# Diagnostic methods and pharmacological management of non-alcoholic fatty liver disease
## There is still room for improvement

**Chrysoula Boutari[1], Panagiotis Pappas[2], Konstantinos Tziomalos[3], Vasileios Athyros[1], Asterios Karagiannis[1]**

[1]Second Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Hippocration Hospital, Thessaloniki, Greece
[2]424 General Military Hospital, Thessaloniki, Greece
[3]First Propedeutic Department of Internal Medicine, Medical School, Aristotle University of Thessaloniki, Thessaloniki, Greece

## ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis in the absence of excessive alcohol consumption and it may progress to non-alcoholic steatohepatitis, cirrhosis, liver failure, and cancer. Despite the alarming rate of the disease observed in the last decades due to the increase in the prevalence of the metabolic syndrome,there are unmet needs in the diagnosis and the management of the disease. The gold standard for the diagnosis of the disease remains the liver biopsy. The ultrasound, the laboratory tests (serum aminotransferases, gamma-glutamyl transpeptidase, and ferritin levels, cytokeratin-18 fragment and fibrosis growth factor 21), the clinical (Fatty Liver Index [FLI], NAFLD fibrosis score [NFS] and Fibrosis 4 calculator [FIB-4]) and the non-invasive scores (NIS-4, FS3) are being developed and evaluated and they are very promising for both detecting the presence and assessing the severity of the disease. For the management of NASH, no pharmacological treatment has been approved. Vitamin E, thiazolidinediones, sodium-glucose transport protein-2 (SGLT-2) inhibitors, inhibitors of dipeptidyl peptidase 4 (DPP-4), glucagon-like peptide-1 receptor agonists (GLP-1 RAs), statins, and many other agents under investigation may have a role in the management of NAFLD alongside their primary indications.

**KEY WORDS:** *Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis, non-invasive diagnostic methods, pharmacotherapy*

**Corresponding author:**
Chrysoula Boutari, MD, PhD
Second Propedeutic Department of Internal Medicine
Hippocration Hospital
49 Konstantinoupoleos str., 54642, Thessaloniki, Greece
Tel.: +30 2310 892071; Fax: +30 2310 992834
E-mail: chrisoulabgr@yahoo.gr

## 1. INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is characterized by hepatic steatosis in the absence of excessive alcohol consumption. It may progress to nonalcoholic

steatohepatitis (NASH, characterized by the presence of varying degrees of inflammation and fibrosis) and cirrhosis[1,2]. Simple steatosis is generally considered as a benign condition, whereas steatohepatitis may progress to cirrhosis, liver failure, and cancer.

The overall prevalence of the disease worldwide is 25-30% in the general population, reaching 70-90% in specific populations, e.g. patients with type 2 diabetes mellitus (T2DM) and morbidly obese individuals[3]. The overall prevalence of NAFLD is 15-40% in western countries while 9-40% in Asian countries[4]. Its pathogenesis is related to several factors like genetic polymorphisms, unhealthy dietary patterns, lack of physical activity, insulin resistance (IR), dysbiosis of gut microbiota, and endocrine abnormalities[3]. Thus, the global increase in its prevalence comes as a result of the rapid increase in the prevalence of obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS)[3]. However, the exact pathophysiology is not clear, and this may be one reason why there is no established treatment for such an emerging epidemic.

Patients with NAFLD (particularly those with NASH) often have one or more components of the MetS, such as obesity, systemic hypertension, dyslipidemia, IR or overt diabetes. There is also a variety of conditions that have been associated with NAFLD, independent of their associations with obesity, including polycystic ovary syndrome, hypothyroidism, obstructive sleep apnea, hypopituitarism, and hypogonadism[5].

Despite the alarming rate of NAFLD, there are limitations in knowledge and unmet needs in the diagnosis and the management of the disease among medical providers. Completed and ongoing clinical trials have mainly focused on the timely and safe diagnosis and the treatment of NASH, since this has been mainly associated with higher morbidity and mortality[6].

