Clinical and Experimental HEPATOLOGY

Clin Exp HEPATOL 2018; 4, 3: 165–174
DOI: https://doi.org/10.5114/ceh.2018.78120
Received: 19.03.2018, **Accepted:** 11.08.2018, **Published:** 05.09.2018

Review paper

A systematic review of the present and future of non-alcoholic fatty liver disease

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the Western world. Ongoing research has furthered our understanding of NAFLD, the nature of progression of this disease, and its impact on morbidity and mortality. An active form of NAFLD is non-alcoholic steatohepatitis (NASH); it is the most severe subtype, without any current recommended therapies, according to the European Medicines Agency. The development of new therapies presents challenges, notably due to the slow progression of NASH and the clinically relevant endpoints.

Correlating new data with effective treatment regimens is an emerging challenge, which will increase our understanding of the factors affecting the NAFLD course. This can enable more appropriate non-invasive prognostic assessments, which can focus on specifically at-risk NAFLD populations for tailored individual treatment. This review article aims to highlight the current developments in the field of NAFLD: pathogenesis, epidemiology, diagnosis, clinical features, and available treatment, including novel targets and therapies.

Key words: NAFLD, NASH, hepatology, novel treatment, review.

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is estimated to affect up to 30% of the population in developed countries [1]. This makes it the most common cause of chronic liver disease in the Western world, and it is now projected to become a leading indication for liver transplantation, superseding hepatitis C [2].

NAFLD is a clinico-histopathological entity that includes a spectrum of conditions that are histologically characterized by macrovesicular hepatic steatosis. NAFLD, as the name suggests, is caused by factors other than excessive alcohol consumption.

Non-alcoholic steatohepatitis (NASH), an active form of NAFLD, comprises hepatocellular necrosis, liver inflammation, and tissue injury. NASH is associated with rapid progression of fibrosis, which is initially accentuated in zone 3 of the centrilobular location; this pattern of fibrosis is known as chicken wire fibrosis. Eventually, this can progress to the development of cirrhosis, with the potential of a hepatic malignancy, hepatocellular carcinoma (HCC) [3].

Currently, the European Medicines Agency does not have a recommended drug therapy regimen for the management of NASH. The development of new drugs presents challenges due to the slow progression of NASH, the clinically relevant endpoints, and the duration of observation. Therefore, lifestyle modifications remain the first line of therapy, since the reversal of overnutrition and metabolic obesity is known to improve the liver's condition.

This review article aims to highlight the current developments of NAFLD with regards to pathogenesis, epidemiology, diagnosis, clinical features, and available treatment, including novel targets and therapies.

Pathogenesis

Although the pathogenesis of NAFLD is not yet fully understood, it is believed to be a multifactorial process, which develops in genetically predisposed individuals. NAFLD is attributed to insulin resistance, hepatic lipid accumulation, gut microbiota, sedentary lifestyle, a high fat diet, and obesity [4].

Currently, the multiple parallel-hit hypothesis is used to explain the progression of NAFLD to NASH; this theory focuses on many hits that act in parallel from the gut or adipose tissue to promote liver inflammation. The first hit is insulin resistance, which is associated with metabolic disturbances.

Overnutrition induces chronic inflammation, and also allows for hepatic de-novo lipogenesis that leads to accumulation of excessive triglycerides and free fatty acids, leading to hepatic steatosis [5]. There is an increase in the fatty acid de-novo biosynthesis owing to the liver X receptor (LXR) nuclear receptor; which is a fundamental regulator of cholesterol, fatty acid and glucose homeostasis. LXR induces the expression of target genes such as carbohydrate regulatory element binding protein (ChREBP), sterol regulatory element binding protein 1c (SREBP-1c), and fatty acid synthase [6, 7].

The presence of insulin resistance is an independent predictor of advanced fibrosis in patients with NAFLD; it causes increased cellular uptake of free fatty acids via the CD36 receptor. CD36 is a member of the class B scavenger receptors, and it binds to various ligands such as long chain fatty acids, oxidized low-density lipoproteins and native lipoproteins [8]. The downregulation of the production and secretion of adipokines and inflammatory cytokines is a consequence of adipose tissue dysfunction, resulting from insulin resistance [9].

