REVIEW

DOI: http://dx.doi.org/10.15403/jgld.2014.1121.274.quo

# Nonalcoholic Fatty Liver Disease: Status Quo

**Ioan Sporea[1], Alina Popescu[1], Dan Dumitrașcu[2], Ciprian Brisc[3], Laurențiu Nedelcu[4], Anca Trifan[5], Liana Gheorghe[6], Carmen Fierbințeanu Braticevici[7]**

1) Department of Gastroenterology and Hepatology, Victor Babeș University of Medicine and Pharmacy, Timișoara
2) 2nd Department of Internal Medicine, Iuliu Hațieganu University of Medicine and Pharmacy, Cluj-Napoca
3) Department of Medical Disciplines, University of Oradea
4) Department of Internal Medicine, University Transilvania Brașov
5) Institute of Gastroenterology and Hepatology Iași, Grigore T. Popa University of Medicine and Pharmacy, Iași
6) Center for Gastroenterology and Hepatology, Fundeni Clinical Institute, Carol Davila University of Medicine and Pharmacy, Bucharest
7) Department of Gastroenterology, University Hospital, Carol Davila University of Medicine and Pharmacy, Bucharest, Romania

***Address for correspondence:***
**Laurentiu Nedelcu**
Transilvania University, Brașov, Romania
laurentiu.nedelcu@unitbv.ro

Received: 10.10.2018
Accepted: 30.11.2018

## ABSTRACT

Nonalcoholic liver disease (NAFLD) is a hot topic for gastroenterologists and hepatologists and clinical practitioners must be kept abreast with the rapid progress of knowledge in this field. The Romanian Society of Gastroenterology and Hepatology (RSGH) has elaborated this review dedicated to evidence-based data on pathogenesis, diagnosis and therapy of this condition.

The term NAFLD includes two distinct conditions, with different histologic features and prognosis: non-alcoholic fatty liver (NAFL) and non-alcoholic steatohepatitis (NASH), the second with the highest risk of evolution to cirrhosis and its complications, including hepatocellular carcinoma (HCC). Non-alcoholic fatty liver disease is considered the hepatic manifestation of the metabolic syndrome. Therefore, NAFLD is associated not only with an increase of liver-related mortality, but also of the overall mortality, especially cardiovascular and malignancies.

Noninvasive techniques, such as biological tests and elastography can be used for the evaluation of NAFLD patients. Liver biopsy should be recommended in selected cases, for diagnostic, therapeutic and prognostic purposes. Patients with NAFLD would benefit from their lifestyle changes by progressive weight loss through exercise and low fat and sugar diet. Pharmacotherapy should be reserved for patients with NASH, particularly for those with significant fibrosis. Until now, there are no FDA approved therapies for NASH.

**Key words:** fatty liver – metabolic syndrome – nonalcoholic fatty liver disease – nonalcoholic steatohepatitis.

**Abbreviations**: CAP: Controlled Attenuation Parameter; FFAs: free fatty acids; HCC: hepatocellular carcinoma; LB: liver biopsy; MRI: magnetic resonance imaging; MR-E: magnetic resonance imaging based elastography; NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis; PPAR: peroxisome proliferator-activated receptor PUFA: n-3 polyunsaturated fatty acids; RSGH: Romanian Society of Gastroenterology and Hepatology; SWE: Shear Wave Elastography; T2DM: type 2 diabetes mellitus; TE: Transient Elastography; UDCA: ursodeoxycolic acid; US: ultrasound.

## INTRODUCTION

Nonalcoholic Fatty Liver Disease (NAFLD) represents the excessive accumulation of fat in the hepatic parenchyma, in the absence of excessive alcohol consumption. It has become a very frequent pathology in developed countries [1] and has been increasing for decades. At this moment, when we have drugs to cure HCV chronic infection in 8-12 weeks or to control HBV chronic infection with one tablet daily, the interest of hepatologists is focused on evaluating the severity of NAFLD in practice and on its treatment.

For a long time, fatty liver was considered in practice to be a mild disease and there was not too much interest on this pathology. Presently, the risk of progression to severe fibrosis and cirrhosis is well recognized in patients with nonalcoholic steatohepatitis (NASH), in whom inflammation accompanies steatosis [2].

It is difficult to detect the population at risk for progression to advanced fibrosis, as 20-30% of European Union citizens have liver steatosis (approx. 116 million inhabitants) [3, 4] and 30% of USA population [5, 6]. An epidemiological study involving 8,515,431 subjects from 22 countries showed a global prevalence of NAFLD of 25.2% (95%CI: 22.10-28.65) [2]. The main risk factors for this disease are obesity, type 2 diabetes mellitus (T2DM), sedentarism and dyslipidemia [1]. Currently,

there are about 1 billion obese people in the world and about 380 millions have diabetes.

The diagnostic strategy starts with simple and inexpensive tests and can lead to more expensive or invasive procedures. There is not a perfect consensus between the practitioners regarding the methodology to conduct a specific case, taking into consideration age, comorbidities and the preference of the patient.

The aim of this paper is to establish a strategy for early and simple diagnosis of NAFLD/NASH, that can be used in practice by the hepatologists, internal medicine doctors, diabetologists and general practitioners. At the same time, we highlight the need of screening the risk population (e.g. T2DM patients) for an early diagnosis, as NAFLD can progress to advanced fibrosis. The Romanian Society of Gastroenterology and Hepatology (RSGH) has comissioned a group of experts to elaborate an update on NAFLD. Leaders of opinion wrote paragraphs involving their field of interest and then circulated the manuscript amongst themselves. The final text was read and approved by all contributors.

## EPIDEMIOLOGY OF NAFLD AND NASH; NATURAL HISTORY

### Epidemiology of NAFLD and NASH

Globalization of western lifestyle with pandemic obesity subsequently increases the prevalence of metabolic syndrome and T2DM and leads to a growing prevalence of NAFLD, which has become the leading cause of chronic liver disease. The exact incidence of the disease is difficult to be estimated.

The data on prevalence varies greatly depending on the definition used, the population (general population, adults, children or adolescents or high-risk populations) and the method for diagnosis (aminotransferase level, ultrasounds - US examination, liver biopsy - LB), etc.). The estimated global prevalence in the general population is reported between 6-35%, with a median of 20% [7]. In Europe, the median prevalence in the general adult population is 25-26% [8]. The estimated prevalence of NASH ranges between 3 to 5 %, whereas data about NASH cirrhosis is scarce [9]. In high risk groups, the prevalence of NAFLD has increased, as expected: in patients with morbid obesity, NAFLD was reported in more than 90% of cases and unexpected cirrhosis in 5% [10, 11]; in T2DM the prevalence of NAFLD varies between 42.6 and 69% [12, 13] and in individuals with dyslipidemia it was reported to be 50% [14].

In Romania, the largest published study evaluated the presence of NAFLD in 3005 hospitalized patients, without known liver diseases, using US examination and reported it as 20%, which was similar to the reported prevalence for the European general population [15]. Another Romanian study, analyzed the prevalence and the predictive factors of NAFLD defined by the fatty liver index in T2DM patients and reported the presence of NAFLD in 79% [16]. In patients with morbid obesity, a small histological study showed the presence of NAFLD in 100% of patients, and of NASH in 58% of cases [17]. The prevalence of NAFLD in Romania seems to be similar with that reported in Western countries. A recent study conducted on 2,861 subjects found the prevalence of overweight in 34.7%, obesity in 31.9% (abdominal obesity 73.9%) and metabolic syndrome in 38.5% [18].

