Recent advances in understanding/management of non-alcoholic steatohepatitis

Tommy Pacana and Arun J. Sanyal*

Address: Division of Gastroenterology, Hepatology and Nutrition, Department of Internal Medicine, Virginia Commonwealth University School of Medicine, Richmond, VA 23298, USA

* Corresponding author: Arun J. Sanyal (asanyal@mcvh-vcu.edu)

Abstract

Non-alcoholic steatohepatitis (NASH) can lead to advanced fibrosis, hepatocellular carcinoma, and end-stage liver disease requiring liver transplantation. A myriad of pathways and genetic influence contribute to NASH pathogenesis and liver disease progression. Diagnosing patients with NASH and advanced fibrosis is critical prior to treatment and prognostication. There has been ongoing interest in developing non-invasive biomarkers and tools for identifying NASH and advanced fibrosis. To date, there has been no approved therapy for NASH. Recently, the FLINT (Farnesoid X Receptor [FXR] Ligand Obeticholic Acid in NASH Treatment) trial provided promising results of the efficacy of obeticholic acid, a farnesoid X receptor agonist, in improving histological features of NASH and fibrosis. Long-term studies are needed to assess the safety of obeticholic acid and its effects on liver- and cardiovascular-related outcomes.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in North America and has emerged as a serious public health burden. The clinical-histological phenotype of NAFLD extends from non-alcoholic fatty liver (NAFL) to NASH. The estimated worldwide pre-valences of NAFLD and NASH range from 6.3%-33% and 3%-5%, respectively [1,2]. Whereas NAFL can progress to cirrhosis in 2% to 3%, NASH has an increased risk for the progression to cirrhosis at 15% to 20% and predisposes patients to the development of hepatocellular carcinoma and increased mortality [3,4]. Although extensive research in understanding the disease pathogenesis and numerous clinical trials aimed at halting disease progression have been performed in the last two decades, optimal therapy is still lacking. The aims of this article are to provide a short overview of important mechanisms and genetic factors influencing NAFLD disease progression and to present strategies in the diagnostic and therapeutic management of NASH.

Pathogenesis of non-alcoholic steatohepatitis

The histological hallmark of NAFLD is hepatic steatosis. Dysfunctional adipocytes secrete cytokines and chemokines, and this perpetuating inflammatory cycle induces a state of insulin resistance, resulting in failure to suppress lipolysis in the adipocytes [5]. This leads to release of free fatty acids (FFAs) to the circulation and is followed by uptake of these FFAs to the liver. A carbohydrate-rich diet and hyperinsulinemia activate carbohydrate-related element-binding protein (ChREBP) and sterol regulatory element-binding protein-1 (SREBP-1), respectively, and promote hepatic *de novo* lipogenesis [6]. The imbalance between triglyceride acquisition and removal results in excess triglycerides stored as lipid droplets.

Several mechanisms have been implicated in the progression from steatosis to NASH. Key players include lipotoxicity, oxidative stress, endoplasmic reticulum (ER) stress, activation of the innate immune activity, and cytokine-mediated cellular damage. It has been demonstrated that triglyceride synthesis is not harmful but protective against