Adipokines have an important role in the homeostasis of glucose and lipid metabolism. When there is resistance to leptin, an adipokine, there is associated triglyceride accumulation in non-adipose organs such as the liver, muscle and pancreas. Adiponectin levels correlate positively with high-density lipoprotein cholesterol (HDL-C) and negatively with triglycerides. Hypoadiponectinemia leads to a raised level of several inflammatory markers as well as causing the promotion of visceral fat accumulation. As the severity of the NAFLD increases, there is an increase in leptin levels and a decrease in adiponectin levels, leading to liver fat loss, which is seen in cirrhosis and advanced fibrosis [10].

Hepatokines are secreted by the liver and play an important role in NAFLD; usually in NAFLD patients, there is increased secretion of hepatokines with upregulation of gluconeogenesis, decreased glycogen synthesis and inhibition of insulin signaling. The most important hepatokines include fetuin-A, fibroblast growth factor-21 (FGF-21), selenoprotein P, sex hormone-binding globulin (SHBG), angiopoietin-related growth factor, and leukocyte derived chemotaxin 2 (LECT2). Fetuin-A is a biomarker of NAFLD, and is increased in NAFLD patients, this hepatokine suppresses the autophosphorylation of insulin receptors in the skeletal muscles and liver, as well as promoting inflammatory cytokines in monocytes and adipocytes whilst simultaneously activating Toll-like receptors (TLRs) 4 [11].

Bile acids are amphipathic steroid molecules which emulsify and absorb dietary fat, cholesterol, and fat soluble vitamins. The farnesoid X receptor (FXR), mainly expressed in the liver, intestine, kidney and adipose tissue, is involved in the regulation of cholesterol metabolism via regulation of bile acid synthesis and conjugation transport; bile acids are endogenous FXR ligands with a high affinity to FXR.

Toll-like receptors and NLRP3 inflammasomes are implicated in inducing liver inflammation via the non-specific immune system. TLRs are predominantly expressed on sentinel cells, with TLR4 mediating the recruitment of monocytes and macrophages to the liver [12]. Subsequent initiation of the inflammatory cascade stimulates hepatic cells to produce proinflammatory cytokines. NLRP3 is activated by lysosomal damage, mitochondrial dysfunction, and oxidative stress via the recruitment of an apoptosis-associated protein. Oxidative stress is one of the major mechanisms that lead to NASH, due to the elevated production of reactive oxygen species (ROS). ROS induce lipid peroxidation causing inflammation and fibrogenesis via activation of hepatic stellate cells, as well as inhibiting hepatocyte secretion of very-low-density lipoproteins (LDL); this results in liver fat accumulation. NASH with fibrosis is a more severe subset of NAFLD and is associated with a worse prognosis of liver failure [13, 14].

There is a buildup of damaged cellular products deposited in the hepatocytes due to the defective autophagic function. This promotes inflammation and endoplasmic reticulum (ER) stress. ER stress constitutes a cellular process that is triggered by pathologies which disturb the folding of proteins [15]. Hepatocyte apoptosis is a consequence of liver injury in NASH, and therefore the extent of apoptotic cell death is closely associated with the severity of NASH. This hence enables the development of biomarkers that reflect this process [16].

NAFLD is a polygenic and heritable disease according to evidence from population-based and familial-aggregation studies, including twin studies. Single nucleotide polymorphisms have been identified at several

loci, and there has been extensive knowledge acquired on the role of patatin-like phospholipase domain-containing protein 3 (PNPLA3). PNPLA3 is believed to play a role in the export of very-low-density lipoprotein (vLDL), and hepatic cell lipid droplet remodeling; this gene may have a polymorphism that results in the conversion of isoleucine into methionine amino acid [17].