### Natural history of NAFLD and prognosis

The natural history of NAFLD is still unknown and unpredictable. Over the years, the whole spectrum of NAFLD has varied, steatosis with nonspecific inflammation being the last condition included alongside simple steatosis and NASH [19, 20].

The natural history of NAFLD has been assessed based on clinical evolution from long-term prospective follow-up studies and on histological progression, using serial biopsy probes. Most data revealed that NAFLD patients with the highest risk of disease evolution are those with NASH [2].

Non-alcoholic steatohepatitis is a main cause of liver cirrhosis in Western countries, and it is likely that in the next 30 years NASH will become the foremost cause of advanced liver disease [21]. However, recent data indicates that some patients with NASH and fibrosis can regress while a small proportion of patients with NAFLD develop NASH (mainly those with nonspecific inflammation) [22]. A recent meta-analysis of 11 paired-biopsies studies showed that the annual fibrosis progression rate was significantly higher in patients with NASH, 0.14 stages versus 0.07 stages for NAFLD. This was interpreted into one stage of progression every 14 years for NAFLD and one stage of progression every 7 years for patients with NASH [23].

Nowadays, it is clear that hepatocellular carcinoma (HCC) is part of the clinical spectrum of liver disease in NAFLD and it should be considered as a subdivision of the natural history of progressive NAFLD. A recent meta-analysis specified an incidence of HCC among NAFLD patients of 0.44 per 1,000 person-years [2]. Remarkably, recent data showed that HCC can develop in NAFLD patients without cirrhosis, especially in the presence of features of metabolic syndrome [24].

NAFLD is also associated with an increase in overall mortality, not only of liver-related [2]. Usually, liver-specific complications are the third leading cause of death, whereas the main causes of death are attributed to cardiovascular events and extra-hepatic malignancies [25]. It is not surprising, taking into account that NAFLD is the hepatic manifestation of metabolic syndrome. The risk factors independently associated with disease progression together with genetic polymorphism (*PNPLA3* gene variant) are, from a clinical perspective, T2DM, arterial hypertension, obesity [26] and certainly, significant fibrosis portends worse prognosis [27].

## RISK FACTORS AND PATHOGENESIS

The common risk factors associated with NAFLD are obesity, T2DM, dyslipidemia and metabolic syndrome [28]. The entire spectrum of obesity, from overweight to morbid obesity, is associated with NAFLD. Almost 50% of diabetic patients develop NAFLD [29]. Dyslipidemic patients, especially those with high triglyceride levels and low HDL-cholesterol levels, develop NAFLD. Other risk factors associated with NAFLD include: metabolic syndrome, polycystic ovary syndrome, sleep apnea and endocrine diseases (hypothyroidism, hypogonadism and hypopituitarism).

Pathogenesis of non-alcoholic fatty liver is complex. Even if only two stages (steatosis, followed sometimes by steato-hepatitis) are usually described, the pathophysiological mechanisms of cellular injury are present in both NAFLD and NASH. The pathogenic elements of NAFLD represented by the processes of lipogenesis and lipolysis lead to the ectopic fat in hepatocytes [30].

The liver is the main storage of numerous lipids: triglycerides, free fatty acids (FFAs), free cholesterol and cholesterol esters, phospholipids, diacylglycerol, ceramide. Free fatty acids sources are non-esterified fatty acids (60%), *de novo* lipogenesis (25%) and dietary fatty acids (15%) in the form of chylomicron lipoproteins. In the liver, FFAs can follow three pathways: mitochondrial oxidation, assembly and export of very-low-density lipoprotein (VLDL) and synthesis of triglycerides with their storage as lipid droplets. In this way, FFAs concentrations function as a regulator of lipogenesis. The potential pathogenic mechanisms of NAFLD might be an increased endogenous synthesis of FFAs, decreased B-mitochondrial oxidation of fats, deficient export of VLDL and finally the increase of triglyceride deposits.

In obesity, there are some conditions that are responsible for the appearance of a fatty liver. Because of the adipose tissue resistance to insulin, there is an increase in FFAs release in the liver. Hyperinsulinemia and excess of carbohydrates also lead to *de novo* lipogenesis. The compensatory increase of VLDL is not sufficient to cover the excess of triglyceride formation [31]. Initially, it was considered that triglycerides excessively accumulated in steatosis are relatively inert, potentially benign, but nowadays it is recognized that the hepatocellular injuries are determined by the hepatotoxicity of FFAs, their derivatives as well as the overload of mitochondrial capacity [31]. The abundant accumulation of triglycerides and excessive lipid drops storage are responsible for the progression to NAFLD. The pathogenic processes of NAFLD and its progression are multifactorial and are influenced by many factors: diet composition, genetic aspects, and intestinal genome [31]. These factors explain the great variety of NAFLD patients.

Oxidative stress, as a result of the lack of balance between pro and antioxidant activity of the body, is the key mechanism of NASH genesis. Excessive accumulation of adipocytes is responsible for increased oxidative stress and the release of proinflammatory cytokines: tumor necrosis factor alpha, interleukin 6 and resistin [32]. Progression of inflammation is facilitated by the immune system and gut microbiome. Activation of macrophages and lymphocytes leads to the release of proinflammatory cytokines associated with the resistance to insulin. Bacterial endotoxins that pass through portal blood to the liver play a role in generating inflammation [33]. Hepatocellular injury and activation of immune cells lead to activation of hepatic stellate cells with fibrosis and disorganization of liver architecture [34].

In patients without an evidence of risk factors, genetic polymorphism and histocompatibility antigens are susceptible to develop NAFLD [35]. The data was confirmed by family, twins, and epidemiological studies. A common 148M *PNPLA3* gene variant (patatin-like phospholipase domain containing 3) was associated with the accumulation of triglycerides in the liver [36].

Psychosocial factors contribute also to the pathogenesis of NAFLD [37] (Table I).

The association is explained by common risk factors: life style and diet, microbiota, systemic inflammation, association with obesity and diabetes, which all induce cognitive alterations, etc. [38, 39].

**Table I.** Psychosocial factors associated with NAFLD

| Type of factor | Psychosocial factor | Clinical influence |
|---|---|---|
| Emotional | anxiety | severity |
| | depression | progression |
| Cognitive | confidence in exercise, perceived benefit of exercise, readiness to change (in contemplative stage); cognitive dysfunctions: memory impairment, attention deficit | resistance to therapy |

Not only psychosocial factors are involved in fatty liver, but the brain may suffer changes in this condition. Indeed, brain imaging techniques have shown that brain is ageing earlier in NAFLD and brain volume is reduced. This reduction is however independent of visceral adiposity and other components of the metabolic syndrome. This brain volume reduction is associated with cognitive impairment [40, 41].

## NONINVASIVE EVALUATION OF NAFLD PATIENTS

**a) Biological tests**

Biological tests should be useful in NAFLD to discriminate those patients with NASH vs. steatosis, to assess the severity of the disease by assessing the fibrosis (the most important prognostic factor in NAFLD), and to identify the patients with worse prognosis for the follow up.

No biological test is validated until now for the diagnosis of NASH [42]. Cytokeratin-18 fragments were expected to be a good marker for NASH diagnosis, but the available data showed a modest accuracy (66% sensitivity and 82% specificity) [43, 44]. The most used biological tests for predicting fibrosis in NAFLD are presented in Table II [45].