## 2. DIAGNOSTIC METHODS

The gold standard for the diagnosis of NAFLD remains the liver biopsy. This method permits both the evaluation of the determination of the severity and the staging of the liver injury[7]. This method is an invasive procedure, limited by cost, sampling error, and occasional morbidity and mortality. For these reasons, the non-invasive diagnostic methods seem very attractive and useful for the diagnosis of NAFLD.

The ultrasound is a low-cost imaging method that is broadly available[7]. It often reveals a hyperechoic texture or a bright liver because of diffuse fatty infiltration[8]. However, the specificity and the sensitivity of this technique appears to be decreased and, thus, it cannot be utilized as a diagnostic tool for monitoring the progression of the disease[9]. Magnetic resonance spectroscopy (MRS) allows a non-invasive and real-time detection of hepatic fat, cell membrane and energy metabolism and it is an effective tool to quantify liver steatosis and assess hepatic lipid composition[10], although it is not sensitive enough to detect inflammation or fibrosis[11] and not widely available[10]. Vibration controlled transient elastography measures the speed of a mechanically generated shear wave across the liver to derive a liver stiffness measurement (LSM), a marker of hepatic fibrosis. Measuring the attenuation of ultrasound signal through the liver is used to derive the Controlled Attenuation Parameter (CAP), which is measured simultaneously with LSM as a marker of hepatic steatosis. It is a low cost and routinely used tool, which is used to grade fibrosis based on liver stiffness. It is also able to grade hepatic steatosis but the recognition of early fibrosis stages or the detection of hepatic necroinflammatory changes seems to be limited[12].

As regards the laboratory tests, although the serum aminotransferases, gamma-glutamyl transpeptidase, and ferritin levels are often abnormal in NAFLD, this does not always occur in NAFLD and sometimes they may be normal[13]. Other extensively examined biomarkers like cytokeratin-18 fragment and fibrosis growth factor 21, they are considered as indices which are impaired in NAFLD, since they are related to hepatocellular apoptotic activity, oxidative stress, and inflammation[14]. Nevertheless, they are not able to differentiate steatohepatitis from simple steatosis[15,16].

Currently, the clinical scores, under development and evaluation, are very promising and may be used not only to detect the presence but also to assess the severity of the disease. To assess the presence of steatosis the European Association for the Study of the Liver (EASL) guidelines[7] mention the Fatty Liver Index (FLI) and the NAFLD liver fat score. Both of these scores result from common blood tests and clinical information. Specifically, FLI is calculated from serum triglyceride, body mass index, waist circumference, and gamma-glutamyltransferase[17], and NAFLD liver fat score is calculated evaluating the presence/absence of MetS and T2DM, fasting serum insulin, and aminotransferases[18]. They have been validated in a cohort of severely obese patients and the general population, reliably predicting the presence of steatosis, but not its severity[19]. Nevertheless, according to the American Association for the Study of Liver Diseases (AASLD) guidelines, only inflammation and fibrosis determine the prognosis of NAFLD patients, and highlight the lack of evidence of the usefulness of quantifying hepatic steatosis in the routine clinical setting. Instead, they recommend that the simultaneous presence of several metabolic diseases is the most potent predictor of hepatic inflammation and

adverse outcome in patients with NAFLD[20].

Liver fibrosis determines the liver-related outcomes and, thus, patients with advanced fibrosis need closer monitoring. For this purpose, the enhanced liver fibrosis (ELF) blood test, which combines three serum biomarkers: hyaluronic acid (HA), procollagen III amino terminal peptide (PIIINP), and tissue inhibitor of metalloproteinase 1 (TIMP-1), has been recommended to detect advanced fibrosis[21]. NAFLD fibrosis score (NFS) (a non-invasive scoring system based on age, body mass index, impaired fasting glucose or T2DM, aspartate/alanine aminotransferase (AST/ALT) ratio, platelets and albumin) and Fibrosis 4 calculator (FIB-4) (which helps to estimate the amount of scarring in the liver using age, AST and ALT levels and platelet count) have shown the best predictive value for advanced fibrosis among histological proven NAFLD patients in comparison with other scores[22]. However, these scores are useful for excluding the presence of significant fibrosis and not for grading that. It is also noteworthy that the results of these scores vary with age and are not so accurate in patients with diabetes or subjects with reduced body mass index[22].