fatty acid-induced lipotoxicity [7]. It is known that FFAs can directly activate inflammatory pathways, ER stress, and the innate immune system via Toll-like receptors [8]. Recently, many aspects of lipid metabolism were reported to be altered in NAFLD [9]. The presence of biologically active lipid molecules such as free cholesterol, diacylglycerol, lysophosphatidylcholine, and ceramides can affect lipogenesis, insulin signaling, and cellular injury and contribute to NAFLD phenotype and disease progression [10–12]. Once the capacity of the liver to store triglycerides is overwhelmed, there is increased compensation by the mitochondria and peroxisomes to oxidize fatty acids. The oxidative capacity of these organelles becomes impaired and can lead to overproduction of reactive oxygen species [13]. The consequent cytokine production and lipid peroxidation have the potential to induce apoptosis, inflammation, and liver fibrosis. In response to ER stress, the unfolded protein response is activated to restore homeostasis as an adaptive mechanism [14]. However, pathways leading to apoptosis are initiated if homeostasis is compromised. Moreover, the gut microbiome has been more recognized to play an important role in the pathogenesis of NASH. Changes in the microbiota can alter intestinal permeability and promote translocation of microbes into the portal circulation. The gut-derived microbial products, including lipopolysaccharide and bacterial DNA, can enter the liver and induce inflammation by activating Toll-like receptors in Kupffer cells and hepatocytes [15]. In addition, the innate immune system is activated in the adipocytes, and the release of various adipokines (interleukin-6 and tumor necrosis factor-alpha) can contribute to hepatic inflammation [16]. Combined with the host genetic background, the complex interplay of these mechanisms can produce inflammation, hepatocellular injury, and cell death and activate fibrogenesis.

Genetic variants in non-alcoholic fatty liver disease

Phenotypic variations in NAFLD with similar risk factors implicate a genetic contribution. The genetic susceptibility was first demonstrated by the first genome-wide association study (GWAS) on NAFLD in a population of Hispanic, African American, and European American individuals [17]. Variations in palatin-like phospholipase domain-containing 3 (PNPLA3) were shown to influence ancestry-related and inter-individual difference in hepatic fat content and susceptibility to NAFLD. Specifically, the allele rs738409 encoding for the isoleucine-to-methionine variant at protein position 148 (I148M) was strongly linked to hepatic fat accumulation and inflammation. This finding was replicated in several studies across different populations and found to be associated with NAFLD disease severity [18–24].

Through a similar GWAS strategy, other single-nucleotide polymorphisms (SNPs) were identified and associated with various aspects of NAFLD phenotype [25]. Variants near PNPLA3, neurocan (NCAN), and protein phosphatase 1, regulatory (inhibitor) subunit 3B (PPP1R3B) were associated with computed tomography-measured hepatic steatosis from several large-population studies. These SNPs were subsequently genotyped in biopsy-proven NAFLD from the NASH Clinical Research Network and showed that variants in or near NCAN, glucokinase regulator (GCKR), lysophospholipase-like 1 (LYPLAL1), and PNPLA3, but not PPP1R3B, were associated with histologic lobular inflammation or fibrosis or both. These variants have distinct effects on various metabolic pathways indicative of genetic heterogeneity in NAFLD pathogenesis. More recently, Kozlitina and colleagues [26] reported the association of a transmembrane 6 superfamily member 2 (TM6SF2) variant with hepatic triglyceride content in an exome-wide association study of patients derived from the Dallas Heart Study. The gene is more common in individuals of European ancestry (7.2%) than in African Americans (3.4%) or Hispanics (4.7%). The TM6SF2 variant encoding p.Glu167Lys correlated with elevated serum alanine aminotransferase (ALT) and reductions in serum alkaline phosphatase and plasma levels of triglycerides and low-density lipoprotein cholesterol in three large cohorts. Although the exact function of the gene is unknown, it has been proposed that the TM6SF2 acts to promote very-low-density lipoprotein secretion. In another study, the TM6SF2 rs58542926 was found to be associated with NASH severity and advanced liver fibrosis [27].

Diagnosis

Whereas NAFL is characterized by the presence of hepatic steatosis with no evidence of hepatocellular injury, NASH is characterized histologically by the presence of ballooned hepatocytes and lobular inflammation with or without perisinusoidal fibrosis in addition to steatosis [28]. Diagnosing NASH and advanced fibrosis is crucial for prognostication and before starting therapy. Although liver biopsy is considered the gold standard to diagnose and stage NASH, it is invasive and has several limitations, including cost and sampling error, and is accompanied by risks (bleeding, pain, perforation, infection, and rarely death) [29,30]. The metabolic syndrome and diabetes are associated with increased risk for NASH and can be used in selecting those patients for liver biopsy [31,32]. The levels of aminotransferases are not reliable, and imaging studies have not been accurate in the diagnosis of NASH and this is because the histologic features of apoptosis, ballooning, and cell injury that characterize NASH are not detectable by currently available modalities.