The advantages of these tests are their high applicability (> 95%) [46], high feasibility, good interlaboratory reproducibility [47] and broad availability for non-patented tests. Some of them showed acceptable diagnostic accuracy with AUROC>0.8 (APRI 0.82, BARD 0.81, FIB-4 0.80, NFS 0.88, Fibrotest 0.81-0.92) [48], but more importantly they have good negative predictive values for excluding advanced fibrosis (APRI 95%, BARD 96%, FIB-4 90%, NFS 93%, Fibrotest 98%) [48].

**b) Ultrasound, Controled Attenuation Parameter, Liver Elastography**

**Ultrasound (US)** evaluation of the liver represents the most common way for the detection of fatty liver. The presence of „bright liver" with posterior attenuation represents the major sign. Increased difference between liver and right kidney echogenity is a supplementary indicator of steatosis [49]. A semi-quantitative apreciation of the severity of steatosis can be performed using US with a classification in mild, moderate or severe steatosis (S1, S2, S3). Ultrasound sensitivity for the

**Table II.** Biological tests and the biomarkers included in their formula and their diagnostic accuracy for predicting advanced fibrosis (F3-F4). Adapted from Castera et al [48]

| Biological test and the biomarkers included in their formula | AUROC | Cut offs | PPV | NPV |
|---|---|---|---|---|
| APRI= AST (/ULN)/platelet (109 /L) x 100 | 0.82 | 1.0 | 31% | 95% |
| BARD score (BMI≥28=1, AST/ALT≥0,8=2, DM=1; scor≥2 – odds ratio for advanced fibrosis =17) | 0.81 | <2 | - | 96% |
| FIB-4 = age (yr) x AST [U/L]/(platelets [109 /L] x √ALT [U/L]) | 0.80 | <1.30<br>>2.67 | 43%<br>80% | 90%<br>83% |
| NAFLD fibrosis score (NFS) = (-1,675 + 0,037 x age (years) x BMI (kg/m$^2$) + 1,13 x IFG/DM (yes=1, no=0) + 0,9 9 x AST/ALT ratio - 0,013 x platelet count (x10$^9$/l - 0,66 x albumin [g/dl]) | 0.88 | <-1.455<br>>0.676 | 56%<br>90% | 93%<br>85% |
| Fibrotest = Fibrotest® (Biopredictive, Paris, France) patented formula combining α-2-macroglobulin, GGT, apolipoprotein A1, haptoglobin, total bilirubin, age and gender | 0.81-0.92 | >0.30<br>>0.70 | 33%<br>60% | 98%<br>89% |

ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under receiver operator characteristic curve; BMI, body mass index; DM, diabetes mellitus; GGT, γ glutamyl transpeptidase; IFG, impaired fasting glucose; NPV, negative predictive value; PPV, positive predictive value.

detection of steatosis ranges between 60-94%, with a specificity between 88-95% (thus a very good specificity) [50]. Sensitivity of US for the diagnosis of steatosis increases along with the severity, being more than 80% in severe steatosis [51]. These results are confirmed by a meta-analysis [52], including 49 studies and 4,720 subjects, where sensitivity of US for the diagnosis of moderate/severe steatosis was 84.8% (95% CI: 79.5-88.9%), with a specificity of 93.6% (95% CI: 87.2-97.0) in comparison with liver biopsy. In these conditions, considering the low cost, absence of radiation and "point of care" use of US, the recommendation is that this method will be used for liver steatosis assessment in clinical settings and population studies.

**Controled attenuation parameter (CAP)** is a technique implemented in FibroScan (EchoSens), that can make an objective evaluation of liver steatosis by measuring the attenuation of US beam during liver passage (attenuation increases with the severity of steatosis). A meta-analysis showed AUROCs between 0.823 (95%CI: 0.809-0.837) and 0.865 (95%CI: 0.850-0.880) for the prediction of moderate and severe steatosis [53-55]. Some cut-off values were proposed for S1, S2, S3: 250, 270 and 290 dB/m, respectively [55]. More recent, CAP was implemented in M and XL probes (for normal and obese subjects).

Controled Attenuation Parameter was more accurate for detecting hepatic steatosis in comparison with US [54, 56]. A meta-analysis identified as factors that could increase the CAP values: NAFLD, T2DM and obesity [55], and then a correction of the obtained values in CAP was proposed, by deducting 10 dB/m for NAFLD and T2DM patients and 4.4 dB/m for every unit in BMI for >25 kg/m$^2$ [57].

**Liver Elastography** can be divided into US or magnetic resonance imaging (MRI) based [45]. *Ultrasound based elastography* is frequently used in practice, using either Transient Elastography (TE) with M or XL probes, point Shear Wave Elastography (point SWE) or 2D-SWE [58].

For TE, the most used and validated method for liver stiffness evaluation, the proposed cut-off values are different for the XL probe (most often used in obese patients) (F≥2: 6.2 kPa, for F≥3: 7.2 kPa and for F=4: 7.9 kPa) and M probe (F≥2: 7 kPa, for F≥3: 8.7 kPa and for F=4: 10.3 kPa) [59, 60]. In a meta-analysis [44], the sensitivity and specificity of TE for the assessment of liver stiffness in NAFLD patients were for F≥2: 79% and 75%, respectively; for F≥3: 85% and 85%, respectively; and for cirrhosis: 92% and 92%, respectively (showing an increasing performance with the severity of fibrosis).

For 2D-SWE, in a study performed in comparison with LB [61], the AUROCs were 85.5% for severe fibrosis and 91.7% for cirrhosis.

A comparative study between 2D-SWE (SSI), TE and point SWE (VTQ) [62], in a cohort of 291 NAFLD patients with LB, showed AUROC for SSI, TE, and VTQ of 0.86, 0.82, and 0.77 for diagnosis of ≥F2; 0.89, 0.86, and 0.84 for ≥F3; and 0.88, 0.87, and 0.84 for F4, respectively.

*Magnetic resonance imaging based elastography (MR-E)* was predominantly used in the USA for liver stiffness assessment. Meta-analyses of MR-E have reported diagnostic accuracies of 93–98% for the diagnosis of advanced liver fibrosis (F≥3), with sensitivities of 85–92% and specificities of 85–96%, respectively [63, 64]. Magnetic resonance imaging based elastography in NALFD patients showed a diagnostic accuracy of 92 % for diagnosing significant fibrosis [65].

## LIVER BIOPSY

In the era when LB has been quite completely replaced by non-invasive tests for evaluation of viral hepatitis [66], this procedure is still considered the "gold standard" for NAFLD. It can reliably differentiate NASH from NAFLD, assess the severity of steatosis, the activity (ballooning and lobular inflammation) and the fibrosis. It depicts other histological features related to NAFLD, identifies other possible etiologies for the liver disease and most importantly, provides prognostic factors [67]. However, the huge number of patients estimated to have NAFLD make the indication for LB in all patients impossible and probably without benefit for every individual case. The international guidelines recommend LB in selected cases, in those who would benefit the most from diagnostic, therapeutic, and prognostic perspective [68]. The EASL–EASD–EASO Clinical Practice Guidelines recommend that NASH be diagnosed by LB that depicts steatosis, hepatocyte ballooning and lobular inflammation. On the other hand, these guidelines strongly recommend LB when serum biomarkers/scores and/or elastography indicate advanced fibrosis and consider a repeat LB at 5 years in patients with high probability for progression of fibrosis [42]. The recommended score to be used in histological evaluation of NAFLD is SAF score (steatosis, activity and fibrosis) [67]. Despite the recommendations of clinical practice guidelines that LB must be performed to accurately diagnose NASH, not all the clinicians follow this rule. In fact, between 31%- 57% of health care providers in

the United States and 62% of French gastroenterologists are performing LB in NAFLD [69], whereas in Romania a lower percentage (17.6%) would perform a LB in the case of steatosis at ultrasound and persistent hepatocytolysis [70]. Moreover, in Romania a higher proportion of patients with NAFLD are reluctant to accept LB (refusal rate 60%), in comparison to only 22% in French patients [71].