Recently, non-invasive scores have been developed and validated. The NIS4 is a score based on miR-34a, Alpha2-macroglobulin, YKL-40, and HbA1c, used to detect patients with active NASH and significant fibrosis[22]. The FS3 combines two physical parameters (vibration controlled transient elastography, VCTE and controlled attenuated parameter, CAP) and a simple circulating parameter reflecting inflammation (aspartate aminotransferase, AST) and it is developed to detect patients with active NASH and advanced fibrosis. Both of them have good performance across the clinical spectrum of NAFLD[23,24].

## 3. PHARMACOLOGICAL MANAGEMENT

### 3.1. Current pharmaceutical treatments

Currently, non-invasive diagnostic methods are required to accurately diagnose and monitor the progression of the disease without the need for performing a liver biopsy. Despite the high prevalence of NAFLD, the existing therapeutic options are not adequate. Although lifestyle changes, such as diet and exercise, are principal suggestions, long-term compliance with them is rarely achieved. Nevertheless, no pharmacological treatment has been approved for the management of NASH by the US Food and Drug Administration or by the European Medicines Agency and all pharmaceutical interventions should be considered as an off-label treatment.

For patients with biopsy-proven NASH and fibrosis stage ≥2 who do not have T2DM, Vitamin E is suggested at a daily dose of 800 IU. Vitamin E is considered to improve steatosis and inflammation. However, in some studies,

its administration was accompanied by an increase in all-cause mortality and the progression of prostate cancer. For this reason, it is not recommended in individuals without fibrosis stage ≥2 and in those with a history of prostate cancer[25].

Glucose lowering therapies were found to be effective in NAFLD/NASH management. Peroxisome proliferator activated receptor (PPAR)-γ agonists, including thiazolidinediones (TZDs; pioglitazone and rosiglitazone) are the most extensively studied drugs for NASH treatment and seem to have promising results[26]. A meta-analysis of four trials that compared PPAR-γ agonists (such as pioglitazone and rosiglitazone) with placebo in patients with NASH showed that, compared with placebo, thiazolidinediones were more likely to improve hepatic histologic lesions like ballooning degeneration, lobular inflammation and steatosis (with combined ORs of 2.11 [95% CI, 1.33-3.36], 2.58 [95% CI, 1.68-3.97] and 3.39 [95% CI, 2.19-5.25] respectively). When pioglitazone (n = 137) was analysed alone, the improvement in fibrosis with pioglitazone (n = 137) vs. placebo (n = 134) (combined OR 1.68 [95% CI, 1.02-2.77]) was statistically significant[27]. Interestingly, pioglitazone resulted in a significant improvement in fibrosis compared with the placebo. Moreover, they cause adipose tissue redistribution from the visceral (and consequently the liver) to the subcutaneous stores, resulting in a considerable increase in adiponectin production and secretion[28].

Sodium-glucose transport protein-2 (SGLT-2) inhibitors decrease body weight and blood glucose levels. They increase glucagon secretion which results in gluconeogenesis and β-oxidation of fatty acids in the liver[29] and, subsequently, they inhibit hepatic fat deposition and inflammatory cytokine expression in the liver[30,31]. A systematic review of eight studies (four randomised controlled trials and four observational studies) and a total of 214 patients showed that SGLT-2 inhibitors led to a significant improvement in hepatic enzymes, liver fat and fibrosis and had a positive effect on the glycaemic and lipidemic profile of patients with T2DM and NAFLD[32]. However, these studies did not use histopathological endpoints.

