biopsy-proven NASH in diabetic and nondiabetic patients Treatment of diabetes in patients with NAFLD	Symptomatic heart failure	May increase risk of bladder cancer	Weight gain, bone loss, GI upset, fatigue, and lower extremity edema
Vitamin E	Primary treatment of biopsy-proven NASH in nondiabetic patients	History of prostate cancer and bleeding disorder	May increase all-cause mortality, risk of prostate cancer Not tested in diabetic patients	Increased risk of bleeding and hemorrhagic stroke
Metformin	Treatment of diabetes and insulin resistance in patients with NAFLD	Renal failure	Not a primary treatment of NASH	Diarrhea, lactic acidosis, and GI upset
Obeticholic acid	Primary treatment of biopsy-proven NASH in diabetic and nondiabetic patients	Not currently commercially available	Not FDA approved or available outside of clinical trials Long-term safety is not known	Pruritus and hypercholesterolemia
Statin	Treatment of hyperlipidemia in patients with NAFLD	Excessive alcohol use and hypersensitivity to statin class	Not a primary treatment of NASH	Myalgia, GI upset, mild transaminitis, rare liver injury, and myopathy

GI = gastrointestinal; FDA = Food and Drug Administration; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis.

fibrosis[3,13,23,24] (Figure 2).[5,12,13,25-27] Although fibrosis regresses in some patients,[6,13,28] others progress to advanced fibrosis or cirrhosis.[13,29] In fact, NASH is the third leading cause of cirrhosis in the United States and the third most common indication for liver transplant.[30,31] In addition to cirrhosis, and the complications that accompany it, NASH places patients at risk for HCC.[32] There have been recent reports of HCC developing in patients with NASH without cirrhosis; however, the risk appears to be low and routine surveillance for HCC in patients with NASH without cirrhosis is not recommended until further evidence is available.[26,27]

PATHOGENESIS

Nonalcoholic fatty liver can be seen in any setting in which there is an excess of energy intake and increased hepatic lipid storage in the form of triglycerides.[33] Steatosis may be worsened by de novo lipogenesis in the liver and decreased export of triglycerides from the liver in the form of very low density lipoproteins.[34] Nonalcoholic steatohepatitis occurs in only a subset of patients with NAFLD. The "two-hit" hypothesis of NAFLD suggests that an increase in oxidative stress leads to overwhelming lipid peroxidation and resultant necroinflammatory injury to the fat-laden hepatocytes.[33,35] A more recent view of NAFLD pathogenesis identifies lipotoxicity as the primary driver of cellular injury and death in NASH.[36] In the lipotoxicity model of NASH, free fatty acid metabolites cause endoplasmic reticular stress, hepatocyte apoptosis, necrosis, and inflammation, leading to the histologic findings that characterize this disease.[37,38] The hepatocellular injury, in turn, triggers fibrogenesis and inflammation, hastening disease progression.[39] In this model, triglyceride accumulation may actually be protective against hepatocellular injury.[36]

Like most chronic medical conditions, NASH involves a complex interplay between genetics and the environment. There are strong correlations among NASH, obesity, diabetes, and metabolic syndrome,[19] suggesting a mechanistic link between these diseases. Recent investigations have revealed an interplay between the liver, innate immune system, gut, and adipose tissue. As a result, the farnesoid X receptor (FXR) has become a target of interest.[40] The FXR plays an important role in modulating metabolism and insulin sensitivity through the binding of lipophilic bile acids.[40,41] Dysregulation of the gut-liver axis has also been implicated as a contributor to NASH pathogenesis through derangement