## TREATMENT OF NAFLD/NASH

**Behavioural therapy**

A comprehensive approach of NAFLD should include from the beginning the behavioral risk factors and the intervention of these. The main behavioral intervention should be advised to sedentary people.

Several studies linked low levels of physical activity to NAFLD and in general with the metabolic syndrome. Indeed, most NAFLD patients do not practice enough sport or physical activities and have difficulties in daily activities [72]. On the other hand, diminished physical activity has a negative effect on NAFLD. Even the naps during the day, as a reflection of sedentarism, are associated with NAFLD [73]. It is important therefore to ask the NAFLD patients to increase progressively their physical activity, in order to reduce their liver steatosis.

Other targets of behavioral therapy are represented by dietary interventions and on smoking and drinking habits. Because the physical condition is associated with psychological factors, it is recommended to associate behavioral therapy also with cognitive interventions [74].

**Non-medical treatment**

Changes in lifestyle are recommended for all patients because an unhealthy lifestyle could lead to NAFLD [42]. Patients with NAFLD would benefit from their lifestyle changes by progressive weight loss through exercise, low fat and sugar diet, also fruit and vegetables intake [75]. In these patients, there is evidence that lifestyle changes may improve liver enzymes and steatosis measurement through US or other imaging methods [68]. Patients with NAFLD should exercise more, because it has a lowering effect on steatosis. One of the causes of NAFLD is insulin resistance. During aerobic exercise, insulin sensitivity is increased at the skeletal muscle, therefore lowering the insulin resistance and steatosis [76, 77].

All patients have to be advised to exercise moderately at least 30 minutes, five times in a week. Resistance training, moderate as well as high intensity training may improve liver enzymes and steatosis, no matter the amount of weight loss, even though the microscopic aspect is still unknown. Patients who are unable to exercise, are recommended to increase their daily footsteps up to 10,000 using a pedometer [78]. Physical activity is influencing the gut-liver axis including the enterohepatic flow of bile acids [79].

**Diet**

The best diet approach for NAFLD is still unknown [78]. In order to achieve the target weight, a person should loose 0.5-1 kg per week by consuming 600 Kcal less than his caloric needs in order to maintain his weight [80]. Patients with NAFLD should not consume saturated fats, simple carbohydrates and sugary drinks [81].

Instead of a low fat and high carbohydrate diet, a Mediterranean diet high in monounsaturated fatty acids is preferred, as it has been shown to reduce liver steatosis and improve the insulin sensitivity in non-diabetic patients [82]. A strict and controlled 12-month lifestyle by a dietician is proven better than a standard care regarding weight loss and NAFLD remission respectively [83].

**The role of the gut microbiota**

Gut microbiota is closely related to overweight, insulin resistance and subclinical inflammation [84]. An experimental ob/ob mice (model for NAFLD) study [85], using a combination of eight strains of bacteria did not only reduce liver fibrosis, but also had an antioxidant effect on advanced liver disease, even cirrhosis.

**Medical and surgical treatment (bariatric surgery)**

There are currently no specific pharmacologic therapies for NAFLD/NASH approved by regulatory agencies [86]. Since no medication is currently licensed for this indication, practitioners should be advised to avoid overdiagnosis and overtreatment of NAFLD/NASH, due to predictable negative outcomes, including physical harm through investigation and treatment, and psychosocial harms associated with disease labelling [87].

Pharmacotherapy should be reserved for patients with: 1) NASH and significant fibrosis (≥F2), 2) active NASH (persistently increased ALT, high necroinflammatory activity) [88] and 3) early NASH with risk factors for disease progression (age >50 years, T2DM, metabolic syndrome) [89, 42].

The improvement of histological lesions defining NASH (hepatic necroinflammation and/or fibrosis) is now accepted as a surrogate endpoint [90]. While no firm recommendations for treating NASH can be made, several therapeutic options with varying efficacy are available: insulin sensitizers, antioxidants, lipid-lowering agents, incretin-based drugs, weight loss medication, bariatric surgery and liver transplantation (Table III).

The prospective, randomized, placebo-controlled PIVENS trial found a significant benefit (improvement of steatosis, inflammation and ballooning) with oral *vitamin E* 800 IU daily vs. placebo for 2 years in non-diabetic patients with NASH (43% vs. 19%, p=0.001; number needed to treat, NNT=4.2) [91]. The potential beneficial effects of vitamin E in NASH should be weighted against concerns about long-term safety associated with ≥400 mg/day: increase in overall mortality [92], hemorrhagic stroke [93] and prostate cancer in males older than 50 [94].

*Thiazolidinediones* are peroxisome proliferator-activated receptor (PPAR) γ agonists with insulin-sensitizing effects. The same PIVENS study showed that pioglitazone improved all histological features of NASH, excepting fibrosis (34% vs. 19%, p=0.04, NNT=6.9) [91]. Current data support the use of glitazones to treat selected patients with NASH and T2DM, where the drug is registered. Although metformin use in NAFLD/NASH patients was associated with improvement in insulin resistance and aminotransferase levels, it failed to

improve histological paramenters [95]. However, because of its antidiabetic efficacy, metformin should be considered for patients with T2DM and NAFLD (it is safe even in cirrhotic patients and may protect against the development of HCC).

*Incretin-mimetic* drugs augment the meal-related insulin secretion and its extra-pancreatic effects. High-dose of liraglutide (3 mg daily), approved by FDA and EMA for T2DM and, recently, for primary management of obesity in patients without diabetes, has proven beneficial effects on NASH (ALT improvement and NASH remission without worsening of fibrosis) in a pilot study [96].

Preliminary data from small or uncontrolled studies suggested that n-3 *polyunsaturated fatty acids* (PUFA) might reduce liver fat and improve biochemistry [97], but trials assessing histological outcomes of PUFA therapy were negative [98]. *Statins*, used to reduce LDL-cholesterol and prevent cardiovascular risk, have not been adequately tested for this indication; their use in NASH is safe and significantly reduces aminotransferase levels [99]. *Pentoxifylline* improved steatosis, hepatocyte ballooning and decreased NAS≥2 points (NAFLD Activity Score) in small studies (posibly by reduction in lipid oxidation) and might be of benefit in NASH (38.5% pentoxifyllin vs. 13.8% placebo, p=0.036) [100].

High ferritin levels are commonly seen in NAFLD/NASH patients, in the presence of variable transferrin saturation and independent of gene polymorphisms of familial hemochromatosis. In these patients, *phlebotomy* programs to reduce iron stores met the histological endpoint (improvement in NAS score without worsening fibrosis) [101].

In the phase IIb FLINT trial, a 72-week course of therapy with 25 mg daily of *obeticholic acid*, a synthetic farnesoid X nuclear receptor ligand, improved NASH histology, including fibrosis, in non-cirrhotic NASH patients (45% treated vs. 21% in the placebo group). Main safety signals were increased low-density lipoprotein (LDL)-cholesterol and pruritus [102].

*Elafibranor* (an unlicensed dual agonist of PPARα/δ receptors) 120 mg daily for 1 year has been shown to induce resolution of NASH, without worsening fibrosis, in patients with NAS ≥4, in a phase IIb randomized placebo-controlled trial (20% vs. 11%, p=0.018) (GOLDEN 505). Elafibranor also resulted in the improvement of serum lipid levels and liver enzymes [103].

