World Journal of Hepatology

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World J Hepatol 2017 June 8; 9(16): 715-732

DOI: 10.4254/wjh.v9.i16.715

ISSN 1948-5182 (online)

REVIEW

Non-alcoholic fatty liver disease: An expanded review

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Author contributions: Benedict M wrote the paper; Zhang X edited, revised and contributed with conceptual development.

Conflict-of-interest statement: Authors declare no conflict of interests for this article.

Manuscript source: Invited manuscript

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Received: December 16, 2016
Peer-review started: December 19, 2016
First decision: January 28, 2017
Revised: February 8, 2017
Accepted: April 18, 2017
Article in press: April 20, 2017
Published online: June 8, 2017

Abstract

Non-alcoholic fatty liver disease (NAFLD) encompasses the simple steatosis to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma. NAFLD is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. NAFLD patients are at increased risk of liver-related as well as cardiovascular mortality, and NAFLD is rapidly becoming the leading indication for liver transplantation. Liver biopsy remains the gold standard for definitive diagnosis, but the development of noninvasive advanced imaging, biochemical and genetic tests will no doubt provide future clinicians with a great deal of information and opportunity for enhanced understanding of the pathogenesis and targeted treatment. As it currently stands several medications/supplements are being used in the treatment of NAFLD; however, none seem to be the "magic bullet" in curtailing this growing problem yet. In this review we summarized the current knowledge of NAFLD epidemiology, risk factors, diagnosis, pathogenesis, pathologic changes, natural history, and treatment in order to aid in further understanding this disease and better managing NAFLD patients.

Key words: Non-alcoholic fatty liver disease; Metabolic syndrome; Steatohepatitis; Hepatocellular carcinoma; Steatosis

Core tip: Non-alcoholic fatty liver disease (NAFLD) is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. NAFLD patients are at increased risk of liver-related as well as cardiovascular mortality, and NAFLD is rapidly becoming the leading indication for liver transplantation. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. However, the exact pathogenic mechanism of NAFLD still remains unclear, and there

is no effective treatment yet so far. In this review we summarized the current knowledge of NAFLD epidemiology, risk factors, diagnosis, pathogenesis, pathologic changes, natural history, and treatment.

Benedict M, Zhang X. Non-alcoholic fatty liver disease: An expanded review. *World J Hepatol* 2017; 9(16): 715-732 Available from: URL: http://www.wjgnet.com/1948-5182/full/v9/i16/715.htm DOI: http://dx.doi.org/10.4254/wjh.v9.i16.715

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is an umbrella term and encompasses the simple deposition of adipose tissue in the liver to more progressive steatosis with associated hepatitis, fibrosis, cirrhosis, and in some cases hepatocellular carcinoma (HCC)[1]. For the sake of terminology, NAFLD is comprised of non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH)[1]. NAFL is characterized by steatosis of the liver, involving greater than 5% of parenchyma, with no evidence of hepatocyte injury[2]. Whereas, NASH is defined by histologic terms, that is a necroinflammatory process whereby the liver cells become injured in a background of steatosis[2]. Although the natural history of NAFLD remains incompletely characterized, what is clear from the published data is a risk of progression to cirrhosis and HCC[3-7]. However, whether there is a clear progression of NAFL to NASH is under active investigation, but early evidence suggests this could be the case[1]. In terms of epidemiology, several studies have tried to quantify the true worldwide incidence of NAFL/NASH; however, due to extreme variations in study parameters and available testing, a clear and reliable occurrence rate is not currently available[1]. With that being said, estimates have been posited suggesting the incidence of NAFLD to be 20%-30% in Western countries and 5%-18% in Asia[1]. It is no surprise that the prevalence of NAFLD is increasing worldwide with each passing year, given the current trends in dietary irresponsibility and preponderance of a sedentary lifestyle[1]. Additionally, there has been a linear rise of NAFLD with that of diabetes and metabolic syndrome[3]. In one study from the United States, it was shown that the incidence of NAFLD was 10% higher in overweight individuals compared to lean persons[8]. In fact, NAFLD has been projected, within the next 20 years, to become the major cause of liver related morbidity and mortality as well as a leading indication for liver transplantation[3]. As it currently stands, NAFLD represents the second most common reason to be listed for a liver transplant[9]. Additionally, not only does NAFLD place a strain on the medical system and its resources, it also is associated with a 34%-69% chance of dying over the next 15 years when compared with the general population[9]. The pathogenetic processes that underscore NAFLD typically lead to death by cardiovascular disease with liver related mortality only accounting for 5% in these individuals[9,10]. In the forthcoming sections we will provide context for how and why NAFLD develops, current genetic proposals, histologic criteria, differential diagnoses, and prognosis of this very important disease affecting not only the United States but much of the world.

RISK FACTORS AND ETIOLOGY

Metabolic syndrome and type 2 diabetes mellitus

Metabolic syndrome is a conglomerate of cardiovascular risk factors which predispose a person to developing type Ⅱ diabetes and cardiovascular disease[2]. The current diagnostic criteria require having 3 of 5 of the following factors: Triglycerides 150 mg/dL or greater, high-density lipoprotein-cholesterol of less than 40 mg/dL in men and less than 50 mg/dL in women, hyperglycemia (fasting glucose of 100 g/dL or greater), an increased waist circumference (defined by population specific data), and hypertension (systolic blood pressure of 130 mmHg or greater or diastolic blood pressure of 85 mmHg or greater)[2]. As previously mentioned the incidence of NAFLD has been increasing in concert with the rising rates of metabolic syndrome. In fact it has been stated that the incidence of NAFLD increases with increasing number of metabolic syndrome criteria met[2]. When compared to non-diabetic patients (matched for age, sex, and body weight), type 2 diabetes mellitus (T2DM) patients have liver fat contents that are 80% higher[11]. Interestingly, it has been shown that T2DM patients with NAFLD can have normal liver function tests, which may lead one to believe that the prevalence of NAFLD in T2DM patients is much higher than reported in this patient population[11]. Additionally, T2DM patients display a very high risk of developing NASH as well as a two-to-four-fold increased risk of fatty liver associated complications[11,12].

Ethnic differences

The rate at which NAFLD develops has been shown to be greatest in Hispanic patients[13]. Also, NAFLD in the Asian population has been increasing, and interestingly, can be seen in those who have a normal body mass index[13]. In a United States based study, the investigators found a lower degree of steatosis in African Americans when compared to whites and also showed a higher degree of NAFLD findings in Asians and Hispanics[14]. The Hispanic population also has been shown to have a higher occurrence of steatohepatitis and cirrhosis, while those who are African American enjoy a decreased chance of developing liver failure[15]. With further genetic investigation by genome wide association, it was noted that Hispanics had a twofold higher liver fat content if they possessed the homozygous PNPLA3 allele (patatin-like phospholipase domain-containing protein 3 rs738409)[15]. The PNPLA3 gene family has been shown to affect lipid metabolism and patients who harbor this polymorphism were found to have increased hepatic fat content, triglyceride stores,

and inflammation[13]. In fact, the mutation of *PNPLA3 rs738409* gene (encoding I148M) has revealed more severe histologic features of NAFLD in those carrying the mutation[13]. More information on the genetic basis for NAFLD can be found under the "genetics" heading.