As regards the incretin-associated drugs, they include the inhibitors of dipeptidyl peptidase 4 (DPP-4) which cause rapid degradation of the GLP-1, an incretin hormone originated from the proglucagon polyprotein which turns to glucagon[33]. However, the studies that examined their effects are small and the majority of them did not use histological findings[34,35]. Besides, there are some others that did not show any positive effects of these agents on the liver fat content or liver enzymes[36,37]. For instance, a randomized, double-blind, placebo-controlled study demonstrated that sitagliptin was not better than placebo in diminishing hepatic fat in patients with NAFLD

and diabetes or pre-diabetes (mean difference between sitagliptin and placebo arms: -1.3%, p=0.4)[36].

The GLP-1 RAs provoke glucose-dependent insulin secretion, prevent glucagon release, delay gastric emptying and increase satiety[38,39]. They also inhibit de novo lipogenesis and cause fatty acid oxidation[40]. Liraglutide is a representative of this category and various studies have evaluated its efficacy in patients with NAFLD. In a trial including 52 patients with NASH who received liraglutide or placebo for 48 weeks, a biopsy was performed at the end of the treatment in 23 patients from the liraglutide group and 22 patients from the placebo group[41]. Finally, NASH resolved in 39% of patients who received liraglutide and only 9% of them who received placebo. Moreover, patients who received the GLP1 RA were less likely to have a progression of fibrosis (9% vs 36%; RR 0.2; 95% CI 0.1-1.0).

Many patients with NAFLD have also dyslipidemia and increased cardiovascular (CV) risk. However, due to their harmful effects on the liver, their use in patients with elevated liver enzymes have been restricted[42]. Nevertheless, accumulating data derived from post-hoc analyses of large survival studies and some smaller trials[43,44] support the beneficial effects of statins in patients with NAFLD, suggesting that they are safe to be prescribed in those patients. The post-hoc analyses of the GREACE (the Greek Atorvastatin and Coronary Heart Disease Evaluation)[45], the IDEAL (Incremental Decrease in End Points Through Aggressive Lipid Lowering)[46], and the ATTEMPT (Assessing the treatment effect in metabolic syndrome without perceptible diabetes)[47] study showed that statins significantly reduced CV events and normalized the liver enzymes. Particularly, the GREACE study[45] suggested that statins significantly reduced CV events especially in patients with impaired liver function tests LFTs, such as NAFLD patients. The post-hoc analysis of the IDEAL trial[46] compared the effects of atorvastatin or simvastatin in a large Scandinavian population with established CV disease and found that atorvastatin normalized liver enzymes among patients with elevated levels and resulted in a greater reduction in the risk for major CV events, compared to simvastatin. Finally, the sub-analysis of the ATTEMPT study[47] showed that atorvastatin normalized the liver enzymes and ultrasonographic findings among patients with metabolic syndrome.

Fenofibrate, by activating PPARα, effectively improves the atherogenic lipid profile associated with T2DM and MetS may play a role in NAFLD prevention and management too. Fenofibrate-related PPARα activation may enhance the expression of genes promoting hepatic FA β-oxidation[48]. Furthermore, fenofibrate reduces hepatic IR[49]. It also inhibits the expression of inflammatory mediators involved in NASH pathogenesis[49,50]. These include tumor necrosis factor-α, intercellular cell adhesion molecule-1, vascular cell adhesion molecule-1 and monocyte chemoattractant protein-1. Other liver-protective effects include decreased oxidative stress and improved liver microvasculature function[51]. Interestingly, fenofibrate seems to limit liver steatosis associated with high-fat diet, T2DM and obesity-related IR[52,53].

Studies also have shown a benefit of omega-3 fatty acids in patients with NAFLD. In a meta-analysis of nine studies with more than 350 patients receiving omega-3 polyunsaturated fatty acid (PUFA) supplementation or control treatment, omega-3 PUFA improved hepatic steatosis compared to control treatment (effect size=-0.97, 95% CI: -0.58 to -1.35, p<0.001)[54]. Given that NAFLD patients are at high CV risk, statins and omega-3 fatty acids should be used to treat dyslipidemia and hypertriglyceridemia, respectively[55].