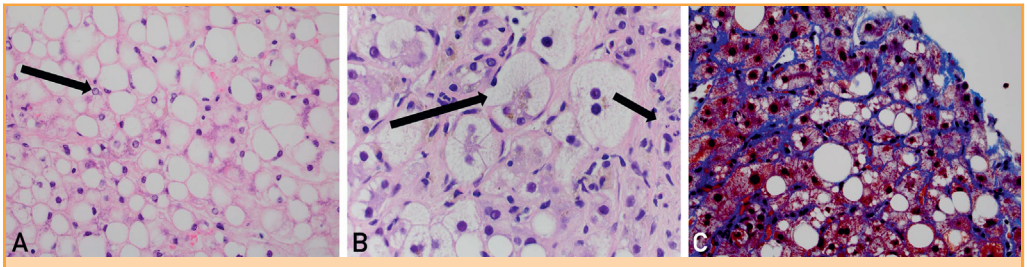

FIGURE 1. Histopathology of NAFLD. A, Image of NAFL (H&E, original magnification 40×). Note the severe fatty change, numerous glycogenated nuclei (arrow), and lack of inflammation or balloon degeneration. B, Image of NASH (H&E, original magnification 60×). In addition to significant steatosis, there is evidence of ballooned hepatocytes (long arrow) and mixed inflammation including neutrophils (short arrow). C, Image of NASH (Klatskin trichrome stain, original magnification 40×). On this Klatskin stain, the sinusoidal fibrosis characteristic of NASH is evident (zone 3). H&E = hematoxylin and eosin; NAFL = nonalcoholic fatty liver; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis.

of the intestinal microbiome, production of gut-derived endotoxin, and altered intestinal permeability.[42-44] Chemokines, such as adiponectin[45,46] and tumor necrosis factor α,[47,48] may be mediators of some of these systemic changes. A thorough discussion of NAFLD pathogenesis is outside the scope of this review, but is addressed in several recent publications.[39,49]

CLINICAL PRESENTATION

Nonalcoholic fatty liver disease is most commonly asymptomatic, although some patients have nonspecific symptoms, such as fatigue, right upper quadrant discomfort, or epigastric fullness. In the absence of advanced liver disease, hepatomegaly may be the only physical finding. Once cirrhosis develops, splenomegaly, spider angiomata, palmar erythema, or ascites may be identified. Nonalcoholic fatty liver disease is most commonly recognized through abnormal liver chemistries or incidental ultrasound findings and should be considered in the differential of any patient with elevated transaminase levels.[50,51] Most commonly, patients have a mildly elevated aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) level, with an AST/ALT ratio of less than 1.[50] In later stages of the disease this ratio may reverse, so AST/ALT greater than 1 does not exclude NAFLD.[52] Although patients with NASH commonly come to medical attention because of an elevated ALT level, a normal or near-normal ALT level does not exclude NASH.[53] The alkaline phosphatase and/or gamma-glutamyltransferase level may be mildly elevated, but the bilirubin level typically remains normal unless advanced disease is present. Increased international normalized ratio, hypoalbuminemia, or thrombocytopenia often indicate cirrhosis or portal hypertension and may be the primary laboratory findings in patients with advanced fibrosis.

SCREENING, DIAGNOSIS, AND INITIAL MANAGEMENT

The prevalence of NAFLD and NASH is high in the general population, but there are no accepted screening regimens, even in high-risk patients. Despite poor sensitivity and specificity, serum ALT and AST levels are the most readily available and commonly used tests to evaluate for asymptomatic liver disease. Obtaining a random ALT and AST level in patients with metabolic syndrome or diabetes may be reasonable, given the high disease burden in this population. Although 50% of patients with NAFLD have normal liver chemistries, up to 80% of patients with NASH may be identified on the basis of elevated transaminase levels.[53]

There are several noninvasive scoring systems designed to increase the detection of NAFLD and advanced fibrosis,[54,55] but the best way to incorporate these into a screening model is not clear. The fatty liver index, for example, uses triglycerides, waist circumference, body mass index (BMI; calculated as the weight in

Mayo Clin Proc. ■ September 2015;90(9):1233-1246 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.013
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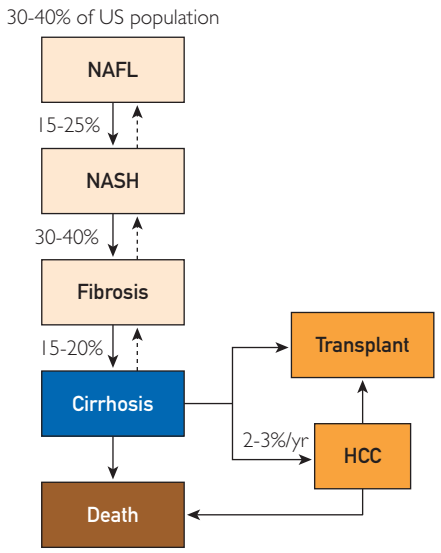

FIGURE 2. Epidemiology of NAFLD. It is estimated that 3% to 12% of the US population is affected by NASH.[5] Although patients with NASH have a higher risk of progression, some patients may progress directly from NAFL to advanced fibrosis.[12,13] Approximately 2% to 3% of patients with NASH cirrhosis will develop HCC each year.[25] Some patients with NASH without cirrhosis may develop HCC, but the magnitude of risk is not clear.[26,27] HCC = hepatocellular carcinoma; NAFL = nonalcoholic fatty liver; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis.