A variant of PNPLA3 is rs738409; it is associated with accumulation of fat in the liver, and GG homozygotes have a 73% increase of lipid fat content [18]. This genotype is linked to a 3.24-fold increased risk of a more pronounced necroinflammatory score, and a 3.2-fold increased risk of developing fibrosis [19]. Rs738409 explains a fraction of the sexual dimorphism associated with NAFLD, as a remarkably higher effect was demonstrated in women. Transmembrane 6 superfamily 2 (TM6SF2) affects the secretion of triglyceride-rich lipoproteins (TRLs) and the hepatic lipid droplet content and also aids in liver fat metabolism [20]. The variant rs58542926, on the C allele, exerts a notable effect in modulating lipid traits such as total cholesterol, circulating low-density lipoprotein cholesterol (LDL-C), and triglycerides; these are associated with a higher cardiovascular risk [21]. The T allele of this genotype is particularly associated with NAFLD [22].

There are 7 categories of genes linked with NAFLD which are demonstrated in Table 1 [23, 24].

Patatin-like phospholipase domain-containing protein 3 (PNPLA3), transmembrane 6 superfamily 2 (TM6SF2), nuclear receptor subfamily 1 group I member 2 (NR1I2), peroxisome proliferator-activated receptor alpha (PPAR-alpha), phosphatidylethanolamine N-methyltransferase (PEMT), microsomal triglyceride transfer protein (MTTP), apolipoprotein C-III (APOC3), apolipoprotein E (ApoE), ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1), insulin receptor substrate 1 (IRS-1), glucokinase regulator (GCKR), solute carrier family 2, facilitated glucose transporter member 1 (SLC2A1), ghrelin O acyl transferase (GOAT), transcription factor 7-like 2 (TCF7L2), peroxisome proliferator-activated receptor gamma (PPARG), bile acyl-CoA synthetase (SLC27A5), fatty acid desaturase 1 (FADS1), lipin-1 (LPIN1), human hemochromatosis protein (HFE), glutamate-cysteine ligase catalytic (GCLC), glutamate-cysteine ligase regulatory subunit (GCLM), ATP-binding cassette sub-family C member 2 (ABCC2), superoxide dismutase 2, mitochondrial (SOD2), Toll-like receptor 4 (TLR4), cluster of differentiation 14 (CD14), tumor necrosis factor (TNF), interleukin 6 (IL-6), angiotensin II receptor type 1 (AGTR1), Kruppel-like factor 6 (KLF6).

Epidemiology

Currently, due to the clinical nature of NAFLD, there is limited availability of epidemiological data to determine its incidence rates. Nevertheless, NAFLD is one of the most common causes of chronic liver diseases and it is a growing problem in the Western world.

In a major European study, a prevalence rate in those with both type 2 diabetes mellitus (T2DM) and NAFLD was reported to be 42.6-69.5% [25]. There are limited studies investigating the incident rates of NAFLD in general populations. A study in Italy conducted over an 8.5-year follow-up period diagnosed NAFLD via ultrasonography at a rate of 18.5 per 1000 person-years [26]. In a UK study, NAFLD accounted for 26.4% of cases, and it was found to be the most common cause of having asymptomatic abnormal liver biochemistry [27]. A study in Greece revealed that in autopsied cases of ischemic heart disease or deaths due to traffic accidents, when hepatitis B and any known liver diseases were excluded, NAFLD was discovered in 31% of cases, with 40% of cases having NASH [28].

In the general population of Poland, NAFLD is documented at a rate of 37.2%, with prevalence rates peaking at 51.4% in those aged 65-70; the prevalence subsequently decreases with advancing age [29]. A further study conducted in Poland observed NAFLD in 78% of all obese individuals and documented an increasing trend. This study concluded that there was a strong indication to raise awareness amongst obese individuals about the influence of their lifestyle on their health status [30].