Promising novel agents with anti-inflammatory, antifibrotic or insulin sensitizing properties (dual PPARα/δ agonists, dual chemokine receptor CCR2/CCR5 antagonists and fatty acid/bile acid conjugates) and antifibrotic drugs (anti-lysyl oxidase-like [anti-LOXL2] monoclonal antibodies) are also being tested in late-phase ongoing randomised controlled trials in NASH.

Given the increasing prevalence and public health implications of NAFLD/NASH, although there are not licensed pharamacologic therapies, SRGH strongly recommends the use of *off-label* medication with beneficial effects and a good safety profile in non-cirrhotic NASH patients with/at risk of significant fibrosis and progression to cirrhosis. Vitamin E and pioglitazone are the only recommended therapies in selected patients according to guidelines. Additionally, SRGH suggests the use in clinical practice of some agents that are not recommended for NASH treatment in the current guidelines, such as liraglutide, metformin, pentoxifylline, UDCA, statins and ezetimibe, orlistat, but have shown good biochemical and histological response in selected patients and a good safety profile (Table III). Clinical efficacy and long-term safety of novel agents are pending and they should be incorporated in clinical practice as soon as positive results of phase III clinical trials will be available.

**Table III**. Pharmacologic treatment options in patients with NASH

| Cathegory | Drug | Mechanism of action | Benefits/recommendation |
|---|---|---|---|
| Biguanide | Metformin | Improve insulin sensitivity | Recommended for T2DM and NASH |
| Thiazolidinediones | Pioglitazone | Improve tissue insulin sensitivity through PPAR | (+) Recommneded for T2DM and NASH |
| Glucagon-like peptide-1 analogues | Liraglutide | Suppress appetite, promote weight loss and enhances endogeneous insulin production | Recommended in obese patients with T2DM and NASH |
| Antioxidants | Vitamin E | Reduce oxidative stress | (+) Recommended in NASH patients without diabetes |
| Phosphodiesterase inhibitor | Pentoxifylline | Raise c-AMP and reduces TNFα | Suggested in NASH |
| Bile acids | UDCA | Antioxidative efficacy | (-) Suggested |
| Statins | Atorvastatin | Lower plasma lipids | Suggested in patients with dislipidemia and NASH/NAFLD |
| Lipase inhibitors | Orlistat | Decreases fat absorbtion and reduces body weight | (-) Suggested in obese patients with NAFLD/NASH |
| Farnesoid X receptor agonists | *Obeticholic acid | Alters hepatic lipogenesis and reduces steatosis and inflammation | Suggested in NASH patients |
| PPARα/δ agonists | *Elafibranor | Reduces steatosis, inflammation and fibrosis | Suggested in NASH patients |

Based on the quality of evidence-based data, the strength of recommendations are as follows: Recommended denotes clear recommendation for selected patients with NAFLD/NASH (moderate quality of data, large number of patients, good safety profile); (+) Recommended denotes strong recommendation for selected patients with NAFLD/NASH (low-moderate quality of data, good safety profile, limited number of patients); Suggested denotes weak recommendation (low quality of evidence, low number of patients); (-) Suggested denotes very weak recommendation (low quality of evidence, low number of patients, inconclusive/conflictual results).*Ongoing phase III clinical trials.

In patients unresponsive to lifestyle changes and intensive pharmacotherapy, *bariatric surgery* can be effective in improving NASH, reducing weight and obesity-related metabolic complications, with stable results in the long term [104, 105]. Despite these results, bariatric surgery is currently only indicated for the management of obesity; the cost and invasiveness limits its evaluation as a primary treatment modality for NASH.

## CONCLUSIONS AND CONSENSUS HIGHLIGHTS

• The term NAFLD includes two distinct conditions with different histologic features and prognosis: NAFL and NASH, the second one with the highest risk of disease evolution to cirrhosis and its complications, including HCC.

• NAFL pathogenesis is complex. Insulin resistance triggers hepatotoxic insults (oxidative stress, lipotoxicity and mitochondrial dysfunction) that lead to hepatocellular injuries, inflammatory activation and fibrogenesis.

• NAFLD is considered the hepatic manifestation of metabolic syndrome. Therefore, NAFLD is associated not only with an increase of liver-related mortality, but also of overall mortality, especially cardiovascular and malignancies.

• Noninvasive techniques, such as biological tests and elastography, are used for the evaluation of NAFLD patients.

• Liver biopsy should be recommended in selected cases. Patients with NASH should be diagnosed by LB if it shows steatosis, hepatocyte ballooning and lobular inflammation. Liver biopsy is indicated when serum biomarkers/scores and/or elastography indicate advanced fibrosis and should be repeated at 5 years, in cases with high probability for progression of fibrosis.

• Patients with NAFLD would benefit from their lifestyle changes, by progressive weight loss through exercise and low fat and sugar diet.

• Pharmacotherapy should be reserved for patients with NASH, particularly for those with significant fibrosis. Until now, there are no FDA approved therapies for NASH. Available drugs (off-label use) are: vitamin E, pioglitazone, liraglutide, pentoxifilline, obeticholic acid. Bariatric surgery is a solution only for morbidly obese patients.

**Conflicts of interest**: I.S.: speaker or advisory board: Philips, General Electric, Siemens, Canon Toshiba, Worwag Pharma, AbbVie, BMS, Janssen, MSD, Sanofi, Neola; AP: speaker Philips, General Electric, Abbvie, AstraZeneca, Zentiva, Neola, Dr. Reddy, BMS, MSD, Mylan. D.D.: speaker or advisory board: Sanofi, Worwag Pharma; C.B.: speaker or advisory board Abbvie, Zentiva, Roche, Bayer,MSD, Gilead; L.N.: no conflict of interest; A.T.:No conflict of interest; L.G.: speaker or advisory board Sanofi; CFB: speaker or advisory board Sanofi, Worwag Pharma.

**Authors' contribution**: I.S. gave the idea, distributed the tasks, corrected the draft; A.P., D.L.D., C.B., L.N., A.T., L.G., C.F.B. wrote each a chapter of this review and contributed to the editing of the main manuscript; all authors approved the final version.