The poor performance of liver biopsy has led to significant interest in developing non-invasive biomarkers for identifying steatohepatitis or fibrosis (or both) in patients with NAFLD. Multiple biomarkers and predictive models have been suggested to predict NASH but with varying degrees of success, and the majority require external validation [33]. Circulating keratin 18 (CK18), a marker for hepatocyte apoptosis, is the most validated biomarker that is reproduced in several studies for differentiating NASH from simple steatosis [34–37]. In a recent meta-analysis, plasma CK18 levels had a sensitivity of 78%, specificity of 87%, and an area under the receiver operating curve (AUROC) of 0.82 for identifying steatohepatitis. Though fairly specific, this biomarker lacks the sensitivity needed for it to be an adequate screening test to stage NASH [38].

The NAFLD fibrosis score and enhanced liver fibrosis (ELF) panel are non-invasive tools that facilitate the identification of the majority of advanced fibrosis patients but without the need of a liver biopsy [28]. The NAFLD fibrosis score is a scoring tool calculated by incorporating clinical parameters that include age, glycemia, body mass index, platelet count, albumin, and aspartate transaminase-to-ALT ratio. This has been validated in many studies and confirmed by a recent meta-analysis to predict advanced fibrosis with an AUROC of 0.85 [39]. The ELF combines three serum markers of matrix turnover (TIMP 1, hyaluronic acid, and P3NP) and can identify advanced fibrosis in NASH patients with an AUROC of 0.87 [40]. However, both NAFLD fibrosis score and ELF perform poorly in early and intermediate stages.

Modalities that measure liver stiffness were developed to assess liver fibrosis. Transient elastography (FibroScan®) was first evaluated in a Japanese study that reported a stepwise increase in liver stiffness with increased severity of liver fibrosis [41]. A meta-analysis showed a pooled AUROC of 0.94 with 94% sensitivity and 95% specificity for advanced fibrosis [39]. A major drawback of this modality is its inconsistency in obtaining measurements in obese patients [42]. Using a new XL probe has enabled the examination in obese patients with diagnostic accuracy comparable to that of the standard probe [43]. More recently, studies have shown promise of acoustic radiation force impulse (ARFI) sonoelastography for the assessment of liver stiffness [44–46]. Aside from having the capability to be integrated in a standard liver ultrasound, this modality can evaluate large parts of the liver in one examination and is more reliable than transient elastography in obese patients. In a study of 57 patients with NAFLD, ARFI showed a diagnostic

accuracy similar to that of transient elastography for advanced fibrosis when compared with liver histology [45]. A recent meta-analysis of various chronic liver diseases confirmed this finding, although only a small proportion of patients were in the NAFLD group [47]. Therefore, more studies are needed to validate this modality in NAFLD. Another imaging modality is magnetic resonance elastography, which allows evaluation of the whole liver and shows good accuracy for detecting severity of liver fibrosis [48]. Its major limitations are cost and lack of wide availability. More studies are needed to assess the role of these imaging tools in assessing changes in fibrosis in response to treatment.

Treatment

Meaningful therapy for NASH should target reduction of mortality, development of metabolic comorbidities, liver-related and cardiovascular outcomes, and improvement of symptoms and quality of life. Despite an increasing understanding of the mechanisms of NAFLD pathogenesis, approved therapy is lacking.

Lifestyle modifications (diet and regular exercise) remain the initial therapeutic strategy for NASH. This simple yet challenging approach has been shown to correlate with NASH improvement histologically [49–51]. The majority of the patients with NASH have concurrent metabolic comorbidities, and not surprisingly the leading cause of their mortality is cardiovascular disease [52]. The presence of multiple features of the metabolic syndrome is associated with progressive liver disease [53]. Therefore, regardless of NASH diagnosis by liver biopsy, the treatment of obesity, diabetes, dyslipidemia, and hypertension should be optimized in NAFLD to reduce the risks for cardiovascular disease and liver disease progression.