Table 1. Seven categories of genes linked with non-alcoholic fatty liver disease [23, 24]

Hepatic lipid export/oxidation in steatosis	PNPLA3, TM6SF2, NR1I2, PPAR-alpha, PEMT, MTTP, APOC3 and APOE
Glucose metabolism and insulin resistance	ENPP1/IRS1, GCKR, SLC2A1, GOAT, TCF7L2, PPARG
Steatosis-hepatic lipid import or synthesis	SLC27A5, FADS1, LPIN1
Steatohepatitis oxidative stress	HFE, GCLC/GCLM, ABCC2, SOD2
Steatohepatitis-endotoxin response	TLR4 and CD14
Cytokines	TNF and IL6
Fibrosis	AGTR1 and KLF6

Risk factors

In 1980, the term NASH was first described by Ludwig and his team when a group of morbidly obese patients developed liver failure following surgical jejunoileal bypass [31]. NAFLD is now recognized as a hepatic manifestation of the metabolic syndrome, which is in turn the most common risk factor for NAFLD. Metabolic syndrome is a cluster of cardiovascular risk factors that include the presence of 3 or more of the following criteria which can be seen in Table 2 [32].

Western diets are associated with a greater likelihood for the development of metabolic syndrome, and progression to NAFLD. This diet may include high intakes of red and processed meats, refined sugars, saturated fats, refined grains, high-fat dairy, and sugar-laden fructose drinks [33, 34]. Statistically, 30% of obese patients have a fatty liver, and up to 90% of morbidly obese patients with a BMI > 35 are diagnosed with NAFLD [35]. Obstructive sleep apnea is associated with obesity, and it is hypothesized that intermittent hypoxia causes NASH [36]. The severity of NAFLD is augmented through reduced physical activity, since sedentary behavior is associated with the development of NAFLD and NASH [37]. A further lifestyle risk factor is smoking, as tobacco is known to increase susceptibility to the development of insulin resistance [38].

Sexual dimorphism of NAFLD exists with a higher incidence in males as well as postmenopausal females. Additionally, early menarche may lead to a higher risk, which is primarily due to the accumulation of adipocytes. Whilst fertile, the risk of liver fibrosis in females is reduced, but there is an increased risk of severe hepatocyte injury and inflammation, in comparison to men and postmenopausal status. During the female reproductive years, a common syndrome that encompasses insulin resistance and obesity is polycystic ovarian syndrome (PCOS); PCOS sufferers can be hyperandrogenic, which leads to a higher risk of developing NAFLD [39].

Moreover, ethnicity is seen as a risk factor, with Hispanic individuals being more susceptible than their Caucasian counterparts. The lowest susceptibility of NAFLD is observed in black individuals [40].

Additionally, NAFLD can be caused by medications, which include amiodarone, nucleoside analogues, aspirin, oestrogens, glucocorticosteroids, methotrexate, tamoxifen and tetracycline [41]. Other risk factors include total parenteral nutrition, severe anemia, inflammatory bowel disease, inborn errors of metabolism, barium salts, chromates, thallium, and phosphorus.

Table 2. Diagnostic criteria for metabolic syndrome [32]

Hypertension (resting blood pressure)	⩾ 130 mmHg for systolic ⩾ 90 mmHg for diastolic
Triglycerides (fasting serum)	⩾ 1.5 g/l
High-density lipoprotein cholesterol	< 400 mg/l in males < 500 mg/l in females
Hyperglycemia (fasting)	⩾ 1.1 g/l
Waist circumference	102 cm in males 88 cm in females

Diagnosis

Typically, NAFLD is clinically silent, with diagnoses being incidental due to abnormal liver enzymes or imaging; most commonly it results from steatosis. Once NAFLD is suspected, the diagnosis is confirmed after other potential causes of steatosis have been excluded; alcoholic hepatitis is clinically indistinguishable from NASH, and therefore it must be ruled out in order to establish a diagnosis. It is necessary to exclude any significant alcohol consumption, which is usually considered as consumption of more than 20 g daily [42]. The clinical history, dietary history, medication use, occupational exposure to organic solvents and a family history of liver disease should all be investigated.

Anti-smooth muscle antibodies and antinuclear antibody (ANA) are often seen in patients with NASH. This often represents a nonspecific antibody response that is not related to the severity or pattern of injury on liver biopsy [43].

Transabdominal ultrasonography is the preferred first line imaging investigation for the diagnosis of hepatic steatosis due to it being inexpensive, non-invasive and widely accessible. On the ultrasound, there is normally a visual decrease of the vascular margins, a loss of definition of the diaphragm, hepatomegaly, and hyperechogenicity of the liver parenchyma, in addition to focal fat deposition on the hyperechoic area. Transabdominal ultrasonography is very effective if the steatosis of hepatocytes is not less than 31% [44].