Gender and age

Unfortunately, the role of gender in the development of NAFLD has been met with differing conclusions in the literature. Several studies provide data to suggest a higher prevalence in males while others proposed the opposite[1]. However, according to Lonardo *et al*[11] epidemiological review, NAFLD is more common in men and has been shown to increase in those who are younger to middle aged with a decline noted after the age of 50-60 years. In contrast, NAFLD has been shown to spare those women who are pre-menopausal and then a rise in incidence occurs after the age of 50 with a peak at 60-69 years, and the preponderance of evidence does seem to suggest that NASH is histologically more severe in women when compared to men[11]. It has been reported that the prevalence of NAFLD increases with age (20% in people younger than age 20) to greater than 40% in those who are older than 60 years of age[16]. Not only does the prevalence of NAFLD increase with increasing age, but the incidence of NASH and cirrhosis also increases in those patients who are 50 years of age or greater compared with younger age groups[1]. Notably, it has been suggested that NAFLD begins in utero based on several studies, using magnetic resonance spectroscopy, showing steatosis in infants born to mothers with gestational diabetes (GD)[17]. In a study using hepatic fat fraction (HFF), performed at 1-3 wk of age in neonates born to normal mothers compared to those with gestational diabetes, neonates born to obese mothers with GD had a mean HFF that was 68% higher than those born to normal weight mothers[18]. In another study by Patel *et al*[19], 33 stillborn babies of diabetic mothers were compared with 48 stillborn babies of mothers without diabetes and there was a markedly increased rate of hepatic steatosis in neonates born to mothers with diabetes (79%) *vs* controls (17%). A study with 191 Italian children with biopsy confirmed NAFLD, showed hepatic steatosis, inflammation, hepatocyte ballooning, and fibrosis were worse in those children who were not breast-fed compared to those who were[20]. Similar to what has been observed in adults, obesity is a considerable risk factor for the development of NAFLD in children[21]. According to the Study of Child and Adolescent Liver Epidemiology, approximately one-third of obese children have NAFLD[22]. With that being said, a fatty liver is the most common liver abnormality found in children aged 2-19 years[22]. Again like that seen in adulthood, there is also an association of pediatric NAFLD and cardiovascular disease with higher levels of total cholesterol, LDL, triglycerides, and systolic blood pressure reported[21]. As it currently stands the incidence of HCC in the pediatric population with NALFD is not known but thought to be rare[17]. Only one case report of HCC with concurrent NAFLD in a 7-year-old boy has been reported[23]. Longitudinal outcomes are sparse for pediatric patients with NAFLD; however, what is known is that children can present with cirrhosis at diagnosis and may progress from NASH to cirrhosis[24].

Diet, smoking and life style

Diet has been thought of as an independent risk factor for the development of NAFLD, specifically, a diet high in fats[15]. It has been shown, through energy restriction and manipulation of dietary macronutrients, namely, restriction of carbohydrates, fat, or enrichment with monounsaturated fatty acids, that dietary modifications can reduce metabolic syndrome[25,26]. Diets that model after a Westernized pattern, such as those high in red meat consumption, refined grains, pastries, and sugar laden beverages are associated with a greater likelihood for the development of metabolic syndrome and subsequent NAFLD[15]. In a retrospective study with 2029 participants, cigarette smoking was found to be an independent risk factor for the onset of NAFLD[27]. The use of tobacco predisposes a person for the development of insulin resistance[28-30]. Additionally, in a study looking at adolescents in the United States, passive and active smoke exposure are strong independent predictors of metabolic syndrome[31]. As to life style, associations have been shown between a person's fitness and sedentary behavior with the risk of developing NAFLD and NASH; the severity of NAFLD also intensifies with lower physical activity[15]. In fact, as part of the EASL-EASD-EASO Clinical practice guidelines for the management of NAFLD, a recommendation for the assessment of physical activity habits should be included as part of a comprehensive NAFLD screening exam[32]. Additionally, part of the treatment regimen for NAFLD incorporates diet and physical activity to address obesity and insulin resistance. Several studies have evaluated the effect of a balanced diet with gradual weight reduction and their effects of NAFLD biologic parameters. Overwhelmingly, gradual weight reduction through diet, with or without exercise, have shown improvements in serum liver enzymes, reduced hepatic fatty infiltration, decreased hepatic inflammation and reduced levels of fibrosis[33]. Also there is a clear benefit of exercise on hepatic fatty infiltration; this benefit is even evident with minimal or no weight loss and exercise levels that fall below those which are recommended for obesity management[34]. According to a systematic review, NAFLD is also improved with resistance exercise (as opposed to the therapeutic benefits of aerobic activities such as running), which may be more tolerable for the NAFLD patients who suffer from poor cardiorespiratory fitness and cannot tolerate intense aerobic exercise[35].

Polycystic ovarian syndrome

Polycystic ovarian syndrome (PCOS) is a common endocrine disorder in reproductive aged women and is typically

characterized by obesity and insulin resistance[36]. Hence, women with PCOS are at a heightened risk of developing T2DM[36]. In a study that evaluated 600 women with PCOS and 125 body mass index (BMI)-matched healthy control women, the prevalence of NAFLD was found to be higher in those with PCOS[36]. Insulin resistance and obesity, as have been previously examined in this paper, are known to contribute to the development of NAFLD. Women with PCOS are typically hyperandrogenemic and insulin resistance worsens the hyperandrogenemia by increasing ovarian androgen synthesis and decreasing liver SHBG production, which results in elevated circulating levels of free androgens[36]. The subsequent hyperandrogenemia is associated with a more prominent insulin resistance in patients with PCOS, which endangers these patients for developing NAFLD[36]. Numerous other investigations into the association of PCOS and NAFLD have been performed and similar results were obtained[37-40].

Obstructive sleep apnea

Obstructive sleep apnea (OSA) is characterized by complete or partial airway obstruction caused by pharyngeal collapse during sleep[41]. A budding association of OSA with diabetes mellitus, metabolic syndrome, and cardiovascular disease has started to appear in the last few years[41]. In the general population, obstructive sleep apnea has a prevalence of around 4% with that number jumping to 35%-45% in obese individuals[15]. In a study performed by Tanné *et al*[42], patients with severe OSA were found to be more insulin resistant and had a higher percentage of steatosis as well as increased necrosis and fibrosis scores (on liver biopsy) when compared to those patients without OSA and a similar BMI. The pathogenic mechanisms that underpin this association is believed to be due to the alteration of gas exchange (repetitive hypoxemic and hypercapnic events), termed chronic intermittent hypoxia, which can lead to an increase in proinflammatory cytokines, endothelial dysfunction, oxidative stress, metabolic dysregulation, and finally insulin resistance[41]. Interestingly, OSA may be one of the elements promoting the evolution of NAFLD from steatosis to NASH[41]. Additionally, using animal models, OSA was shown to promote the digression of NAFLD to NASH[15]. Investigational evidence has suggested that chronic intermittent hypoxia may trigger liver injury, inflammation, and fibrogenesis with several studies showing an intriguing relationship between OSA and NASH[41,43-48].