Furthermore, pentoxifylline has been suggested as a potentially beneficial therapy for NASH since it exerts anti-inflammatory and anti-oxidative effects. Two meta-analyses proved its beneficial effects on liver enzymes, weight loss and histology among patients with NAFLD/NASH[56,57].

## 3.2. Agents under investigation

Currently, several investigational pharmacotherapies are under investigation. More than 60 phase 2 trials are planned or ongoing and agents like cenicriviroc, elafibranor, obeticholic acid, and selonsertib are in phase 3 trials. Particularly, cenicriviroc is an antagonist of C-C motif chemokine receptor (CCR) types 2 and 5, which promote anti-inflammatory and antifibrotic effects in the liver[39,58] and it is expected to reduce hepatic fibrosis (CENTAUR study, NCT02217475). CENTAUR ("Cenicriviroc for the Treatment of NASH in Adult Participants With Liver Fibrosis"; NCT02217475), a 2-year, phase 2b study of cenicriviroc (150 mg daily) in 289 patients with F1-F3 fibrosis, is currently ongoing. In the 1-year interim analysis, cenicriviroc did not met the primary endpoint, being an at least 2-point improvement in NASH without worsening of fibrosis[59]. However, cenicriviroc meet the endpoint in a subset of patients with more severe NASH (defined as NAS ≥5)[59]. Importantly, more patients on cenicriviroc (20%) than on placebo (10%) met the secondary endpoint, being improvement in fibrosis by at least one stage without worsening of NASH. Hepatocellular ballooning was improved only in a subset of patients with prominent baseline ballooning (grade 2). LFTs and hepatic steatosis were not improved by cenicriviroc[59]. Elafibranor is a dual antagonist for PPARα and δ, which are ligand-stimulated nuclear receptors that control gene expression and im-

prove insulin sensitivity, glucose homeostasis, and lipid metabolism, and attenuates hepatic inflammation[60]. Following favorable results in rodents[61], a 8-week treatment with elafibranor (80 mg/d) reduced LFTs, triglycerides, LDL-C and hepatic IR in obese individuals[62]. Of note, target genes were not induced in the skeletal muscle, thus elafibranor possibly showing a hepatic selectivity[62]. Furthermore, obeticholic acid (OCA) has been shown to improve liver histology compared with placebo (45% vs 21% had improved liver histology, relative risk 1.9, 95% CI 1.3 to 2.8; p=0.0002)[63]. A post-hoc analysis of that trial showed that OCA specifically increases small very low-density lipoprotein-cholesterol (VLDL-C) particles, large and small LDL-C particles, and decreases HDL-C particles at 12 weeks[64]. For this reason, another RCT, named "Clinical Study Investigating the Effects of Obeticholic Acid and Atorvastatin Treatment on Lipoprotein Metabolism in Subjects With Nonalcoholic Steatohepatitis" (CONTROL), showed that the co-administration of atorvastatin 10 mg with OCA may mitigate the unfavorable effect of OCA on LDL-C, but not on HDL-C[65]. Finally, selonsertib an oral inhibitor of apoptosis signal-regulating kinase 1 (ASK1) reduced hepatic collagen content compared with simtuzumab at week 24 and a lower percentage of patients progressed to cirrhosis[66]. There was no additive effect of simtuzumab on selonsertibe. A given limitation of this study is the lack of placebo group, even if the effect of simtuzumab on NASH is minimal, as above mentioned. Based on these findings, phase 3 trials evaluating selonsertib among patients with NASH (STELLAR3; NCT03053050) or NASH-related cirrhosis (STELLAR4; NCT03053063) are ongoing.

## 4. CONCLUSIONS

The early, accurate, and safe diagnosis of NAFLD is of high importance. The investigation of non-invasive methods is suitable for close monitoring of the disease progress. Lifestyle changes targeting weight loss are the cornerstone of NAFLD management and pharmacotherapy should only be considered when lifestyle changes do not present the expected results. Both the novel therapeutic options and the non-invasive methods that are under investigation and evaluation promise a new era in the management of NAFLD, since there is currently no approved medication for its prevention and treatment, and thus there is still room for improvement and progress.