Pharmacologic therapy can be considered only in patients with biopsy-proven NASH until reliable biomarkers are available. Insulin sensitizers, angiotensin-converting enzyme inhibitors and receptor blockers, anti-oxidants, bile acids, cytokine-mediated therapy, lipid-lowering agents, and endocannabinoid antagonists have been proposed, but the majority of these agents either lack efficacy or require further studies for validation before definitive recommendations can be made [54]. This article will focus on pharmacologic options for which sufficient evidence is available to make a recommendation.
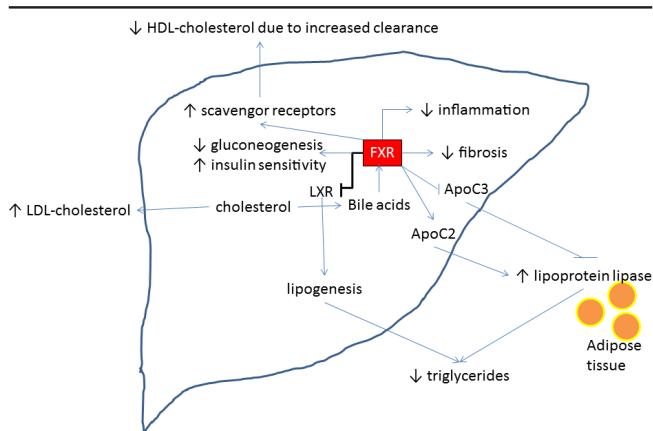
Oxidative stress is a major player in the genesis of NASH. Vitamin E is an antioxidant that suppresses lipid peroxidation, helps replete glutathione stores, decreases

cytokine production, and inhibits profibrotic activity [55–58]. To date, the Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis (PIVENS) trial is the largest randomized controlled trial comparing vitamin E (800 IU/day) and pioglitazone (30 mg/day) with placebo for 96 weeks in non-diabetic and non-cirrhotic adult patients with NASH [59]. Vitamin E was superior to placebo in improving NASH histology, but no significant improvement in fibrosis was observed. There are insufficient data to recommend vitamin E for NASH patients with concomitant diabetes or cirrhosis, and long-term efficacy and safety of vitamin E remain uncertain. It is important to note that vitamin E at high doses has been implicated in increased all-cause mortality [60]. Other safety concerns of vitamin E include increased risk for development of hemorrhagic stroke and prostate cancer [61,62]. Therefore, competing risks should be discussed with non-diabetic patients with NASH before starting on therapy.

Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor-gamma agonists that ameliorate insulin resistance in adipose tissues, liver, and muscles. TZD increases adiponectin, reduces fatty acid synthesis, and increases fatty acid oxidation by activating 5′ adenosine monophosphate-activated protein kinase (AMPK) and counteracts pro-inflammatory cytokines [6,63,64]. Belfort and colleagues [65] showed that pioglitazone improved liver histology by improving steatosis, inflammation, and ballooning in patients with NASH who have impaired glucose tolerance or diabetes. In the largest clinical trial (PIVENS), although pioglitazone did not achieve the primary endpoint as assessed by the NAFLD activity score (NAS), resolution of NASH was achieved in a higher percentage of non-diabetic patients receiving pioglitazone versus placebo (47% versus 21%, P = 0.001) [59]. A recent meta-analysis suggested that pioglitazone may improve liver histology and fibrosis in patients with NASH [66]. In terms of safety, treatment with pioglitazone may not be without adverse effects, and its long-term use has not been studied. Potential adverse effects include weight gain, higher rate of heart failure, bone loss, and rarely bladder cancer [67–70]. Overall, pioglitazone is an option in patients with biopsy-proven NASH, particularly for those with impaired glucose tolerance or diabetes, but this medication must be used with caution.