GENETICS

Data from numerous studies have given evidence for a heritable component to NAFLD and includes: Familial aggregation, twin studies, and interethnic differences in susceptibility[49-57]. Whole exome sequencing studies performed on obese Caucasian participants with NAFLD have revealed deleterious mutations in Bardet-Biedl syndrome 1 gene as well as the Melanocortin 3 receptor gene[58]. In 2008, the first genome wide association study was published; it examined hepatic triacylglycerol (HTAG) accumulation and identified association with increased HTAG and the *PNPLA3* gene[59]. This single nucleotide polymorphism is a nonsynonymous cytosine to guanine nucleotide transversion mutation that results in an isoleucine to methionine amino acid change. Subsequent work has confirmed this variant (PNPLA3 rs738409) in Japanese, Indian, and Chinese NAFLD patients[60-65]. In a meta-analysis of 24 studies with 9915 participants, Singal *et al*[66] found that PNPLA3 was associated with fibrosis severity. Additionally, among nine studies, totaling 2937 participants, the PNPLA3 was again linked with increased risk for the development of HCC in those with cirrhosis[66]. A separate meta-analysis, 16 studies included, revealed the rs738409 GG genotype compared to the CC genotype was linked to a 73% greater liver fat content as well as a 3.24-fold increased risk of more pronounced necroinflammatory scores and a 3.2-fold increased risk of developing fibrosis[67]. Xu *et al*[68], by way of meta-analysis totaling 23 case-control studies (totaling 6071 NAFLD participants and 10366 controls) found the PNPLA3 rs738409 polymorphism to have a significant association with a high cross-ethnicity risk for NAFLD as well as NASH. Genome-wide association study performed on 236 non-Hispanic white women with NAFLD (324623 single nucleotide polymorphisms in total from 22 autosomal chromosomes) found the NAFLD activity score to be associated with the SNP rs2645424, the degree of fibrosis associated with SNP rs343062, lobular inflammation with SNPs rs1227756, rs6591182, and rs887304, increased levels of ALT was associated with SNPs rs2499604, rs6487679, rs1421201, and finally rs2710833[69]. Using exome-wide association, Kozlitina *et al*[70] found three variants to be associated with higher liver fat levels: Two in the aforementioned PNPLA3 and one in the *TM6SF2* gene, which likely is required for normal VLDL secretion. The variant frequency in *TM6SF2* gene was found to be highest in those of European, African-American, and Hispanic ancestry[58]. In a later study by Mahdessian *et al*[71]; the *TM6SF2* gene was found to be a regulator of liver fat metabolism, which influenced triglyceride secretion and hepatic lipid droplet content. As it stands currently, approximately 7 categories of genes have been associated with NAFLD and are broken down as follows: (1) hepatic lipid export/oxidation in steatosis (PNPLA3, TM6SF2, NR1I2, PPAR-alpha, PEMT, MTTP, APOC3 and APOE); (2) glucose metabolism and insulin resistance (ENPP1/IRS1, GCKR, SLC2A1, GOAT, TCF7L2 and PPARG); (3) steatosis-hepatic lipid import or synthesis (SLC27A5, FADS1, and LPIN1); (4) steatohepatitis-oxidative stress (HFE, GCLC/GCLM, ABCC2 and SOD2); (5) steatohepatitis-endotoxin response (TLR4 and CD14); (6) cytokines (TNF and IL6); and (7) fibrosis (AGTR1 and KLF6)[49,72].

PATHOGENESIS

Non-alcoholic fatty liver disease, not surprisingly, as its name implies revolves around the deposition of fat within the liver. Specifically, free fatty acids and triglyceride accumulation is

the hallmark feature and has been attributed, at least in part, to insulin resistance and obesity[73]. With that being said, the pathogenic components of NAFLD are complex and multifactorial with different theories presented in the literature[74]. A two-hit model of NAFLD development has been proposed with the first hit consisting of: Hepatic lipid accumulation, sedentary lifestyle, high fat diet, obesity, and insulin resistance[74]. The second hit activates an inflammatory event with associated fibrogenesis[75]. This two-hit model has lost some favor as it was believed to be too simplistic to fully describe the intricacy of human NAFLD where a multitude of factors are acting in concert with one another in a genetically predisposed individual[74]. As was described in the risk factors, a multitude of factors contribute and have some association with the development of NAFLD[76]. However, it is insulin resistance that plays a key role in the development of steatosis/NASH, which results in hepatic *de novo* lipogenesis and subsequent reduction of adipose tissue lipolysis, with a consequent increase of fatty acids in the liver[77]. Alterations in the production and secretion of adipokines and inflammatory cytokines are a consequence of adipose tissue dysfunction, which is brought about by insulin resistance[78]. The production of reactive oxygen species and endoplasmic reticulum stress coupled with mitochondrial dysfunction occurs as a result of fat accumulations in the liver, specifically in the form of triglycerides[79]. An excess of nutrients essentially overwhelms the endoplasmic reticulum, which then turns on the unfolded protein response and as a consequence, triggers the development of insulin resistance through a number of mechanisms, including c-jun N-terminal kinase activation and inflammation[79]. The gut microbiota has been recognized as one of the key players in the pathogenesis of NAFLD. Gut microbiota not only influences absorption and disposal of nutrients to the liver, but also conditions hepatic inflammation by supplying toll-like receptor ligands, which can stimulate liver cells to produce proinflammatory cytokines. Accordingly, the modification of intestinal bacterial flora by specific probiotics has been proposed as a therapeutic approach for the treatment of NASH[80]. Interestingly, dysfunctional adipose tissue, as seen in obesity, T2DM and NAFLD, impairs glucose and lipid metabolism by two mechanisms: One, by acting as an endocrine organ, which is releasing a number of fat-derived cytokines; and two, by free fatty acid-induced ectopic fat deposition and lipotoxicity[79].

Liver transplantation is performed for a variety of reasons: Liver failure, end-stage liver disease, tumors; however, after surgery these patients often develop an increase in body weight, subsequent insulin resistance, and metabolic perturbations[81]. Additionally, patients who undergo a liver transplant may also fall prey to diabetes mellitus, hyperlipidemia, and arterial hypertension[81]. In part, some the metabolic derangements that occur after liver transplantation are due to medication effects (*i.e.,* corticosteroids, calcineurin inhibitors, and sirolimus promote hyperglycemia, hypertension, and hyperlipidemia)[81]. Many of the effects aforementioned can be found in the diagnostic criteria of metabolic syndrome, and as previously discussed, NAFLD is essentially the liver's manifestation of this syndrome. Hence, it is not surprising to see recurrent or *de novo* NAFLD/NASH after a liver transplant[82]. It is important to note that 15.5% and 26.3% of liver transplant patients, at one and three years, respectively, become clinically obese[83]. Likewise, post-transplant development of DM is reported to range from 10%-64%, although the underlying mechanisms for this is yet to be entirely worked out[84]. However, it does appear that the main risk factors for the development of post liver transplant DM would include: Male gender, obesity, family history, hepatitis C virus (HCV), older age range, and high dose immunosuppresives[84]. Additionally, the rate of metabolic syndrome development post liver transplant is approximately 50%-60%[85]. In a cohort comprising 170 transplant patients followed for two years, the researchers showed the presence of metabolic syndrome in approximately one-third[86]. Not surprisingly, the incidence of NAFLD after having received a liver transplantation ranges from 18%-40% and the incidence of NASH ranges from 9%-13%[87]. Intriguingly, post-transplant NAFLD risk has also been tied to polymorphisms in PNPLA3, which has been shown to mediate triglyceride hydrolysis and is also associated with pretransplant obesity and NAFLD[87]. Overall, the natural history of post-liver transplant NAFLD is incompletely understood, however, it may contribute to increased cardiovascular disease mortality in these patients[87].