### Disclosure Statement

*There are no conflicts of interest*

**ΠΕΡΙΛΗΨΗ**

## Διαγνωστικές μέθοδοι και διαχείριση φαρμακολογικής θεραπείας της μη αλκοολικής λιπώδους νόσου του ήπατος: Εξακολουθεί να υπάρχει περιθώριο βελτίωσης

Χρυσούλα Μπουτάρη[1], Παναγιώτης Παππάς[2], Κωνσταντίνος Τζιώμαλος[3], Βασίλειος Άθυρος[1], Αστέριος Καραγιάννης[1]

[1]Β' Προπαιδευτική Παθολογική Κλινική, Ιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Γ.Ν. Ιπποκράτειο, Θεσσαλονίκη, [2]424 Γενικό Νοσοκομείο Στρατού, Θεσσαλονίκη, [3]Α' Προπαιδευτική Παθολογική Κλινική, Ιατρική Σχολή, Αριστοτέλειο Πανεπιστήμιο Θεσσαλονίκης, Θεσσαλονίκη

Η μη αλκοολική λιπώδης νόσος του ήπατος (NAFLD) χαρακτηρίζεται από ηπατική στεάτωση απουσία υπερβολικής κατανάλωσης αλκοόλ και μπορεί να εξελιχθεί σε μη αλκοολική στεατοηπατίτιδα, κίρρωση, ηπατική ανεπάρκεια και καρκίνο. Λόγω της ανησυχητικής αύξησης της συχνότητας της νόσου κατά τις τελευταίες δεκαετίες εξαιτίας της αύξησης του επιπολασμού του μεταβολικού συνδρόμου, υπάρχει ανάγκη εύρεσης νέων μεθόδων στη διάγνωση και την αντιμετώπιση της νόσου. Η μέθοδος εκλογής για τη διάγνωσή της παραμένει η βιοψία του ήπατος. Το υπερηχογράφημα, οι εργαστηριακές εξετάσεις (αμινοτρανσφεράσες ορού, επίπεδα γ-γλουταμινικής τρανσπεπτιδάσης και επίπεδα φερριτίνης, θραύσμα κυτοκερατίνης-18 και αυξητικός παράγοντας ίνωσης-21), οι κλινικοί (Fatty Liver Index [FLI], NAFLD fibrosis score [NFS] and Fibrosis 4 calculator [FIB-4]) και οι μη επεμβατικοί δείκτες (NIS-4, FS3) στεάτωσης και ίνωσης υπόσχονται ελπιδοφόρα αποτελέσματα τόσο για την ανίχνευση της παρουσίας όσο και για την αξιολόγηση της σοβαρότητας της νόσου. Για τη διαχείριση

της στεατοηπατίτιδας δεν έχει εγκριθεί φαρμακολογική θεραπεία. Η βιταμίνη Ε, οι θειαζολιδινεδιόνες, οι αναστολείς των συμμεταφορέων νατρίου-γλυκόζης (SGLT-2), οι αναστολείς της διπεπτιδυλικής πεπτιδάσης 4 (DPP-4), οι αγωνιστές των GLP-1 υποδοχέων (GLP-1 RAs), οι στατίνες και άλλοι υπό έρευνα παράγοντες μπορεί να διαδραματίσουν σπουδαίο ρόλο στη διαχείριση της μη αλκοολικής λιπώδους νόσου του ήπατος παράλληλα με τις κύριες ενδείξεις τους.

**ΛΕΞΕΙΣ ΚΛΕΙΔΙΑ:** *Μη αλκοολική λιπώδης νόσος του ήπατος, μη αλκοολική στεατοηπατίτιδα, μη επεμβατικές διαγνωστικές μέθοδοι, φαρμακολογική θεραπεία*

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