kilograms divided by the height in meters squared), and gamma-glutamyltransferase to improve the sensitivity and specificity of diagnosing NAFLD,[56] but there is no consensus on which, or if, patients should be screened using this or similar models. Biomarkers, such as cytokeratin 18, currently lack the sensitivity needed to be clinically useful screening tests.[57] A recent study from the NASH Clinical Research Network proposed and validated a model designed to quantify the likelihood of NASH or advanced fibrosis in adult diabetic patients using routinely available clinical variables and laboratories.[58] The model for NASH identification included white race, BMI, waist circumference, ALT level, AST level, albumin level, hemoglobin A_{1c} level, homeostasis model assessment of insulin resistance, and ferritin level. Although sensitivity was poor (56.8%), the model exhibited excellent specificity (90.0%) and had a positive predictive value of 93.2%, making it a helpful clinical tool to predict the presence of NASH in adult diabetic patients. Despite improvements in predictive models, screening is not recommended at this time in any population.

Baseline liver evaluation and cardiovascular risk assessment should be considered in all patients who have a presumptive diagnosis of NAFLD (Table 2).[58] A liver ultrasound, full liver chemistry panel, international normalized ratio, creatinine level, and complete blood cell count should be obtained to characterize the pattern of liver injury and to assess the severity of disease. Other causes of liver disease should be investigated and excluded, and medications known to worsen steatosis should be discontinued.[59] Common comorbidities, such as diabetes and dyslipidemia, should be identified and treated. A higher amount of fat in the liver is associated with an increased risk of cardiovascular disease[15] but there is no consensus on routine cardiovascular screening in this population. Patients with cirrhosis should be screened for HCC and esophageal varices according to the current American Association of Liver Disease guidelines.[29] There are no current HCC screening guidelines for patients with NASH without cirrhosis.[60]

IMAGING

Ultrasonography is the most inexpensive and widely available imaging test for NAFLD.[50,61] Typical sonographic findings of NAFLD are hepatomegaly and increased echogenicity. Unfortunately, ultrasonography is not sensitive if less than 30% of the liver is involved by steatosis.[62] Variability in operator skill and the limiting body habitus of the typical patient with NAFLD can lead to inadequate or inconsistent results.[63] Although changes of cirrhosis and portal hypertension may be identified on ultrasound, it is neither a sensitive nor a specific modality for this diagnosis.[61] Given these limitations, other imaging studies are being investigated for the diagnosis and risk stratification of patients with NAFLD. Magnetic resonance imaging, including magnetic resonance spectroscopy, has shown good sensitivity and specificity in detecting and quantifying steatosis,[64] but it is expensive and not widely used for this purpose.[65-67] Transient elastography has been used with moderate success to evaluate the degree of fibrosis and cirrhosis in patients with

TABLE 2. Recommended Management of Patients With NAFLD[a]

1. Recommend lifestyle modification:
a. Weight loss of at least 5%-10% of the total body weight
b. Aerobic exercise 3-5 times/wk
c. Minimization of alcohol use (no more than 1 drink/d for women or 2 drinks/d for men)
2. Assess cardiovascular risks using lipid profile, fasting glucose and/or hemoglobin A_{1c} level, waist circumference, and BMI
3. Manage comorbidities, including diabetes, dyslipidemia, hypertension, and cardiovascular disease
4. Discontinue medications that may worsen steatosis, including corticosteroids, amiodarone, methotrexate, tamoxifen, estrogens, tetracyclines, and valproic acid
5. Obtain baseline liver evaluation, including liver ultrasound, CBC count, liver panel (AST, ALT, bilirubin, and alkaline phosphatase levels), INR, and creatinine level
6. Consider referral for liver biopsy, if
a. Patient has risk factors for NASH and advanced fibrosis, including diabetes[b] and/or metabolic syndrome
b. Patient has findings concerning for cirrhosis, such as thrombocytopenia, AST level > ALT level, or hypoalbuminemia
c. Patients is undergoing cholecystectomy or bariatric surgery and intraoperative biopsy is low risk
7. Consider pharmacotherapy if patient has biopsy-proven NASH without cirrhosis and no absolute contraindications
8. Obtain appropriate screening if patient has known cirrhosis:
a. Right upper quadrant ultrasound every 6 mo for HCC screening (refer to AASLD guidelines)
b. EGD screening for esophageal varices (refer to AASLD guidelines)
c. Referral to a transplant center when appropriate (refer to AASLD guidelines)