FibroScan is non-invasive ultrasound-based elastography for detecting liver fibrosis in both adults and children; the result is expressed as kilopascals (kPa), and this transient elastography technique takes less than five minutes to complete [45].

A liver biopsy is not always required to diagnose NAFLD, but it still remains the gold standard examination since it can be used to distinguish steatohepatitis from simple steatosis, provide an assessment of the extent of necroinflammatory activity, and visualize fibrotic and architectural alterations. The most widely used histological grading and staging system for NAFLD is the NAFLD Activity Score (NAS), which can be seen in

Table 3 [46]. The SAF score, which includes the assessment of steatosis (S), activity (A) and fibrosis (F), has made it easier to identify a subset of NAFLD: NASH [5]. The histopathological features of NAFLD include lobular and portal inflammation, steatosis, hepatocellular ballooning, glycogenated nuclei, apoptotic hepatocytes (acidophil bodies), deposition, megamitochondria, Mallory-Denk bodies, and fibrosis with the characteristic pattern focused on the perisinusoidal/pericellular area. This fibrotic pattern is known as chicken wire fibrosis, and it typically originates in zone 3 for adults [47].

A score of ≥ 5 with steatosis and hepatocyte ballooning is generally considered diagnostic of NASH, but patients can still have NASH with lower NAS scores if there is presence of steatosis and hepatocyte balloon [46].

Symptoms

In the absence of a chance diagnosis, NAFLD usually presents asymptomatically until liver decompensation commences. However, sometimes signs and symptoms can occur; these are illustrated in Table 4. The most prevalent initial symptoms are fatigue and malaise, which do not seem to correlate with the severity of disease [48]. Hepatosplenomegaly presents in up to 50% of patients. However, as the disease progresses, the liver shrinks in size, whereas the spleen continues to enlarge [49]. Pruritus can also be seen as a result of increased bilirubin levels and jaundice, but pruritus can occur when bilirubin levels are normal. A Cruveilhier-Baumgarten murmur, which is a venous hymn in the epigastric region, may also be heard. There may be presence of hypotension due to reduced total systemic vascular resistance.

Available treatments

A fundamental part of NASH management involves lifestyle modification. The European Association for the Study of the Liver (EASL) recommends that a 7% weight reduction may be adequate in resolving steatosis and inflammation, whereas the American Association for the Study of Liver Diseases (AASLD) states that a decrease of at least 10% may resolve mild to moderate fibrosis, and improve necroinflammation [50, 51]. A large study involving 154 NAFLD patients demonstrated that a lifestyle modification over a one-year period resulted in remissions of 64% in the lifestyle modification group, and only 20% remissions in the control group [52].

One method of lifestyle alteration is via aerobic and resistance training exercise regimens to reduce both liver and visceral fat. Johnson *et al.* discovered that in previously inactive, overweight or obese NAFLD patients, all exercise doses, regardless of the amount or intensity, were capable of scaling down liver fat and visceral fat. In patients with obesity, exercise is further complicated by musculoskeletal problems that manifest biomechanically [53].

Dietary restriction is recommended in studies by Elias *et al.*, and Lin *et al.*, proving that pursuing a hypocaloric diet is key in improving NAFLD; a reduction

Table 3. Non-alcoholic fatty liver disease activity score – NAS [46]

Histological features	Score	Category definition
Steatosis	0	< 5%
	1	5-33%
	2	34-66%
	3	> 66%
	Plus	
Hepatocyte ballooning	0	None
	1	Few
	2	Many
	Plus	
Inflammation	1	1-2 foci per *20 field
	2	2-4 foci per *20 field
	3	> 4 foci per *20 field
NAS total 0-8		
Fibrosis	0	No fibrosis
	1a	Zone 3 mild perisinusoidal fibrosis
	1b	Zone 3 moderate perisinusoidal fibrosis
	1c	Periportal/portal fibrosis only
	2	Zone 3+ periportal/portal fibrosis
	3	Bridging fibrosis
	4	Cirrhosis
Fibrosis score 0-4		