HISTOPATHOLOGY

Non-alcoholic fatty liver disease shows a wide range of histologic manifestations, which can range from a very mild steatosis (5% or more of hepatocytes involved), to more aggressive forms showing lobular and/or portal inflammation, ballooning hepatocytes, fibrosis, and ultimately cirrhosis[88]. The presence of less than 5% of steatosis is not regarded as clinically significant. In adult patients, steatosis typically affects the centrilobular hepatocytes first; whereas in children the periportal or panacinar patterns are more likely seen[89]. Steatosis comes in a few morphologic appearances, the macrovesicular terminology is used when large lipid droplets inhabit the cytoplasm and displace the nucleus[90]. However, macrovesicular steatosis also encompasses small lipid droplets, which varying in size and keep their nuclear central location[90]. Finally, the terminology of microvesicular steatosis denotes the accumulation of innumerable lipid droplets with the hepatocyte nucleus remaining essentially in its original location[90,91]. It is important to note that microvesicular steatosis is rare in isolation but has been reported to occur in a patchy distribution (approximately 10% of NAFLD cases)[90,91]. With that being said the presence of pure microvesicular steatosis has been reported somewhat more commonly in the diagnosis of alcoholic fatty liver disease (so-named alcoholic foamy degeneration)[92]. As was alluded to earlier in this paper,

lipid is a dynamic and metabolically active substance and the same holds true for fatty lipid droplets in the liver. Lipid droplets are comprised of a core of triacylglycerols with or without cholesterol esters and a peripheral monolayer of phospholipids[93]. Inactive PNPLA3 has been shown to accumulate on the surface of lipid droplets and is linked to an increase in macrovesicular steatosis[94]. Recent studies have espoused that the loss of reticulin seen in those patients with extensive steatosis may not be related to the presence of inflammation or fibrosis; the effects of such a loss in connective framework has yet to be determined, however, this finding should be remembered when HCC enters the differential diagnosis[95].

Assessment of the extent of steatosis

With the starting point of at least 5% steatotic involvement being pathologic, the affected parenchyma is then divided into thirds: 5%-33%, 34%-66% and > 66%[96]. The rule of thirds has allowed a three-tiered classification system with 5%-33% designated as mild, 34%-66% designated as moderate, and > 66% corresponding to severe steatosis[96]. Steatosis, when not in abundance, is typically centered in a zone 3 distribution but when prominent can be found in a panacinar location[90]. In a patient who has resolving hepatic steatosis, the fat droplets can be found in an irregular distribution throughout the acinus[90]. In a more rare occurrence, the steatosis may be found in a zone 1 location with disease progression to cirrhosis leading to a more irregular distribution or complete loss of steatotic droplets[90]. There has been a documented tendency to overestimate the degree by which the liver parenchyma is involved by steatosis among pathologists, hence more accurate and objective methods have employed the use of digital imagining analysis[97]. It is important to point out that conventional imaging (ultrasound, computed tomography, or magnetic resonance imaging), are not sensitive enough to detect hepatic steatosis when the percent involvement is less than 30%[91]. More advanced imaging techniques such as controlled attenuation parameter, magnetic resonance imaging-estimated proton density fat fraction, and ^{1}H-magnetic resonance spectroscopy have been shown to correlate well with histologic steatosis assessment in both the adult and pediatric NAFLD populations[98,99].

Steatosis with inflammation and/or fibrosis

In the realm of NAFLD, steatosis rarely is identified as the only finding and is oftentimes accompanied by a chronic inflammatory infiltrate (typically mononuclear) with varied severity, few plasma cells and monocytes may also be encountered[91]. Neutrophils make a rare appearance with occasional eosinophils in the presence of a lipogranuloma (a structure composed of a central steatotic hepatocyte or fat droplet and a peripheral accumulation of mononuclear cells and macrophages)[91]. Kupffer cell density in NAFLD has correlated with the degree of necroinflammatory activity, injury, and degree of fibrosis[100]. In fact, it is the Kupffer cell that is believed to play a commanding role in the pathogenesis of NAFLD with its regulation of hepatic triglyceride storage, mediation of inflammatory activity, and hepatocyte injury to include parenchymal fibrosis[100]. In the strictest and most traditional of viewpoints of NAFLD, the presence of hepatocyte injury and fibrosis were thought to be a product of disease progression to steatohepatitis[89]. However, some mild NAFLD cases encountered in adults have shown a very mild degree of fibrosis, mainly centered on the portal area or occasionally zone 3[91]. A note of clarification is in order due to some confusion which may occur with NASH. In NASH, most experts would agree that the most basic criteria of hepatocyte ballooning in addition to steatosis and inflammation must be met in order to render a diagnosis of NASH[88,101]. It is, as of yet, still unclear whether these patients with NAFLD (*i.e.,* not NASH) and a mild component of inflammation/fibrosis have as benign of a course when compared with those who have steatosis alone[90]. Conflicting reports on progression are found in the literature with some suggesting that these cases may evolve to more severe disease, typically at a slower rate, while others have shown these lesions may stabilize or regress[102,103].

Steatohepatitis

Ballooned hepatocytes with accompanied steatosis and inflammation are typically found in zone 3 of the hepatic microanatomy[91]. Some recent work using immunohistochemistry, specifically CK8/18, have shown that ballooned hepatocytes display significantly decreased expression compared to normal hepatocytes[90]. As it currently stands, the use of immunohistochemical stains for differentiating ballooned hepatocytes is not currently a common practice[90]. Although the exact mechanisms by which a hepatocyte takes on a ballooned appearance are not entirely elucidated, some proposed mechanisms include: Oxidative stress alteration of microtubules, loss of intermediate filament cytoskeleton, retention of fluid, modifications to small droplet fat and endoplasmic reticulum dilatation[104-108]. Mallory-Denk bodies, glycogenated nuclei, acinar lipogranulomas, megamitochondria, pericellular fibrosis, and acidophilic bodies are frequently seen in NASH, but are not required for the diagnosis[101]. Ductular reaction can be seen in NASH as well and is usually associated with fibrosis[90]. It is important to keep in mind that no single feature is entirely specific for the diagnosis of NASH[91].