[a]AASLD = American Association of Liver Disease; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMI = body mass index; CBC = complete blood cell; EGD = esophagoduodenoscopy; HCC = hepatocellular carcinoma; INR = international normalized ratio; NAFLD = nonalcoholic fatty liver disease; NASH = nonalcoholic steatohepatitis.
[b]Biopsy should be considered in diabetic patients with other risk factors for advanced fibrosis.[58]

NAFLD, but is limited by body habitus and degree of steatosis.[68,69] Magnetic resonance elastography (MRE) provides an accurate noninvasive measure of fibrosis in NAFLD and may be particularly helpful in identifying patients with advanced fibrosis.[70,71] Among all noninvasive imaging modalities, MRE appears to be the most accurate test for fibrosis assessment in NAFLD.[71,72] Despite growing evidence of its potential clinical utility, MRE is not widely available and data are needed to show its performance and cost-effectiveness as a fibrosis screening test in routine clinical practice. If validated in prospective studies, MRE may be used to screen for advanced fibrosis in high-risk patients, such as older diabetic patients. A novel quantitative ultrasound technique using ultrasound imaging—based biomarkers is being developed for the diagnosis and quantification of hepatic steatosis.[73] Although not yet commercially available, in the future this could provide a low-cost method of identifying patients with probable NAFLD. As with all diagnostic modalities, advanced imaging studies should be ordered in a facility equipped to accurately perform and interpret the test. With the development of more specialized and complex testing, it is important that the ordering physician understand the indications for the study as well as the clinical application of specific results.

REFERRAL FOR CONSIDERATION OF LIVER BIOPSY

Liver biopsy is the criterion standard for diagnosing NAFL and NASH, but is not indicated in all patients with suspected diseases. It is invasive, expensive, and not without risk. Liver biopsy should be considered in all patients with persistently elevated aminotransferase levels in whom the diagnosis remains uncertain.[59] Biopsy is also important in ruling out the presence of other concomitant liver diseases, such as in the setting of elevated serum ferritin levels or the presence of autoantibodies. Furthermore, biopsy has prognostic value in NAFLD because the presence of NASH and/or fibrosis provides information on the future risk of progression to cirrhosis and the risk of liver-related mortality.

The American Association of Liver Disease practice guidelines for NAFLD recommend considering liver biopsy in patients with NAFLD who are at an increased risk of NASH and advanced fibrosis. These guidelines suggest that the presence of metabolic syndrome and the NAFLD fibrosis score be used to identify these high-risk patients.[1] A recent study by the NASH Clinical Research Network used data on 435 patients with NAFLD and diabetes to develop and verify a model to predict the presence of NASH and advanced fibrosis in diabetic patients with NAFLD.[58] Among this large cohort of patients, 69.2% were found to have NASH and 41.0% were found to have advanced fibrosis,[58] highlighting the high risk of progressive disease in this population. The model used to predict advanced fibrosis in diabetic patients included age, BMI, waist-to-hip ratio, Hispanic ethnicity, hypertension, ALT/AST ratio, bilirubin (total and direct) level, alkaline phosphatase level, isolated abnormal alkaline phosphatase level, globulin level, albumin level, serum insulin level,

Mayo Clin Proc. ■ September 2015;90(9):1233-1246 ■ http://dx.doi.org/10.1016/j.mayocp.2015.06.013
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hematocrit, international normalized ratio, and platelet count.[58] This proposed model predicted advanced fibrosis better than did the NAFLD fibrosis score, and it may be used to guide liver biopsy referral in patients with type 2 diabetes. New evidence suggests that advanced age or a strong family history of NASH may also be associated with a more aggressive disease course,[5] but there are not adequate data to support biopsy in all patients with these risk factors. Patients undergoing bariatric surgery have a high prevalence of NASH and should strongly consider having intraoperative liver biopsy for diagnosis and staging.[74] Laboratory parameters suggesting cirrhosis, such as thrombocytopenia, hypoalbuminemia, and AST level > ALT level, should also prompt consideration of liver biopsy referral.[75]