Table 4. Signs and symptoms [64]

Alopecia	Low blood pressure
Ascites	Malaise
Caput medusae	Muehrcke's lines
Clubbing	Peripheral edema
Cruveilhier-Baumgarten murmur	Pruritus
Dupuytren's contracture	Right upper quadrant discomfort
Fatigue	Spider angioma
Gynecomastia	Terry's nails
Hepatic encephalopathy	Testicular atrophy
Hepatosplenomegaly	Truncal obesity

Table 5. Nutritional recommendations for non-alcoholic fatty liver disease [54, 55]

Low carbohydrate: lowering of simple carbohydrates, especially fructose
Low fat: lowering of saturated fatty acids and trans-fatty acids
Low cholesterol
Low glycemic index
Low protein: reducing red meat consumption

as low as 450 to 1000 kcal/day has proven to be effective and safe. Nutritional recommendations, listed in Table 5, for patients with NAFLD state that carbohydrates should comprise 40-50% of total dietary energy, with an increase in the amount of complex carbohydrates rich in dietary fiber. Furthermore, fat intake should be less than 30% of the daily calorie intake, and there should also be an increase of mono- and polyunsaturated fatty acids [54, 55].

Bariatric surgery (BS) is one of the most reliable options available in yielding a sustained reduction in body weight for individuals classified as severely obese, with a body mass index ≥ 35 kg/m^2. Therefore, patients who have severe obesity and NAFLD could benefit from BS due to improvements in glycemic control, quality of life, and long-term survival. Additionally, BS may aid in alleviating some obesity-related comorbidities, such as sleep apnea, which will also increase patients' exercise tolerance. To date, there have not been any randomized controlled trials examining the effect that BS has on NAFLD patients; thus, BS as an approach for treatment in NAFLD is not definitive. Nonetheless, there has been a meta-analysis including 766 liver biopsies after BS, which showed a resolution rate of 91.6, 81.3 and 65.6% in steatosis, steatohepatitis, and fibrosis respectively. A study involving 109 morbidly obese patients with biopsy-proven NASH, who had undergone BS, showed features of reduced pathology after 1 year of follow-up in 85% of the cohort. Presently, there is a trial observing biomarkers to quantify liver pathology in patients with NASH at baseline and after BS (ClinicalTrials.gov Identifier: NCT03294850) [56].

NAFLD patients have an increased cardiovascular risk (CVR), with the main cause of death in these patients being ischemic heart disease. Therefore, it is paramount to manage and treat any CVR factors; lipid-lowering agents including statins, and some omega-3 polyunsaturated fatty acid supplements are frequently used in patients in order to reduce CVR, hepatic inflammation and low-density lipoprotein cholesterol (LDL-C) levels [57].

The use of statins remains controversial due to statin-induced hepatotoxicity and is sometimes avoided in patients with increased transaminase levels. The Incremental Decrease in End Points Through Aggressive Lipid Lowering (IDEAL) trial, Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) trial, and Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial all demonstrated the improvement of liver enzymes in these patients. These trials endorsed the safe use of statins in compensated cirrhosis patients, since the absence of statins in patients with elevated aminotransferases correlated with the worsening of cardiovascular outcomes [58, 59]. Furthermore, 26 randomized controlled trials combined their results and the meta-analysis including more than 1.4 million patients with 4298 cases of HCC revealed that the use of statin was associated with a 37% reduction in HCC [60].

The usefulness of ezetimibe was examined in a 24-month trial with 45 patients newly diagnosed with NAFLD via liver biopsy. The results demonstrated that the drug significantly improved visceral fat areas, fasting insulin, concentration of triglycerides, total cholesterol, mean levels of small LDL and very small LDL, as well as significantly lowering serum alanine aminotransferase (ALT) and C-reactive protein levels. Moreover, the histological features of steatosis grade necroinflammatory grade, ballooning score and NAFLD activity score were notably improved when compared to the baseline [61].