Fibrosis

The impact of fibrosis cannot be overstated when discussing NAFL/NASH. In fact, literature has shown a substantial impact regarding the stage of fibrosis and overall morality[90]. Fibrosis, when seen in NAFLD, has a characteristic appearance with early lesions showing a perisinusoidal deposition in zone 3[90]. Collagen fibers may be seen to encircle hepatocytes with more progressed lesions[90]. Additionally, pericellular fibrosis has been shown to progress without any appreciable periportal

fibrosis[90]. Periportal fibrosis develops after the perisinusoidal fibrosis and is demonstrated as trapping of hepatocytes around the portal area and extension of short strands of collagen into the parenchyma. Bridging fibrosis may eventually form single bands between the portal area and central vein without hepatocyte trapping or island formation. Evidence suggests that portal fibrosis in association with pericentral fibrosis is a necessary component for bridging fibrosis to develop[90]. Masson trichrome stain can highlight the fibrosis and are useful in identifying early fibrosis of steatohepatitis. Of note, NASH may retain all of the active steatohepatitis changes but the steatosis may decrease below the 5% level. On the other hand, the active steatohepatitis changes may disappear in cirrhosis as well, resulting in a diagnosis of "cryptogenic cirrhosis"[109].

HCC: Steatohepatitic variant

In the United States HCC has increased by 80% in the last twenty years with HCC being the fifth most common malignancy worldwide and the third most common cause of cancer-related death[110,111]. Hepatitis B and C, alcoholic liver disease, hemochromatosis, and several others represent the mainstay of risk factors for the development of HCC; recent studies have reported NAFLD to be an underlying cause of HCC in a number of cases even in the absence of cirrhosis[112-116]. A new variant of HCC has been described, that is the steatohepatitic variant of HCC, which is reminiscent of steatohepatitis (inflammation, hepatocyte ballooning, Mallory-Denk bodies, and pericellular fibrosis), and was first seen in a population of patients with HCV-related HCCs[117]. In one study, examining 118 cases of HCC over a 3.5 year period, 13.5% represented the steatohepatitic HCC with all but one case occurring in patients with underlying steatohepatitis[116]. When examining patient characteristics, the steatohepatitic HCC variant patients showed higher numbers of metabolic syndrome risk factors as well as at least 3 components of metabolic syndrome[116]. In a separate study, Jain *et al*[118] found the steatohepatitic variant of HCC (SH-HCC) in approximately 19% of their cases over a period of 7 years, with 50% of those cases being seen in NAFLD patients and the other 50% were largely of HCV etiology. It is important to note, in a study performed by Yeh *et al*[119], that SH-HCC can occur outside the morphology of that seen in fatty liver disease or metabolic syndrome and was posited to be more likely attributable to genetic changes of shared genes or metabolic pathways. Yeh *et al*[119] also found a loss of 9q12-q31.1 in a subset of cases, in this regard more investigation needs to be done to further ascertain the molecular driver for such a morphologic variant.

Pediatric NAFLD histology

The main histological differences seen in some pediatric NAFLD when compared to adults has been the distribution of hepatocyte lipid droplets, inflammation and fibrosis location[120]. In some pediatric patients with NAFLD, the lipid vacuoles are largest in the periportal hepatocytes and tend to decrease in diameter in pericentral area (zone 3). Similarly, inflammation and fibrosis is also seen around the portal tract (that is zone 1 predominance opposed to zone 3). When bridging fibrosis develops, the bridges connect portal to portal areas, leaving the central veins alone[120]. However, these features are not specific for pediatric NAFLD and many cases have similar picture as that of adult NAFLD.

Grading and staging in NAFLD/NASH

In order to provide a consistent and reproducible assessment of NAFLD, the evaluation of morphological features must be semiquantified *via* an agreed upon scoring system to guide clinical decision making and for use in clinical trials[96,121-124]. Three histological scoring systems are currently in place: NASH clinical research network's NAFLD activity score (NASH CRN-NAS), steatosis, activity, and fibrosis (SAF), and the Brunt staging system[96,121,124]. The NAS uses numerical scores (Table 1) to develop an activity grade, which includes steatosis (0-3 points), hepatocellular ballooning (0-2 points), and acinar inflammation (0-3 points), as well as a separate fibrosis stage (0-4)[121]. Using a threshold of < 3 (activity score), the NAS showed a good correlation with the absence of a histological diagnosis of NASH[121]. Likewise, using a threshold of greater than or equal to 5, the NAS showed good correlation with having a diagnosis of NASH[121]. In validation by Hjelkrem *et al*[125], a total of 386 liver biopsies were evaluated, the sensitivity and specificity were 57% and 95%, respectively, when using a NAS $\geqslant$ 5 (indicating NASH) and NAS < 5 (indicating no NASH). When using an activity score of $\geqslant$ 4, the sensitivity increased to 85% with a slight decrease in specificity to 81%[125]. The $\geqslant$ 4 threshold has been recommended for any admission to an interventional trial for NASH[125]. In contrast, the SAF scoring algorithm (Table 2) was originally intended for the grading and staging of NAFLD in those patients who were morbidly obese about to undergo bariatric surgery[124]. Since then it has been used in patients with metabolic syndrome and concomitant NAFLD[91]. When using the SAF scoring system, the activity score (consisting of ballooning and lobular inflammation), enabled the discrimination of NASH (NASH patients had A > 2, whereas no patients with an A < 2 had NASH)[124]. Finally, the Brunt system uses a three tiered grading system (mild, moderate, and severe) with three parameters under histological investigation: Steatosis, ballooning, and inflammation (Table 3)[96]. The Brunt system also uses a four tiered staging system based on the location and degree of fibrosis (Table 3)[96]. It should be noted that regardless of every effort to devise a scoring system that is standardized and highly reproducible, the classification of NAFLD will always be plagued by observer bias and a lack of complexity which would be necessary to describe an intricate disease process[91].

DIFFERENTIAL DIAGNOSIS

As would be intuitive by the name of the disease, non-

Table 1 Non-alcoholic fatty liver disease activity scoring system[121]

Steatosis, grade (0-3)	
< 5%	0
5%-33%	1
34%-66%	2
> 66%	3
Lobular inflammation	
No foci	0
< 2 foci per 200 × field	1
2-4 foci per 200 × field	2
> 4 foci per 200 × field	3
Hepatocyte ballooning	
None	0
Few balloon cells	1
Many cells/prominent ballooning	2
Fibrosis stage	
None	0
Perisinusoidal or periportal	1
Mild, zone 3, perisinusoidal	1A
Moderate, zone 3, perisinusoidal	1B
Portal/periportal	1C
Perisinusoidal and portal/periportal	2
Bridging fibrosis	3
Cirrhosis	4

Table 2 Steatosis, activity, and fibrosis scoring system[91,124]