In addition to proving a definitive diagnosis, liver biopsy is useful in NAFLD to identify the stage of liver disease and assess the likelihood of progression. Liver biopsies are subject to substantial sampling error that may lead to inaccurate staging or disease classification,[76] but liver biopsy remains the criterion standard for diagnosis and staging. Patients with bland steatosis without necroinflammation on biopsy are unlikely to develop cirrhosis, assuming metabolic parameters remain unchanged. The severity of steatosis, however, may correlate with the development of metabolic syndrome and the risk of cardiovascular disease independent of the presence of NASH.[15] The presence of NASH on index biopsy predicts a more aggressive disease course with a higher rate of progression to cirrhosis.[11] In addition to informing patient discussions on prognosis, this information helps identify which patients should be considered for more aggressive therapy. All currently recommended pharmacologic treatments of NASH require histologic diagnosis before the initiation of therapy.[1]

LIFESTYLE INTERVENTIONS

Weight loss is the cornerstone of NAFLD treatment. Clinical trials have shown that weight loss reduces hepatic steatosis[77] and that exercise itself may improve histology regardless of weight change.[78] Even resistance training led to improvements in hepatic steatosis in a recent randomized controlled trial (RCT).[79] A weight loss of at least 3% to 5% of the total body weight has been found to improve steatosis, and a weight loss of more than 7% of the total body weight is associated with a decrease in necroinflammation.[80] A small RCT of 31 patients reported that an intensive weight loss program led to more weight loss, elevated ALT level, and histology as compared to general education alone.[77] Two-thirds of the patients in the intervention group no longer met the definition of NASH after the 48-week study period. This suggests that patients may reap more benefit from a structured weight loss regimen than from physician education alone. Encouraging patients to seek additional training and assistance with weight loss may be worthwhile.

BARIATRIC SURGERY

Although bariatric surgery has been shown to help morbidly obese patients lose weight, there are limited data supporting its use as a treatment of NASH. A Cochrane review of 21 prospective and retrospective studies reported an improvement in steatosis and/or inflammation scores after bariatric surgery, but the authors were unable to recommend its use for this indication because none of the studies were adequately randomized or controlled.[74] A recent study from France examined prospectively 109 obese patients with biopsy-proven NASH who underwent bariatric surgery.[81] Nearly 85% of patients had histologic resolution of NASH at 1 year. Histologic resolution was most common in patients with mild NASH before surgery and in patients who underwent gastric bypass rather than vertical gastric banding.[81] The study suggests that bariatric surgery may be a promising treatment of NASH, but is limited by the lack of randomization or a control group.

In a large Cochrane review, 4 studies found worsening fibrosis in patients with NAFLD who underwent bariatric surgery.[74] The finding of worsening disease was seen primarily in patients with high BMI or advanced fibrosis,[74] indicating that this surgery may not be safe in all patients. Other studies evaluating the risk of bariatric surgery in patients with NAFLD have found no increase in postoperative complications.[82,83] Mayo Clinic published a review of 14 patients with compensated cirrhosis who underwent bariatric surgery at their center.[84] Although there was no increase in perioperative complications, the authors[84] noted that the results apply only to well-compensated cirrhotic patients treated in a large referral center. Patients with NAFLD who meet other medical criteria for surgery should

be referred for bariatric procedures,[85] but data to support bariatric surgery as a specific treatment of NASH remain limited.[1] A prospective RCT is needed to further investigate the safety and efficacy of this promising intervention.

PIOGLITAZONE

Although the pathogenesis of NASH has not been fully elucidated, there is a clear association with insulin resistance and sensitization of the liver to metabolic injury and inflammation. Several insulin-sensitizing medications have been tested for the treatment of NASH, with mostly limited success. Although no medications are Food and Drug Administration approved for NASH treatment, pioglitazone appears to have a beneficial effect on steatosis, necroinflammation, and possibly even fibrosis.[86,87] Pioglitazone and other thiazolidinediones are selective peroxisome proliferator–activated receptor-gamma agonists. They modify the adipocytokine profile by stimulating adipocyte maturation. This, in turn, increases β-oxidation of fatty acids and reduces proinflammatory cytokines. Peroxisome proliferator–activated receptor-gamma agonists also work at the level of the muscle, liver, and adipose tissue to improve insulin sensitivity.