Obeticholic acid is a synthetic FXR agonist that has been demonstrated to decrease hepatic fat and fibrosis in animal models of NAFLD [71–73]. The potential mechanisms of FXR agonists are represented in Figure 1. More recently, Neuschwander-Tetri and colleagues [74] performed a placebo-controlled, randomized clinical trial (FLINT) that

Figure 1. Potential mechanisms of farnesoid X receptor agonists in non-alcoholic steatohepatitis

APO C2, apolipoprotein C2; APO C3, apolipoprotein C3; FXR, farnesoid X receptor; LDL-cholesterol, low-density lipoprotein cholesterol; HDL-cholesterol, high-density lipoprotein; LXR, liver X receptor.

assessed the efficacy of obeticholic acid (25 mg/day) versus placebo for 72 weeks in patients with non-cirrhotic NASH. The primary outcome was improvement in liver histology, defined as a decrease in NAS by at least 2 points without worsening of fibrosis from baseline to the end of treatment. Treatment with obeticholic acid improved all features of the NAS (steatosis, hepatocellular ballooning, and lobular inflammation) and fibrosis. However, complete resolution of NASH was not achieved in a substantial proportion of patients. Although obeticholic acid was generally well tolerated, it was associated with frequent pruritus, increased serum cholesterol, and changes in the lipid profile. Therefore, long-term studies are needed to assess the potential risk of obeticholic acid for the development of atherosclerosis and cardiovascular disease in these patients.

Conclusions

A better understanding of NASH pathogenesis may help clarify the best approach in managing patients with NASH. More studies are warranted in understanding the influence of genetics associated with disease progression and patient response to treatment. Although liver biopsy is still required, biomarkers to diagnose NASH and tools to predict advanced fibrosis may be helpful, but more studies are needed to validate their use. While obeticholic acid has shown promise in NASH by improving not only liver histology but also fibrosis, not achieved by either vitamin E or pioglitazone, long-term studies are needed to assess the safety of obeticholic acid and its effects on liver- and cardiovascular-related outcomes. The multiplicity of pathways contributing to NASH and fibrosis progression provides a rationale for individualized treatment strategies.

Combination therapy is an option, but large-scale studies are eagerly awaited.

Abbreviations

ALT, alanine aminotransferase; ARFI, acoustic radiation force impulse; AUROC, area under the receiver operating curve; CK18, circulating keratin 18; ELF, enhanced liver fibrosis; ER, endoplasmic reticulum; FFA, free fatty acid; FLINT, Farnesoid X Receptor (FXR) Ligand Obeticholic Acid in NASH Treatment; FXR, farnesoid X receptor; GWAS, genome-wide association study; NAFL, non-alcoholic fatty liver; NAFLD, non-alcoholic fatty liver disease; NAS, non-alcoholic fatty liver disease activity score; NASH, non-alcoholic steatohepatitis; NCAN, neurocan; PIVENS, Pioglitazone versus Vitamin E versus Placebo for the Treatment of Nondiabetic Patients with Nonalcoholic Steatohepatitis; PNPLA3, palatin-like phospholipase domain-containing 3; PPP1R3B, protein phosphatase 1, regulatory (inhibitor) subunit 3B; SNP, single-nucleotide polymorphism; TM6SF2, transmembrane 6 superfamily member 2; TZD, thiazolidinedione.

Disclosures

Arun J. Sanyal is a consulting advisor for Abbot, Genetech, Gilead Sciences, Ikaria, Merck, Norgine, Roche, Salix, Takeda Pharmaceutical Company, Nimbus, Nitto Denko and Bristol-Myers Squibb, receives research grants from Ikaria, Takeda Pharmaceutical Company, Astellas, Novartis and Galectin Therapeutics and receives royalties from Uptodate Inc. Tommy Y. Pacana has no disclosures.

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