## REFERENCES


1. Adams LA, Lindor DL. Nonalcoholic Fatty Liver Disease. Ann Epidemiol 2007;17:863-869. doi:10.1016/j.annepidem.2007.05.013
2. Younossi ZM, Koenig AB, Abdelatif D, Fazel Y, Henry L, Wymer M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence andoutcomes. Hepatology 2016;64:73-84. doi:10.1002/hep.28431
3. NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet 2016;387:1377-1396. doi:10.1016/S0140-6736(16)30054-X
4. Kaswala DH, Lai M, Afdhal NH. Fibrosis Assessment in Nonalcoholic Fatty Liver Disease (NAFLD) in 2016. Dig Dis Sci 2016;61:1356-1364. doi:10.1007/s10620-016-4079-4
5. Lazo M, Clark JM. The epidemiology of nonalcoholic fatty liver disease: a global perspective. Semin Liver Dis 2008;28:339-350. doi:10.1055/s-0028-1091978
6. Szczepaniak LS, Nurenberg P, Leonard D, et al. Magnetic resonance spectroscopy to measure hepatic triglycedide content: prevalence of hepatic steatosis in the general population. Am J Physiol Endocrinol Metab 2005;288:E462-E468. doi:10.1152/ajpendo.00064.2004
7. Younossi ZM, Stepanova M, Afendy M, et al. Changes in the prevalence of the most common causes of chronic liver diseases in the United States from 1988 to 2008. Clin Gastroenterol Hepatol 2011;9:524-530. e1. doi:10.1016/j.cgh.2011.03.020
8. Bellentani S. The epidemiology of non-alcoholic fatty liver disease. Liver Int 2017;37 Suppl 1:81-84. doi:10.1111/liv.13299
9. Vernon G, Baranova A, Younossi ZM. Systematic review: the epidemiology and natural history of non-alcoholic fatty liver disease and nonalcoholic steatohepatitis in adults. Aliment Pharmacol Ther 2011;34:274-285. doi:10.1111/j.1365-2036.2011.04724.x
10. Haentjens P, Massaad D, Reynaert H, et al. Identifying non-alcoholic fatty liver disease among asymptomatic overweight and obese individuals by clinical and biochemical characteristics. Acta Clin Belg 2009;64:483-493. doi: 10.1179/acb.2009.084
11. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol 2006;45:600-606. doi: 10.1016/j.jhep.2006.06.013
12. Prashanth M, Ganesh HK, Vima MV, et al. Prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. J Assoc Physicians India 2009;57:205-210.
13. Williamson RM, Price JF, Glancy S, et al. Prevalence of and risk factors for hepatic steatosis and nonalcoholic fatty liver disease in people with type 2 diabetes: the Edinburgh type 2 diabetes study. Diabetes Care 2011;34:1139-1144. doi:10.2337/dc10-2229
14. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. Dig Dis Sci 2000;45:1929-1934. doi:10.1023/A:1005661516165
15. Radu C, Grigorescu M, Crisan D, Lupsor M, Constantin D, Dina L. Prevalence and associated risk factors of non-alcoholic fatty liver disease in hospitalized patients. J Gastrointestin Liver Dis 2008;17:255-260.
16. Silaghi CA, Silaghi H, Colosi HA, et al. Prevalence and predictors of non-alcoholic fatty liver disease as defined by the fatty liver index in a type 2 diabetes population. Clujul Med 2016;89:82–88. DOI:10.15386/CJMED-544

17. Livadariu R, Timofte D, Danilă R, et al. Nonalcoholic fatty liver disease and its complications – assessing the population at risk. A small series report and literature review. Rev Med Chir Soc Med Nat Iaşi 2015;119:346-352.
18. Popa S, Mota M, Popa A, et al. Prevalence of overweight/obesity, abdominal obesity and metabolic syndrome and atypical cardiometabolic phenotypes in the adult Romanian population: PREDATOR study. J Endocrinol Invest 2016;39:1045-1053. doi:10.1007/s40618-016-0470-4
19. Angulo P. Nonalcoholic fatty liver disease. N Engl J Med 2002;346:1221-1231. doi:10.1056/NEJMra011775
20. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis 2001;21:17-26. doi:10.1055/s-2001-12925
21. Dyson J, Jaques B, Chattopadyhay D, et al. Hepatocellular cancer: the impact of obesity, type 2 diabetes and a multidisciplinary team. J Hepatol 2014;60:110-117. doi:10.1016/j.jhep.2013.08.011
22. McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: Implications for prognosis and clinical management. J Hepatol 2015;62:1148-1155. doi:10.1016/j.jhep.2014.11.034
23. Singh S, Allen AM, Wang Z, Prokop LJ, Murad MH, Loomba R. Fibrosis progression in nonalcoholic fatty liver vs nonalcoholic steatohepatitis: a systematic review and meta-analysis of paired-biopsy studies. Clin Gastroenterol Hepatol 2015;13:643-654.e1-9. doi:10.1016/j.cgh.2014.04.014
24. Perumpail RB, Wong RJ, Ahmed A, Harrison SA. Hepatocellular Carcinoma in the Setting of Non-cirrhotic Nonalcoholic Fatty Liver Disease and the Metabolic Syndrome: US Experience. Dig Dis Sci 2015;60:3142-3148. doi:10.1007/s10620-015-3821-7
25. Ekstedt M, Hagström H, Nasr P, et al. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547-1554. doi:10.1002/hep.27368
26. Stepanova M, Rafiq N, Makhlouf H, et al. Predictors of all-cause mortality and liver-related mortality in patients with non-alcoholic fatty liver disease (NAFLD). Dig Dis Sci 2013;58:3017-1723. doi:10.1007/s10620-013-2743-5
27. Dulai PS, Singh S, Patel J, et al. Increased risk of mortality by fibrosis stage in non-alcoholic fatty liver disease: Systematic review and meta-analysis. Hepatology 2017;65:1557-1565. doi:10.1002/hep.29085
28. Chalasani N, Younossi Z, Lavine JE, et al. The Diagnosis and Management of Nonalcholic Fatty Liver Disease: Practice Guidance from the American Association from the Study of Liver Disease. Hepatology 2018;67:328-357. doi:10.1002/hep.29367
29. Byrne CD, Tangher G. NAFLD a multisystemic disease. J Hepatol 2016;62(1 Suppl):S47-S64. doi:10.1016/j.jhep.2014.12.012
30. Machado MY, Cortez-Pinto H. Non-alcoholic Fatty Liver Disease. What the clinician needs to know. World J Gastroenterol 2014;20:12956-12980. doi:10.3748/wjg.v20.i36.12956
31. Rotman Y, Sanyal AJ. Current and upcoming pharmacotherapy for non-alcoholic fatty liver disease. Gut 2017;66:180-190. doi:10.1136/gutjnl-2016-312431
32. Fernández-Sánchez A, Madrigal-Santillán E, Bautista M, et al. Inflammation, oxidative stress, and obesity. Int J Mol Sci 2011;12:3117–3132. doi:10.3390/ijms12053117
33. Jin X, Yu CH, Lv GC, Li YM. Increased intestinal permeability in pathogenesis and progress of nonalcoholic steatohepatitis in rats. World J Gastroenterol 2007;13:1732-1736. doi:10.3748/wjg.v13.i11.1732
34. Richardson MM, Jonsson JR, Powell EE, et al. Progressive fibrosis in nonalcoholic steatohepatitis: association with altered regeneration and a ductular reaction. Gastroenterology 2007;133:80-90. doi:10.1053/j.gastro.2007.05.012
35. Amzolini AM, Fortofoiu M, Tudorica-Micu SE, et al. Genetic Factors Involved in the Development and Progression of Non-alcoholic Fatty Liver Disease. Curr Health Sci J 2015;41:297-301. doi: 10.12865/CHSJ.41.04.01
36. Sookoian S, Pirola CJ. Genetic predisposition in Non-alcoholic Liver Disease. Clin Mol Hepatol 2017;23:1-12. doi:10.3350/cmh.2016.0109
37. Macavei B, Baban A, Dumitrascu DL. Psychological factors associated with NAFLD/NASH: a systematic review. Eur Rev Med Pharmacol Sci 2016;20:5081-5097.
38. Tilg H. How to approach a Patient with nonalcoholic fatty liver disease. Gastroenterology 2017;153:345-349. doi:10.1053/j.gastro.2017.06.016
39. Lupu D, Albu A, Dumitrascu DL. Low-grade Inflammation: a Linking mechanism between anxiety and metabolic syndrome? In: Dumitrascu DL, Soellner W. (Eds.). Proceedings of the Annual Meeting of the EAPM 2014 (25-28 June 2014, Sibiu, Romania), Medimond Bologna 2014:162-166.
40. Weinstein G, Zelber-Sagi S, Preis SR, et al. Association of nonalcoholic fatty liver disease with lower brain volume in healthy middle-aged adults in the Framingham Study. JAMA Neurol. 2018;75:97-104. doi:10.1001/jamaneurol.2017.3229
41. Filipović B, Marković O, Đurić V, Filipović B. Cognitive Changes and Brain Volume Reduction in Patients with Nonalcoholic Fatty Liver Disease. Can J Gastroenterol Hepatol 2018:9638797. doi:10.1155/2018/9638797
42. European Association for the Study of the Liver (EASL), European Association for the Study of Diabetes (EASD), European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402. doi:10.1016/j.jhep.2015.11.004
43. Cusi K, Chang Z, Harrison S, et al. Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease. J Hepatol 2014;60:167-174. doi:10.1016/j.jhep.2013.07.042
44. Kwok R, Tse YK, Wong GL, et al. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease–the role of transient elastography and plasma cytokeratin-18 fragments. Aliment Pharmacol Ther 2014;39:254-269. doi:10.1111/apt.12569
45. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del Higado. EASL-ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis. European Association for Study of Liver; Asociacion Latinoamericana para el Estudio del Higado. J Hepatol 2015;63:237-264. doi:10.1016/j.jhep.2015.04.006
46. Poynard T, Munteanu M, Deckmyn O, et al. Applicability and precautions of use of liver injury biomarker FibroTest. A reappraisal at 7 years of age. BMC Gastroenterol 2011;11:39. doi:10.1186/1471-230X-11-39
47. Cales P, Veillon P, Konate A, et al. Reproducibility of blood tests of liver fibrosis in clinical practice. Clin Biochem 2008;41:10-18. doi:10.1016/j.clinbiochem.2007.08.009
48. Castera L, Vilgrain V, Angulo P. Noninvasive evaluation of NAFLD. Nat Rev Gastroenterol Hepatol 2013;10:666-675. doi:10.1038/nrgastro.2013.175