The largest RCT of pioglitazone use in NASH is the PIVENS trial. This trial included 247 nondiabetic patients with biopsy-proven NASH randomized to treatment with 96 weeks of placebo, pioglitazone 30 mg, or vitamin E 800 IU.[88] The primary end point was histologic improvement, which required increase by 1 or more points in the hepatocellular ballooning score, no increase in the fibrosis score, and either a decrease in the nonalcoholic fatty liver disease activity score (NAS) to a score of 3 points or less or a decrease in the NAS of at least 2 points, with at least a 1-point decrease in either lobular inflammation or steatosis score.[89] The NAS is calculated using the grade of steatosis (grade 0-3), ballooning (grade 0-2), and lobular inflammation (grade 0-3)[1] and serves as a relatively objective manner of standardizing NASH histology and allowing a comparison of biopsies in clinical trials. Although the pioglitazone arm did not meet the primary end point ($P=.04$), there was a significant reduction in steatosis ($P<.001$) and lobular inflammation in this group ($P=.004$).[88] The most common adverse events were gastrointestinal upset, lower extremity edema, and fatigue.

A meta-analysis by Boettcher et al[86] reviewed 4 good quality RCTs, including the PIVENS trial, addressing treatment of NASH with thiazolidinediones. When both rosiglitazone and pioglitazone were included in the analysis, there was a statistically significant improvement in necroinflammation as compared with the use of placebo, but again with no improvement in fibrosis. A subgroup analysis of pioglitazone vs placebo, however, did show an improvement in fibrosis.[86]

Although pioglitazone treatment has been associated with histologic improvement in NASH, there are drawbacks to its use. Thiazolidinediones commonly cause a significant increase in body weight with an average increase of 4 kg.[86] Rosiglitazone is no longer available in the United States because of an association with coronary events and decompensation of heart failure. Because of similar concerns about pioglitazone, a large meta-analysis including 16,390 patients with type 2 diabetes was performed.[90] Although pioglitazone was associated with a statistically significant increase in congestive heart failure, from 1.8% to 2.3% ($P=.002$), all-cause mortality, myocardial infarction, and stroke were reduced in patients taking pioglitazone.[90] Unfortunately, because only diabetic patients were included in these safety studies, it is not known if these findings are applicable to all patients with NASH. Considering only diabetic patients with NASH for treatment with this agent may be overly restrictive, because all the studies that investigated histologic improvement were performed in nondiabetic patients. However, only patients with biopsy-proven NASH should be considered for treatment with pioglitazone because these are the patients who are at the highest risk of progression and thus most likely to benefit from a pharmacologic intervention.[59] This medication should be avoided in patients with known heart failure, a history of bladder cancer, or an increased risk of bone loss, because its use may increase the risk of these conditions.

VITAMIN E

The pathogenesis of NASH is felt to be at least in part due to damage from oxidative stress induced by reactive oxygen species. Vitamin E (α-tocopherol) is a naturally occurring antioxidant thought to mitigate oxidative stress and reactive oxygen species formation through suppression of lipid peroxidation and has been investigated as a

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possible therapy for NASH. In the TONIC trial, 173 children and adolescents with biopsy-proven NASH were randomized to treatment with vitamin E 800 IU, metformin, or placebo.[91] At the end of the 96-week trial, the vitamin E group had achieved a significant improvement in NAS (P=.02) and hepatocellular ballooning score (P=.006) but no significant change in ALT level (P=.07) as compared with the placebo group. The PIVENS trial, which included only nondiabetic patients with biopsy-proven NASH and no evidence of cirrhosis, remains the largest RCT to investigate the effect of vitamin E on NASH.[88] After 96 weeks of treatment, patients in the vitamin E arm achieved a significant improvement in NASH histology (P=.001). Furthermore, both vitamin E and pioglitazone led to resolution of NASH in approximately one-third of the patients. Vitamin E use was also associated with a decrease in ALT (P=.001) and NAS ($P\leq$.001), but fibrosis scores were not changed as compared with placebo use (P=.24).[88]