Steatosis score (S): Assessed the quantities of large or medium-sized lipid droplets (0-3)
S0: < 5%
S1: 5%-33%
S2: 34%-66%
S3: > 67%
Activity grade (0-4): Sum of scores for ballooning and lobular inflammation
A1: Mild activity
A2: Moderate activity
A3 and A4: Severe activity
Hepatocyte ballooning (0-2)
0: None
1: Foci of hepatocytes with rounded shape, pale or reticulated cytoplasm
2: Foci of hepatocytes with rounded shape, pale or reticulated cytoplasm and enlargement (> 2 × normal size)
Lobular inflammation (0-2)
0: None
1: < 2 foci per 20 × field
2: > 2 foci per 20 × field
Fibrosis stage (F)
F0: No relevant fibrosis
F1: 1a - mild zone 3 perisinusoidal fibrosis
1b - moderate zone 3 perisinusoidal fibrosis
1c - portal fibrosis
F2: Zone 3 perisinusoidal fibrosis with periportal fibrosis
F3: Bridging fibrosis
F4: Cirrhosis

alcoholic fatty liver disease/non-alcoholic steatohepatitis, the presence of alcohol driving these changes must be ruled out. However, many other disease settings are associated with liver injury which may resemble histological changes that are typically observed in NAFLD/NASH[91]. One category that may mimic NAFLD/NASH is termed chemotherapy (CASH)- or drug-associated steatohepatitis[91,126-128].

Alcoholic steatosis, alcoholic steatohepatitis, alcoholic cirrhosis and HCC are the entities that a patient may develop with chronic alcohol use and abuse[129]. The distinction of alcoholic liver disease (ALD) and NASH can simply be made by delving into the history for the affirmation of alcohol use; however, there are histologic features that may help differentiate one form over the other in the absence of being able to obtain a detailed history (Table 4)[129]. The diagnostic criteria for rendering an ALD diagnosis rests on evidence of liver injury and a reported history of alcohol intake[101]. The amount of alcohol ingested is the strongest predictor of ALD development; just 60 g/d of alcohol consumed leads to the develop fatty liver in more than 90% of individuals[130]. In fact, the risk of developing alcohol related cirrhosis increases greatly with consumption of > 60-80 g/d for more 10 years in men, and > 20 g/d in women[130].

There has been a rapid increase in the number of novel cytotoxic chemotherapeutic agents over the last few years and with the liver's role of drug metabolism it is not surprising that these drugs wreak havoc and produce hepatic injury[131]. Hepatotoxicity is neither predictable nor dose-dependent with most drug reactions occurring in an idiosyncratic manner[132]. Drug induced hepatic steatosis is a fairly rare event with several drugs/classes implicated: Methotrexate, amiodarone, tetracycline, glucocorticoids, tamoxifen, chemotherapeutics, and nucleoside analogues to name a few[133]. Drug-induced hepatic steatosis is thought to result from the exuberant accumulation of intracellular phospholipids due in part by a drug therapy that has lasted several weeks to months[133]. Mechanistically, drug-related hepatic injury is due in part to mitochondrial toxicity resulting in inhibition of beta oxidation, oxidative phosphorylation, and mitochondrial respiration[134]. Since beta oxidation is one of the main ways lipids are metabolized, drug induced inhibition results in the accumulation lipids within the hepatocytes[134]. The steatosis that occurs in the setting of drug/chemotherapeutic treatment often resembles that seen in NAFLD with several notable exceptions[91].

As previously outlined, the prevalence of NAFLD is growing and expanding, which allows the likely overlap of this disease with a concurrent disease, specifically: Chronic hepatitis B, chronic hepatitis C, human immunodeficiency virus, autoimmune hepatitis, biliary diseases, or other inherited metabolic disturbances[135-141]. In fact it has been reported that half of patients with human immunodeficiency virus (HIV) who undergo testing for liver test aberrations have concurrent NAFLD, which can result from HIV itself or the HAART therapy used in treatment[138]. In terms of autoimmune hepatitis, routine autoantibodies are present in NAFLD patients 23% of the time, necessitating the need for a liver biopsy for differentiation[139,140]. When looking at virally infected livers, specifically by HCV, hepatic steatosis has been reported in approximately 40%-85% of infected patients[142]. HCV is interesting in terms of its two pathway approach to liver steatosis: Viral and non-viral[142]. HCV, especially genotype 3a, has

Table 3 Brunt grading and staging of nonalcoholic steatohepatitis[96]

Grading	Staging
Mild (Grade 1)	Stage 1
Steatosis (mostly macrovesicular)	Zone 3 perisinusoidal/pericellular fibrosis (focal or extensive)
Involves up to 66% of biopsy	
Occasional ballooned zone 3 hepatocytes	Stage 2
Scattered rare intra-acinar neutrophils with/without associated lymphocytes	Zone 3 perisinusoidal/pericellular fibrosis with associated focal
No/mild portal chronic inflammation	or extensive periportal fibrosis
Moderate (Grade 2)	
Steatosis-any degree	
Ballooning hepatocytes-zone 3	
Intra-acinar neutrophils-may be associated with zone 3 pericellular fibrosis	Stage 3
Portal and intra-acinar chronic inflammation	Zone 3 perisinusoidal/pericellular fibrosis and portal fibrosis
Severe (Grade 3)	with associated focal or extensive bridging fibrosis
Panacinar steatosis	
Ballooning-zone 3	
Intra-acinar inflammation with scattered neutrophils	Stage 4
Neutrophils associated with ballooned hepatocytes with/without chronic inflammation	Cirrhosis
Chronic portal inflammation-mild or moderate	

Table 4 Histologic comparison of non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and alcoholic liver disease[129]

Characteristic	NAFLD and NASH	Alcoholic liver disease
Disease severity	Mild	Varying
Mallory-Denk body	Poorly formed	Well formed
Glycogenated nuclei	Common	Less common
Ductular proliferation	Less prominent	More prominent
Fibrosis/cirrhosis	Less common	More common
Sclerosing hyaline necrosis	None/rare	Present
Phlebosclerosis	None/rare	Present
Canalicular cholestasis	None/rare	Present
Foamy degeneration	None/rare	Present

NASH: Non-alcoholic steatohepatitis; NAFLD: Non-alcoholic fatty liver disease.

been reported to up-regulate the expression of fatty acid synthase in infected hepatocytes leading to increased fatty acids, impaired beta oxidation and reduced export of triglycerides[143]. As a part of its pathogenesis, HCV causes the inhibition of the microsomal triglyceride transfer protein, which is involved in the release of triglycerides from hepatocytes and as a consequence leads to triglyceride accumulation[142]. The non-viral approach to liver steatosis is typified by interference of insulin signaling resulting in insulin resistance[142]. The mode by which hepatitis B virus (HBV) causes hepatic steatosis is not entirely agreed upon[142]. It is postulated that HBV X protein may lead to lipid accumulation in hepatocytes with inhibition of apolipoprotein B secretion while at the same time PPARgamma and SREBP-1c activation with resultant nuclear factor-kappa B activation and TNF production[144].