Studies have shown that NAFLD patients documented a reduction in transaminase levels when taking omega-3 polyunsaturated fatty acid supplements. In the Japan EPA Lipid Intervention Study (JELIS), the study group taking both the statin and omega-3 supplement regimen saw a reduction in CVD events by 19%, compared to those who solely took statins [62]. Nicotinic acid is an alternative, but the Atherothrombosis Intervention in Metabolic Syndrome with Low HDL/High triglycerides: Impact on Global Health Outcomes (AIM-HIGH) study revealed that taking nicotinic acid over a 4-year follow-up showed no difference in the incidence rates of CVD events [63].

Peroxisome proliferator-activated receptors (PPARs) are affiliated with cellular proliferation and nutritional metabolism. The randomized placebo controlled Fatty Liver Improvement With Rosiglitazone Therapy (FLIRT) trial resulted in 47% of NASH patients having a significant (> 30%) reduction in steatosis after a period of 12 months of treatment, with 38% achieving normalization of ALT values [65]. The FLIRT 2 extension trial reviewed the patients after a period of 48 months to observe the long-term effects, and found that the ALT levels remained normal, steatosis improved and insulin levels decreased. Saroglitazar, used for diabetic dyslipidemia treatment in mouse models of NASH, was found to reduce both ALT and steato-

sis, as well as improving liver histology [66]. A phase 2 open-label study of saroglitazar (PRESS VIII) evaluated 32 patients with NASH and after a period of 12 weeks, a result of a 52% decrease in ALT was documented [67]. A prospective experimental dual PPAR α/δ drug is elafibranor, and it has been shown to reverse NASH by preventing the progression of fibrosis; it is currently undergoing a phase III clinical trial, REOLVE-IT, a randomized pivotal double-blind (2 : 1), placebo controlled trial with 250 centers involving 2000 patients worldwide (ClinicalTrials.gov Identifier: NCT02704403). Fibrates are archetypical PPAR-α agonists that have had mainly disappointing results, but fenofibrates are one of the most used fibrates and are used with statins; they have been shown to reduce triglycerides whilst raising HDL-C levels [74].

GLP-1 agonists have been shown to reduce both hepatic steatosis and necroinflammation [66-69]. In the liraglutide safety and efficacy in patients with NAFLD (LEAN) study, 39% of patients who received liraglutide had resolution of definite NASH at 48 weeks, whereas only 9% of the placebo group experienced resolution [70]. Studies have shown that GLP-1 agonists can reverse the progression of NAFLD indirectly through an incretin effect that improves key parameters, but directly through an effect on lipid metabolism of hepatocytes and inflammation in the liver [71]. A recent study using DDP-4 inhibitors on patients with T2DM, over 6 months, demonstrated an improvement in liver dysfunction; although the effects on NAFLD are unclear, they do deserve some further research [72].

NASH is an inflammatory disorder, and therefore potential strategies that may amend or turn off inflammatory triggers such as cytokines, including tumor necrosis factor (TNF) and chemokines should be investigated. In a non-randomized pilot study, pentoxifylline, a phosphodiesterase inhibitor with antioxidant properties, demonstrated improvements in serum aminotransferases and liver histology, but not in serum TNF. Further controlled trials have established that pentoxifylline failed to reduce hepatocyte ballooning, and had no histological differences; therefore, this drug is not recommended in treatment [73, 74].

A range of illnesses such as obesity and metabolic disorders, including NAFLD, have been associated with the gut microbiome. In a study with NASH patients, it was found that a minute bacterial intestinal overgrowth led to larger intestinal permeability due to a disruption of the gap junctions [75]. A choline-deficient diet can lead to a decrease in vLDL levels and hepatic beta oxidation which results in an inflammatory reaction and accumulation of liver fatty acids. Therefore, a potential therapy could be with the use of pre- and probiotics to manipulate the gut microbes. In 4 randomized control trials of 134 NAFLD/NASH patients receiving probiotics, the results showed that compared to a placebo, there was a decrease in total cholesterol, HDL, ALT, AST and TNF, but there was no change in BMI, glucose or LDLs [76].