Vitamin E is well-tolerated with minimal adverse effects, but the safety of long-term use is not known.[92] A large meta-analysis of randomized trials of antioxidant supplements suggested an increase in all-cause mortality in patients taking vitamin E (relative risk [RR], 1.04; 95% CI, 1.01-1.07) or other antioxidant supplements.[93] Vitamin E use is also associated with an increased risk of prostate cancer with an absolute risk of 1.6 per 1000 person-year.[94] A meta-analysis investigating the effect of vitamin E on stroke subtypes found a decrease in ischemic stroke (RR, 0.9; P=.02), but an increase in hemorrhagic stroke (RR, 1.2; P=.05).[95] Although the increase in absolute risk of these conditions remains low, it is recommended that these potential health concerns be discussed with patients before initiating the treatment. At this time, there is no evidence for use in patients with diabetes, cirrhosis, or NAFLD without biopsy-proven NASH. Given the promising study results and lack of alternative therapies, it is reasonable to begin 800 IU of vitamin E in nondiabetic, noncirrhotic patients with biopsy-proven NASH.[59] It is not recommended to treat with vitamin E unless the patient has a biopsy-proven diagnosis of NASH.[1]

EMERGING THERAPIES

Obeticholic acid, a synthetic derivative of the naturally occurring bile salt chenodeoxycholic acid, is an FXR agonist that has recently been evaluated as a potential therapy for NASH.[96] When bound to the FXR, lipophilic bile acids such as obeticholic acid have been shown to decrease hepatic gluconeogenesis and improve insulin sensitivity.[97] In the FLINT trial, 283 patients were randomized to receive obeticholic acid or placebo with biopsies performed at baseline and after 72 weeks of treatment.[41] After a planned interim analysis of histologic changes revealed benefit from treatment, the decision was made to complete the 72-week biopsy in only 200 patients. The final analysis revealed a statistically significant improvement in histologic features of NASH, including ballooning, inflammation, steatosis, and fibrosis.[41] This study included both diabetic and nondiabetic patients, as well as nonresponders to vitamin E who were randomized to either obeticholic acid or placebo. Although nearly a quarter of patients treated with obeticholic acid developed pruritus, only 1 patient had to discontinue therapy owing to severe pruritus. Because of its effect on bile acid synthesis and cholesterol disposal, obeticholic acid was predictably associated with a significant increase in total cholesterol and low-density lipoprotein (LDL) levels, with a decrease in high-density lipoprotein. Future studies are needed to examine the long-term effects of these alterations on the cardiovascular risk in patients with NASH. Mechanistic studies are underway to better understand the pathogenesis of obeticholic acid—induced pruritus and LDL increase and determine whether cotreatment with other pharmacologic agents can modify the pruritus or risks of LDL increase. It is important to note that ursodeoxycholic acid, a bile acid derivative that is currently in clinical use, failed to show convincing benefit in NASH.[98,99] However, unlike obeticholic acid, ursodeoxycholic acid is not an effective FXR agonist.

Pentoxifylline (PTX) is a phosphodiesterase inhibitor that has been shown to inhibit the proinflammatory cytokine tumor necrosis factor α.[100] Tumor necrosis factor α has been implicated in the pathogenesis of NASH in animal models,[44] although its specific contribution in human NASH is not entirely clear.[101] Pentoxifylline also increases hepatic glutathione production, which may contribute to its hepatoprotective effect. Human studies investigating PTX therapy in

NASH are limited. Only 2 RCTs to date have investigated histologic end points of treatment.[102,103] Zein et al[103] conducted a placebo-controlled trial including 55 patients with biopsy-proven NASH who were randomized to either PTX 400 mg 3 times daily orally or placebo for 12 months. Liver histology was assessed at the end of the treatment. The PTX group exhibited significant improvement in serum ALT level, histologic features of NASH, and fibrosis. There was histologic resolution of NASH in 25% of the treatment arm as compared with 3.9% of those receiving placebo.[103] Cirrhotic patients were excluded from the study, and less than 10% of the study group had diabetes. A smaller RCT including 26 patients who were randomized to either PTX or placebo did not show improvement in histology or ALT.[102] Although the dosing and treatment duration were the same as in the other RCT, this study included patients with cirrhosis, which may account for some of the differences in outcomes. Nausea and vomiting were the most common adverse effects in those receiving PTX. Although these studies indicate that PTX may have therapeutic potential, there are insufficient data to recommend its use in NASH at this time. Additional studies investigating the use of PTX are underway.

