Nonalcoholic Fatty Liver Disease: Status Quo

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INTRODUCTION

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: ursodeoxycholic acid; US: ultrasound.
there are about 1 billion obese people in the world and about 380 million 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, internists, 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 commissioned 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 NASH**

The natural history of NAFLD is still unknown and unpredictable. Over the years, the whole spectrum of NAFLD has varied, steatosis with non-specific 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 non-specific 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: lifestyle and diet, microbiota, systemic inflammation, association with obesity and diabetes, which all induce cognitive alterations, etc. [38, 39].

<table>
<thead>
<tr>
<th>Type of factor</th>
<th>Psychosocial factor</th>
<th>Clinical influence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Emotional</td>
<td>anxiety</td>
<td>severity</td>
</tr>
<tr>
<td></td>
<td>depression</td>
<td></td>
</tr>
<tr>
<td>Cognitive</td>
<td>confidence in exercise, perceived benefit of exercise, readiness to change (in contemplative stage); cognitive dysfunctions: memory impairment, attention deficit</td>
<td>resistance to therapy</td>
</tr>
</tbody>
</table>

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 efficacy 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 echogenicity is a supplementary indicator of steatosis [49]. A semi-quantitative appreciation 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]  

<table>
<thead>
<tr>
<th>Biological test and the biomarkers included in their formula</th>
<th>AUROC</th>
<th>Cut offs</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>APRI= AST (/ULN)/platelet (109/L) x 100</td>
<td>0.82</td>
<td>1.0</td>
<td>31%</td>
<td>95%</td>
</tr>
<tr>
<td>BARD score (BMI≥28=1, AST/ALT≥0.8, 2; DM=1; score≥2 – odds ratio for advanced fibrosis =17)</td>
<td>0.81</td>
<td>&lt;2</td>
<td>-</td>
<td>96%</td>
</tr>
<tr>
<td>FIB-4 = age (yr) x AST [UL] / (platelets [109/L] x √ALT [UL])</td>
<td>0.80</td>
<td>&lt;1.30</td>
<td>43%</td>
<td>90%</td>
</tr>
<tr>
<td>(years=1; no=0) + 0.9 9 x AST/ALT ratio - 0.013 x platelet count (x109/L - 0.66 x albumin [g/dl])</td>
<td>0.88</td>
<td>&lt;1.455</td>
<td>56%</td>
<td>93%</td>
</tr>
<tr>
<td>Fibrotest = Fibrotest® (Biopredictive, Paris, France) patented formula combining α-2-microglobulin, GGT, apolipoprotein A1, haptoglobin, total bilirubin, age and gender</td>
<td>0.81-0.92</td>
<td>&gt;0.30</td>
<td>33%</td>
<td>98%</td>
</tr>
<tr>
<td>NAFLD fibrosis score (NFS) = (-1,675 + 0.037 x age (years) x BMI (kg/m²) + 1.13 x IFG/DM (yes=1, no=0) + 0.9 9 x AST/ALT ratio - 0.013 x platelet count (x109/L - 0.66 x albumin [g/dl])</td>
<td>0.88</td>
<td>&gt;0.676</td>
<td>90%</td>
<td>85%</td>
</tr>
</tbody>
</table>

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.

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 NAFLD 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 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.

Controlled 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 objective evaluation of liver steatosis). A recent, CAP was implemented in M and XL probes (for normal and severe steatosis [53-55]. Some cut-off values were proposed 0.865 (95%CI: 0.850-0.880) for the prediction of moderate and severe steatosis [53-55]. 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).

Controlled 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/m² [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: 83% and 85%, respectively; and for cirrhosis: 92% and 92%, respectively (showing an increasing performance with the severity of fibrosis).
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 lose 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

J Gastrointestin Liver Dis, December 2018 Vol. 27 No 4: 439-448
improve histological parameters [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 (possibly 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, antibiotic 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 pharmacologic 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

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

*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.


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.

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J Gastrointestin Liver Dis, December 2018 Vol. 27 No 4: 439-448