Antifibrotic therapy has attained awareness due to fibrosis being a prognostic marker for NAFLD. Lysyl oxidase (LOX) L2 is an enzyme which aids in the cross-linking of extracellular fibrillar collagen I and is thought to be a critical step in the development of fibrosis in the liver [77]. The efficacy and safety of simtuzumab, a human monoclonal anti-LOXL2 antibody, was explored in two phase 2 placebo-controlled studies, which involved patients with NASH cirrhosis, and patients with advanced NASH fibrosis over a 96-week treatment plan. They showed that the drug was safe and well tolerated but had no additional benefits [78].

Another protein that plays a crucial role in organ fibrosis is galectin-3. In an animal model, mice that were treated with this protein became resistant to liver, lung, and kidney fibrosis. In the mouse models with NASH taking a galectin inhibitor, GR-MD-02, the study concluded that there was a significant decrease in fibrosis, and therefore a phase 1 trial for galectin-3 antagonist is being undertaken [79].

Pathological increases in hepatocyte apoptosis in the liver and peripheral tissues have emerged as an important mechanism in the development of NASH. In phase II clinical trials on 38 patients, emricasan, a pan-caspase inhibitor, lowered serum aminotransferase activity, liver injury and fibrosis by inhibiting hepatocytes apoptosis. A placebo-controlled, multicenter, double-blind, randomized trial with NAFLD patients with raised transaminases found the drug to be well tolerated, and the results suggested the use of the drug as a treatment option for NAFLD/NASH patients [80, 81]. Currently, a 72-week phase II trial in NASH patients with fibrosis is being initiated to verify whether there is any histological improvement of NASH resolution with emricasan (ClinicalTrials.gov Identifier: NCT02686762).

Another controversial but attractive new drug is obeticholic acid (OCA), which is a selective agonist of the FXRs due to its 100-fold-higher affinity to FXR in comparison to chenodeoxycholic acid. A recent randomized placebo-controlled trial called FXR Ligand Obeticholic Acid in NASH Treatment (FLINT) demonstrated an improvement in NAFLD patients' liver histology. The trial showed a decrease in the NAFLD activity score by at least 2 points without a deterioration of fibrosis; 50 patients out of 110 met the primary endpoint at 72 weeks, compared to 23 out of 109 patients

in the placebo group. OCA appears to improve hepatic steatosis, inflammation, and fibrosis, and helps to reduce body weight; however, OCA has been shown to aggravate insulin resistance and dyslipidemia. During treatment, pruritus is commonly witnessed, and furthermore, higher rates of pruritus are reported in patients taking OCA therapy for other liver diseases [82].

Due to the increase in the rates and impact of NASH, there is an urgent requirement to develop more effective management strategies, as well as a need for the discovery of biomarkers to aid in the diagnosis and monitoring of NASH patients. A current trial is investigating the HepQuant SHUNT diagnostic kit to look at the monitoring and treatment of NASH (ClinicalTrials.gov identifier NCT03294941). Other studies are investigating the efficacy of Tropifexor (LJN452) in patients with NASH (ClinicalTrials.gov Identifier: NCT02855164).

Conclusions

NAFLD is one of the most common diseases in the general population, and currently it is the most common cause of chronic liver disease in the Western world; NASH is a severe form of NAFLD. Whilst ongoing research has furthered our understanding of NAFLD, the nature of progression of the disease, as well as its impact on morbidity and mortality, is still highly disparate. Furthermore, screening recommendations for NAFLD and NASH are not available due to a lack of a nationally accepted guideline, but they should be considered in those with risk factors for development of the disease. Additionally, available treatments which have been discussed in this systematic review are of limited efficacy, durability and applicability, due to the intricacy of this disease. Today's emerging challenge is the race to correlate new data with effective treatment regimens to establish a greater understanding of the factors that control the course of NAFLD, and thus enable more appropriate non-invasive prognostic assessments with the ability to focus on at-risk NAFLD populations for tailored individual treatment. In patients with NAFLD, there is also a need for surveillance and management for any complication of cirrhosis which may include HCC. Moreover, the implementation of lifestyle changes is necessary in this population of patients, but this comes with its own difficulties, considering that a rebound to prior unhealthy eating habits and inactivity is common.

Disclosure

The authors report no conflict of interest.

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