Metformin increases peripheral glucose and fatty acid uptake while inhibiting lipogenesis and gluconeogenesis. It has been shown to improve insulin resistance and transaminitis while inducing weight loss.[104] In 2 pilot studies metformin improved liver histology,[105,106] but subsequent RCTs did not support this finding.[91,106-108] Metformin has been shown to be effective in treating diabetes and insulin resistance and is safe for use in patients with NAFLD. Physicians are encouraged to use metformin for the management of prediabetes or diabetes in those who have coexisting NAFLD. Recent case-control and cohort studies have shown the benefit of metformin in reducing the risk of liver decompensation, death, and HCC in patients with cirrhosis.[109] Further RCTs are needed to assess the effect of metformin in reducing the risk of hepatic decompensation and HCC in patients with NASH cirrhosis. There are several other therapies outside the scope of this review that are in clinical trials for the treatment of NAFLD and NASH.[110] Descriptions of ongoing trials are available through the National Institutes of Health website at ClinicalTrials.gov.

REFERRAL FOR CONSIDERATION OF LIVER TRANSPLANT

Despite optimal medical management, many patients with NASH will develop cirrhosis. In fact, NASH often goes unrecognized until patients present with symptoms of decompensated liver disease. Cirrhosis due to NASH is currently the third most common indication for liver transplant in the United States and the second most common indication for liver transplant listing.[31] United Network for Organ Sharing data have shown that the number of patients with NASH listed for liver transplant has tripled in the past 10 years.[31] Nonalcoholic steatohepatitis is also the most rapidly growing etiology of liver disease in patients transplanted for HCC.[30] Despite their increased BMIs and medical comorbidities, patients transplanted for NASH cirrhosis have no worse 90-day survival than do those transplanted for hepatitis C or alcoholic liver disease.[31] Studies evaluating long-term posttransplant survival in patients with NASH found that survival is similar, or even superior, to that in those transplanted for other etiologies.[111,112] Although graft loss is significantly lower (OR, 0.21; 95% CI, 0.05-0.89; P=.03) in patients with NASH, risk of cardiovascular death is higher (OR, 1.65; 95% CI, 1.01-2.70; P=.05),[112] highlighting the importance of excellent preventive care after transplant. Patients transplanted for NASH cirrhosis also have a higher rate of chronic kidney disease after transplant.[113] Any patient developing signs of decompensated cirrhosis, such as ascites, hepatic encephalopathy, or variceal bleeding, and/or a model for end-stage liver disease score of 10 or more should be referred to a transplant center for evaluation.[114]

FUTURE DIRECTIONS AND CONCLUSION

Nonalcoholic fatty liver disease is one of the leading causes of chronic liver disease worldwide. The obesity epidemic has led to an ever-increasing number of patients at risk for NAFLD and NASH. Liver biopsy is expensive and invasive, but it remains the only definitive means to diagnose NASH. Therefore, the development of noninvasive methods of diagnosing NASH is a major unmet need in the field. Although weight loss and other lifestyle modifications remain the foundation of

treatment, there are now good data to support the use of pioglitazone and vitamin E in specific patients. Unfortunately, fewer than 50% of patients with NASH respond to current therapies, which underlines the importance of continued research and the need for improved therapies for NASH and NASH-related fibrosis. There are several promising therapeutic modalities and targets currently under investigation. The field is at a tipping point of discovery and innovation. With continued interest in research efforts, improvements in the diagnosis and management of NAFLD and NASH will continue in coming years.

ACKNOWLEDGMENT

We thank Leana Guerin, MD, Department of Pathology, University of Iowa Hospitals and Clinics, for preparing the histology figures.

Abbreviations and Acronyms: **ALT** = alanine aminotransferase; **AST** = aspartate aminotransferase; **BMI** = body mass index; **EGD** = esophagoduodenoscopy; **FXR** = farnesoid X receptor; **HCC** = hepatocellular carcinoma; **LDL** = low-density lipoprotein; **MRE** = magnetic resonance elastography; **NAFL** = nonalcoholic fatty liver; **NAFLD** = nonalcoholic fatty liver disease; **NAS** = nonalcoholic fatty liver disease activity score; **NASH** = nonalcoholic steatohepatitis; **PTX** = pentoxifylline; **RCT** = randomized controlled trial; **RR** = relative risk

Grant Support: The study was supported by Atlantic Philanthropies, Inc, the John A. Hartford Foundation, the Association of Specialty Professors, and the American Gastroenterological Association and by grant K23-DK090303-02 (R.L.). The study sponsors had no role in the study design; collection, analysis, interpretation of the data; and/or drafting of the manuscript.

Potential Competing Interests: Dr Loomba is partly supported by the American Gastroenterological Association Foundation—Sucampo—ASP Designated Research Award in Geriatric Gastroenterology and by a T. Franklin Williams Scholarship Award.

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