49. Borges VF, Diniz AL, Cotrim HP, Rocha HL, Andrade NB. Sonographic hepatorenal ratio: a noninvasive method to diagnose nonalcoholic steatosis. J Clin Ultrasound 2013;41:18-25. doi:10.1002/jcu.21994
50. Younossi ZM, Gramlich T, Matteoni CA, Boparai N, McCullough AJ. Nonalcoholic fatty liver disease in patients with type 2 diabetes. Clin Gastroenterol Hepatol 2004;2:262-265. doi:10.1016/S1542-3565(04)00014-X
51. Palmentieri B, de Sio I, La Mura V et al. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. Dig Liver Dis 2006;38:485-489. doi:10.1016/j.dld.2006.03.021
52. Hernaez R, Lazo M, Bonekamp S, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082-1090. doi:10.1002/hep.24452
53. Lupşor-Platon M, Feier D, Stefănescu H, et al. Diagnostic accuracy of controlled attenuation parameter measurement by transient elastography for non-invasive assessment of liver steatosis: a prospective study. J Gastrointestin Liver Dis 2015;24:35-42. doi:10.15403/jgld.2014.1121.mlp
54. de Ledinghen V, Vergniol J, Foucher J, Merrouche W, le Bail B. Non-invasive diagnosis of liver steatosis using controlled attenuation parameter (CAP) and transient elastography. Liver Int 2012;32:911-918. doi:10.1111/j.1478-3231.2012.02820.x
55. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. J Hepatol 2017;66:1022-1030. doi:10.1016/j.jhep.2016.12.022
56. Xu L, Lu W, Li P, Shen F, Mi YQ, Fan JG. A comparison of hepatic steatosis index, controlled attenuation parameter and ultrasound as noninvasive diagnostic tools for steatosis in chronic hepatitis B. Dig Liver Dis 2017;49:910-917. doi:10.1016/j.dld.2017.03.013
57. Romero-Gómez M, Cortez-Pinto H. Detecting liver fat from viscoelasticity: How good is CAP in clinical practice? The need for universal cut-offs. J Hepatol 2017;66:886-887. doi:10.1016/j.jhep.2017.01.029
58. Dietrich CF, Bamber J, Berzigotti A, et al. EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, update 2017 (Long Version). Ultraschall Med 2017;38:e16-e47. doi:10.1055/s-0043-103952
59. Wong VW, Vergniol J, Wong GL, et al. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. Hepatology 2010;51:454-462. doi:10.1002/hep.23312
60. Wong VW, Vergniol J, Wong GL, et al. Liver stiffness measurement using XL probe in patients with nonalcoholic fatty liver disease. Am J Gastroenterol 2012;107:1862-1871. doi:10.1038/ajg.2012.331
61. Hermann E, de Lédinghen V, Cassinotto C, et al. Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis. Hepatology 2018;67:260-272. doi:10.1002/hep.29179
62. Cassinotto CH, Boursier J, de Lédinghen V, et al. Liver stiffness in nonalcoholic fatty liver disease: A comparison of Supersonic Shear Imaging, FibroScan and ARFI with liver biopsy. Hepatology 2016;63:1817-1827. doi:10.1002/hep.28394
63. Wang QB, Zhu H, Liu HL, Zhang B. Performance of magnetic resonance elastography and diffusion-weighted imaging for the staging of hepatic fibrosis: A meta-analysis. Hepatology 2012;56:239-247. doi:10.1002/hep.25610
64. Singh S, Venkatesh SK, Wang Z, et al. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. Clin Gastroenterol Hepatol 2015;13:440-451.e6. doi:10.1016/j.cgh.2014.09.046
65. Loomba R, Wolfson T, Ang B, et al. Magnetic resonance elastography predicts advanced fibrosis in patients with nonalcoholic fatty liver disease: a prospective study. Hepatology 2014;60:1920-1928. doi:10.1002/hep.27362
66. Trifan A, Stanciu C. Checkmate to liver biopsy in chronic hepatitis C? World J Gastroenterol 2012;18:5514-5520. doi:10.3748/wjg.v18.i39.5514
67. Bedossa P, FLIP Pathology Consortium. Utility and appropriateness of the fatty liver inhibition of progression (FLIP) algorithm and steatosis, activity, and fibrosis (SAF) score in the evaluation of biopsies of nonalcoholic fatty liver disease. Hepatology 2014;60:565-575. doi:10.1002/hep.27173
68. Chalasani N, Younossi Z, Lavine JE, et al. The Diagnosis and Management of Non-Alcoholic Fatty Liver Disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology 2012;55:2005-2023. doi:10.1002/hep.25762
69. Harrison SA, NASH management. Managing liver disease-from the clinic to the community. AASLD Postgraduate Course 2015:34-39.
70. Iacob S, Ester C, Lita M, Ratziu V, Gheorghe L. Real-life Perception and Practice Patterns of NAFLD/NASH in Romania: Results of a Survey Completed by 102 Board-certified Gastroenterologists. J Gastrointestin Liver Dis 2016;25:183-189. doi:10.15403/jgld.2014.1121.252.naf
71. Ratziu V, Cadranel JF, Serfaty L, et al. A survey of patterns of practice and perception of NAFLD in a large sample of practicing gastroenterologists in France. J Hepatol 2012;57:376-383. doi:10.1016/j.jhep.2012.03.019
72. Elliott C, Frith J, Day CP, Jones DE, Newton JL. Functional impairment in alcoholic liver disease and non-alcoholic fatty liver disease is significant and persists over 3 years of follow-up. Dig Dis Sci 2013;58:2383-2391. doi:10.1007/s10620-013-2657-2
73. Qu H, Wang H, Deng M, Wei H, Deng H. Associations between longer habitual day napping and non-alcoholic fatty liver disease in an elderly Chinese population. PLoS One 2014;9:e105583. doi:10.1371/journal.pone.0105583
74. Surdea-Blaga T, Dumitrașcu DL. Depression and anxiety in nonalcoholic steatohepatitis: is there any association? Rom J Intern Med 2011;49:273-280.
75. Borrelli A, Bonelli P, Tuccillo FM, et al. Role of gut microbiota and oxidative stress in the progression of nonalcoholic fatty liver disease to hepatocarcinoma: Current and innovative therapeutic approaches. Redox Biol 2018;15:467-479. doi:10.1016/j.redox.2018.01.009
76. Kirwan JP, Solomon TP, Wojta DM, Staten MA, Holloszy JO. Effects of 7 days of exercise training on insulin sensitivity and responsiveness in type 2 diabetes mellitus. Am J Physiol Endocrinol Metab 2009;297:E151-E156. doi:10.1152/ajpendo.00210.2009
77. Van Der Heijden GJ, Wang ZJ, Chu Z, et al. Strength exercise improves muscle mass and hepatic insulin sensitivity in obese youth. Med Sci Sport Exerc 2010;42:1973-1980. doi:10.1249/MSS.0b013e3181df16d9
78. Dyson JK, Anstee QM, McPherson S. Non-alcoholic fatty liver disease: a practical approach to treatment. Frontline Gastroenterol 2014;5:277-286. doi:10.1136/flgastro-2013-100404
79. Molina-Molina E, Lunardi Baccetto R, Wang DQ, de Bari O, Krawczyk M, Portincasa P. Exercising the hepatobiliary-gut axis. The impact of physical activity performance. Eur J Clin Invest 2018;48:e12958. doi:10.1111/eci.12958
80. NICE. NICE Clinical guidelines. CG43 Obesity: the prevention, identification, assessment and management of overweight and obesity in adults and children. In: Care NIfHaCEaNCCfP, ed. 2006.