PROGNOSIS, PROGRESSION AND CLINICAL COURSE

Numerous studies have tracked the progression of steatosis, steatohepatitis, and fibrosis in NAFLD patients through paired liver biopsies[103,145-151]. Wong *et al*[145], *via* a prospective longitudinal hospital based cohort study, found that of patients with simple steatosis, 39% developed a borderline NASH picture and 23% developed full blown NASH. In another study, totaling 108 patients (81 with NASH and 27 with NAFL), 42% had fibrosis progression, 40% had no change in fibrosis, and 18% had fibrosis regression[103]. Interestingly, 22% of patients with NAFL at baseline developed stage 3 fibrosis at follow-up biopsy (median biopsy interval 6.6 years, range of 1.3-22.6)[103]. Overall, when evaluating the bulk of progression data it appears as though 33% of patients with NAFL and NASH will progress to fibrosis and up to 20% may have some regression of their disease[3]. Progressive fibrosis in NASH has been shown to be as high as 2 times that of NAFL and some patients with NASH and NAFL may progress rapidly from no fibrosis to severe fibrosis over the course of several years[102]. Clinically, cirrhosis and liver decompensation in NAFLD patients has been shown to be on the order of 3.1% over a mean 7.6 years[152]. The development of complications, specifically portal hypertension, with the development of cirrhosis is 17% (at one year), 23% (at three years), and 52% (at 10 years)[153]. A median survival of two years is seen in those patients with NASH who have experienced decompensation[154].

Several investigations have found that men, postmenopausal women, those who underwent early menopause, and duration of menopause have an increased chance of fibrosis[155,156]. Although Hispanic patients have an increase prevalence of NAFLD, this feature does not seem to confer an increased risk of progression of their disease[57,157]. In contrast, Asian patients have been shown in some studies to have a more severe histologic picture[14]. Single nucleotide polymorphisms, namely, PNPLA3 rs738409 and rs58542926 are associated with severe histology to include NASH and cirrhosis[158,159]. Although increasing age is shown to be prone for the development of more severe fibrosis in NASH, it is

unclear whether this finding just underscores the fact that these patients have cumulative metabolic insults and a longer duration of disease exposure[160]. Additionally, higher rates of fibrosis progression have been seen in diabetics, those who are obese, hypertension (although several studies looking at NASH patients found no increased risk of progression due to hypertension), and degree of inflammation found on biopsy[102,103,145,147,161].

In studies where biopsies were taken at the time of bariatric surgery and after subsequent weight loss, changes in hepatic histology were reported to improve[162,163]. However, some degree of worsening of either the fibrosis or steatosis has also been documented[164]. In an extreme case, one patient was reported to progress from mild fatty change before surgery to severe NASH and death due to liver failure[165]. The obvious mechanisms by which bariatric surgery improved the features of NAFLD would be related to weight loss, improvements in T2DM, reduced insulin resistance, reduced hyperlipidemia, and improved components of metabolic syndrome[162]. Other proposed mechanisms would include the altered route of food delivery, which results in changes to the release of gut and pancreatic hormones, changes in fat distribution, hepatic insulin and free fatty acid metabolism, and changes in adipocytokines and other cytokines[166]. These alterations in hormone secretion affect carbohydrate and lipid metabolism and interfere with hepatic glucose release[166]. Changes in gene expression may also play a pivotal role. In a study of 28 severely obese participants, PNPLA3 expression was measured by rtPCR before and after gastric banding-induced weight loss with the results showing a restoration of PNPLA3 expression in adipose tissue, but not in liver specimens[167].

A study, evaluating NASH and steatosis improvement by weight loss, found that NASH resolution was obtained in 25% and NAS score improvement was seen in 47% of participants[168]. Likewise, 48% had improvement of their steatosis, 39% reduced the ballooning hepatocyte score and 50% showed improved lobular inflammation[168]. In terms of fibrosis, 65% had no change, 19% showed improvement, and 16% progressed[168]. Not altogether surprising, those participants who had the greatest weight loss also showed the most improvement of their histologic endpoint[168]. In another study with 180 participants, those who showed weight reduction had a 18.37-fold increase in the odds of NAFLD resolution[169]. One recommendation is a weight loss of at least 5% to decrease the burden of steatosis and 10% weight reduction to have an effect on liver necroinflammation[170].

Investigations have proposed a link between metabolic syndrome, T2DM, obesity and the development of HCC[171,172]. NAFLD, even in the absence of fibrosis, provides a nurturing environment for the development of HCC with insulin resistance and steatosis providing the inflammation, adipokines, oxidative stress, and lipotoxicity needed for hepatocellular carcinogenesis[172,173]. In a study examining 1500 American veterans, NASH was found to be the third most common risk factor for the development of HCC[174]. With that being said, the appearance of HCC is relatively rare in NAFLD, on the order of 0.2% (after eight year follow-up); however, the development of HCC in NASH cirrhosis ranges from 2.4% and 12.8% over a 3.2 and 7.2-year period, respectively[175,176]. In fact, once HCC develops in these cirrhotic patients their survival appears to be shorter than that seen in patients with HCV induced HCC[114].

DIAGNOSIS, TREATMENT AND SCREENING

Non-alcoholic fatty liver disease, in most instances, represents an incidental diagnosis due to alterations noted on a chemistry profile or when imaging for other purposes finds a steatosis pattern in the liver[9]. In the absence of incidental discovery, often patients are asymptomatic until liver decompensation occurs; however, if the evaluation of the patient reveals such factors as insulin resistance, obesity, or factors associated with metabolic syndrome, the diagnosis can be achieved much earlier than decompensation[9]. In the physical evaluation of the patient, BMI and visceral adiposity are helpful clues to the possible presence of NAFLD; however, in lean patients the diagnosis becomes much more challenging[9]. Screening of patients who are at risk for the development of NAFLD seems to be a worthy undertaking, but liver function tests can be in the normal range in patients with NAFLD/NASH and ultrasound is too expensive and burdensome for use in screening large portions of a population (although it is a good starting point when suspicion is high)[177]. The diagnosis of NAFLD is a four-pronged approach (Table 5): (1) hepatic steatosis (*via* imaging or histology); (2) alcohol consumption is ruled out; (3) there are no rival etiologies; and (4) no other causes for chronic liver disease are identified[177]. The entities discussed in the differential diagnosis section of this paper should be ruled out, namely, alcohol use, chronic hepatitis B and C, medication use, parenteral nutrition, Wilson's disease, biliary disease, autoimmune hepatitis, and malnutrition to name a few of the major considerations. Although mild elevations in serum ferritin can be seen in NAFLD, marked increases should be worked-up for hemochromatosis and *HFE* gene mutations (*i.e.,* C282Y)[177]. As mentioned previously, NAFLD patients may have elevations in serum autoantibodies; however, increased serum autoantibodies in the presence of features to suggest an autoimmune liver disease should result in a more complete work-up for autoimmune disease/autoimmune liver disease[177]. Biomarker development in NAFLD has been a topic of great interest and research. Numerous potential biomarkers have been investigated, for example, cytokeratin 18 fragments were evaluated in potential NAFLD patients at the time of liver biopsy and then correlated with histologic findings[178]. In this study, CK18 fragments found in the plasma showed a significant ($P < 0.001$) and marked increase in patients with NASH when compared with those having steatosis