81. Zivkovic AM, German JB, Sanyal AJ. Comparative review of diets for the metabolic syndrome: implications for nonalcoholic fatty liver disease. Am J Clin Nutr 2007;86:285-300. doi:10.1093/ajcn/86.2.285
82. Ryan MC, Itsiopoulos C, Thodis T, et al. The Mediterranean diet improves hepatic steatosis and insulin sensitivity in individuals with non-alcoholic fatty liver disease. J Hepatol 2013;59:138-143. doi:10.1016/j.jhep.2013.02.012
83. Wong VW, Chan RS, Wong GL, et al. Community-based lifestyle modification programme for non-alcoholic fatty liver disease: a randomized controlled trial. J Hepatol 2013;59:536-542. doi:10.1016/j.jhep.2013.04.013
84. Backhed F, Ding H, Wang T, et al. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004;101:15718-1523. doi:10.1073/pnas.0407076101
85. Li Z, Yang S, Lin H, et al. Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343-350. doi:10.1053/jhep.2003.50048
86. Ratziu V, Goodman Z, Sanyal A. Current efforts and trends in the treatment of NASH. J Hepatol 2015;62(1 Suppl):S65-S75. doi:10.1016/j.jhep.2015.02.041
87. Rowe IA. Too much medicine: overdiagnosis and overtreatment of non-alcoholic fatty liver disease. Lancet Gastroenterol Hepatol 2018;3:66-72. doi:10.1016/S2468-1253(17)30142-5
88. Adams LA, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. J Hepatol 2005;42:132-138. doi:10.1016/j.jhep.2004.09.012
89. Sanyal AJ, Friedman SL, McCullough AJ, Dimick-Santos L; American Association for the Study of Liver Diseases; United States Food and Drug Administration. Challenges and opportunities in drug and biomarker development for nonalcoholic steatohepatitis: findings and recommendations from an American Association for the Study of Liver Diseases – U.S. Food and Drug Administration Joint Workshop. Hepatology 2015;61:1392-1405. doi:10.1002/hep.27678
90. Ratziu V. A critical review of endpoints for non-cirrhotic NASH therapeutic trials. J Hepatol 2018;68:353-361. doi:10.1016/j.jhep.2017.12.001
91. Sanyal AJ, Chalasani N, Kowdley KV, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675-1685. doi:10.1056/NEJMoa0907929
92. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. JAMA 2007;297:842-857. doi:10.1001/jama.297.8.842
93. Schurks M, Glynn RJ, Rist PM, Tzourio C. Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomized controlled trials. BMJ 2010;341:c5702. doi:10.1136/bmj.c5702
94. Klein EA, Thompson IM Jr, Tangen CM, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). JAMA 2011;306:1549-1556. doi:10.1001/jama.2011.1437
95. Li Y, Liu L, Wang B, Wang J, Chen D. Metformin in non-alcoholic fatty liver disease: A systematic review and meta-analysis. Biomed Rep 2013;1:57-64. doi:10.3892/br.2012.18
96. Armstrong MJ, Gaunt P, Aithal GP, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, placebo-controlled phase 2 study. Lancet 2016;387:679-690. doi:10.1016/S0140-6736(15)00803-X
97. Parker HM, Johnson NA, Burdon CA, Cohn JS, O'Connor HT, George J. Omega-3 supplementation and non-alcoholic fatty liver disease: a systematic review and meta-analysis. J Hepatol 2012;56:944-951. doi:10.1016/j.jhep.2011.08.018
98. Argo CK, Patrie JT, Lackner C, et al. Effects of n-3 fish oil on metabolic and histological parameters in NASH: a double-blind, randomized, placebo-controlled trial. J Hepatol 2015;62:190-197. doi:10.1016/j.jhep.2014.08.036
99. Dongiovanni P, Petra S, Mannisto V, et al. Statin use and nonalcoholic steatohepatitis in at risk individuals. J Hepatol 2015;63:705-712. doi:10.1016/j.jhep.2015.05.006
100. Zein CO, Yerian LM, Gogate P, et al. Pentoxifylline improves nonalcoholic steatohepatitis: a randomized placebo-controlled trial. Hepatology 2011;54:1610-1619. doi:10.1002/hep.24544
101. Valenti L, Fracanzani AL, Dongiovanni P, et al. A randomized trial of iron depletion in patients with nonalcoholic fatty liver disease and hyperferritinemia. World J Gastroenterol 2014;20:3002-3010. doi:10.3748/wjg.v20.i11.3002
102. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhostic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015;385:956-65. doi:10.1016/S0140-6736(14)61933-4
103. Ratziu V, Harrison SA, Francque S, et al; GOLDEN-505 Investigator Study Group. Elafibranor, an agonist of the peroxisome proliferator-activated receptor-alpha and –delta, induces resolution of nonalcoholic steatohepatitis without fibrosis worsening. Gastroenterology 2016;150:1147-1159.e5. doi:10.1053/j.gastro.2016.01.038
104. Schauer PR, Bhatt DL, Kirwan JP, et al. Bariatric surgery versus intensive medical therapy for diabetes: 3-year outcomes. N Engl J Med 2014;370):2002-2013. doi:10.1056/NEJMoa1401329
105. Taitano AA, Markow M, Finan JE, Wheeler DE, Gonzalvo JP, Murr MM. Bariatric surgery improves histological features of nonalcoholic fatty liver disease and liver fibrosis. J Gastrointest Surg 2015;19:429-436. doi:10.1007/s11605-014-2678-y