Table 5 Factors to be assessed in the evaluation of a patient with suspected non-alcoholic fatty liver disease[32]

Factor
Personal and family history of diabetes, hypertension and CVD
Alcohol use: < 20 g/d (women), < 30 g/d (man)
Waist circumference, BMI, change in body weight
Hepatitis B/C infection
Liver enzymes
History of steatosis-associated drug use
Fast blood glucose, hemoglobin A1c
Serum total and HDL-cholesterol, triacylglycerol, uric acid
Undertaken due to clinical suspicion
Ultrasound
Hemochromatosis testing: Ferritin and transferrin saturation
Celiac disease: IgA and tissue transglutaminase
Thyroid disease: TSH level (T3/T4)
Polycystic ovarian syndrome
Wilson's disease: Ceruloplasmin
Autoimmune disease: ANA, AMA, SMA
Alpha-1 antitrypsin deficiency: Alpha-1-antitrypsin level

ANA: Anti-nuclear antibody; AMA: Anti-mitochondrial antibody; SMA: Anti-smooth muscle antibody; CVD: Cardiovascular disease; BMI: Body mass index; HDL: High density lipoprotein; TSH: Thyroid stimulating hormone.

or normal findings (median 765.7 U/L *vs* 202.4 U/L *vs* 215.5 U/L, respectively)[178]. These findings were further investigated by several subsequent studies and a meta-analysis revealed CK18 fragment levels to have a sensitivity and specificity of 78% and 87%, respectively, for steatohepatitis in those with NAFLD[179]. Other studies have offered insight into miRNAs as a biomarker for NAFLD and HCC spectrum; however, more investigation is needed to determine its true place in the diagnostic algorithm of NAFLD[180]. Extracellular vesicles shed from the liver have also caught the attention of many investigators and they are being actively researched for a possible role in NAFLD detection[181].

Perhaps the most important treatment option, lifestyle modification (to include diet and exercise), as well as surgical interventions for the treatment of NAFLD have already been discussed. Medications and supplements are also part of the treatment consideration when dealing with NAFLD. Hence, there are four main pathways currently available in the treatment of NAFLD. First, targeting hepatic fat accumulation (pioglitazone, elafibranor, saroglitazar), bile acid-farnesoid X receptor axis (obeticholic acid), *de novo* lipogenesis inhibitors (aramchol, NDI-010976), incretins (liraglutide) and fibroblast growth factor FGF-21 or FGF-19 analogues[182]. Second, oxidative stress alle*via*tion through the use of antioxidants and medications that target the tumor necrosis factor alpha pathway (emricasan, pentoxyifylline) as well as immune modulators (amlexanox, cenicriviroc)[182]. Third, antiobesity medications such as orlistat and finally antifibrotics (simtuzumab and GR-MD-02) will be important players in therapeutic management of NAFLD[182]. Insulin resistance, as a major player in the pathogenesis of NAFLD, is an obvious target of therapeutic intervention by way of insulin sensitizing agents[177]. With that being said, several studies have looked at the effects of metformin on liver function test levels and histology in those with NASH. In initial work, use of metformin showed a reduction in insulin resistance and aminotransferase levels; however, no changes were noted in the participants liver histology[183,184]. A meta-analysis found that in combination with lifestyle changes, metformin did not improve liver function test profiles or liver histology compared with lifestyle modification alone[177]. Although some evidence exists of NASH's histological improvement by metformin intervention (study confounded by weight loss), the current AASLD practice guideline recommendation is not to use metformin for the specific treatment of liver disease in adults with NASH[177,185]. The thiazolidinediones (TZDs), specifically pioglitazone, was shown in meta-analysis to improve steatosis and inflammation but not fibrosis with the caveat that TZDs long term safety profile is still under investigation[177]. The current recommendation, according to the AASLD Practice Guideline for NAFLD, Pioglitazone can be used in the treatment of steatohepatitis in those who have biopsy confirmed NASH with the understanding that trials were conducted in NASH patients without diabetes[177]. Vitamin E, an anti-oxidant, has been investigated for use in the treatment of NASH as oxidative stress is considered to be a major player in hepatocyte injury and disease progression[186,187]. Several studies have produced data to suggest that the use of vitamin E leads to improved steatosis, reduced inflammation and ballooning, decreased liver function test values, resolution of steatohepatitis with no effect on hepatic fibrosis[177]. However, concerns over the use of vitamin E and associated increases in all-cause mortality and an increased risk of prostate cancer in men have been raised[188,189]. As it currently stands, vitamin E should be considered in the therapeutic regimen of patients with biopsy proven NASH who also are non-diabetics[177]. Other therapies such as Pentoxifylline (shown to improve hepatic steatosis with no effect on insulin resistance), obeticholic acid (improves insulin resistance, hepatic steatosis, hepatic inflammation, and hepatic fibrosis), Orlistat (improves insulin resistance), ursodeoxycholic acid (improves insulin resistance and hepatic steatosis), Statins (improves hepatic steatosis), and Omega-3 (improves hepatic steatosis), and glucagon-like peptide 1 receptor agonists (improves hepatic steatosis) have been investigated and have shown varying and often limited benefit[190]. Finally, up and coming agents to be aware of: PPARα/δ agonists, chemokine receptor (CCR)2/CCR5 antagonists and numerous fatty acid/bile acid conjugates and antifibrotic agents are being investigated for use in NASH and the results of these studies/trials will reveal what benefit if any they will have on the NALFD landscape[32].

According to the most recent American College of Gastroenterology and American Gastroenterological Association guidelines, the screening of adults in primary care clinics or high-risk groups (*i.e.,* those attending diabetes or obesity clinics) for NAFLD is not recommended and the

systematic screening of family members for NAFLD is also discouraged[191]. This due to the lack of evidence or current understanding regarding the long-term benefits and cost effectiveness of screening and the current uncertainties related to diagnostic tests and treatment options[191]. However, other screening guidelines suggest the implementation of a screening policy in those who are at high risk for NAFLD identified by the presence of metabolic risk factors and/or IR[191].

CONCLUSION

NAFLD is a growing epidemic, not only in the United States, but worldwide in part due to obesity and insulin resistance leading to liver accumulation of triglycerides and free fatty acids. Liver steatosis may be innocuous in most occasions but the progression and development of fibrosis is not and often heralds a poor prognosis. Numerous risk factors for the development of NAFLD have been espoused with most having some form of metabolic derangement or insulin resistance at the core of its pathophysiology. Additionally, access and decreasing cost for high quality and powered genetic scrutiny will no doubt provide future clinicians with a great deal of information and opportunity for enhanced targeted treatment. The same can be said for the development of advanced imaging and biochemical tests. As it currently stands several medications/supplements may be used in the treatment of NAFLD; however, none seem to be the "magic bullet" in curtailing this growing problem. Not enough can be said about the importance of lifestyle coupled with proper diet and appropriate exercise in the defense of developing NAFLD.

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P- Reviewer: Borzio M, Fan XM, Marchesini GM, Sinakos E, Zheng SJ
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