

A New Endemic of Concomitant Nonalcoholic Fatty Liver Disease and Chronic Hepatitis B

Hira Hanif^{1*}, Muzammil M. Khan^{1*}, Mukarram J. Ali¹, Pir A. Shah^{1,2}, Jinendra Satiya¹

Daryl T.Y. Lau^{1**}, Aysha Aslam^{3**}

** equal first author contribution*

*** equal senior author contribution*

¹Liver Center, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA

²Department of Internal Medicine, University of Texas, San Antonio, TX, USA

³Department of Medicine, Louis A Weiss Memorial Hospital, Chicago, IL, USA

Short Title: Concurrent NAFLD and chronic hepatitis B

Key words: Chronic hepatitis B, Nonalcoholic fatty liver disease (NAFLD), nonalcoholic steatohepatitis (NASH), serum biomarkers, Controlled attenuation parameter (CAP), transient elastography (Fibroscan), magnetic resonance technology, NAFLD therapy

Corresponding Author:

Daryl T.Y. Lau, MD, M.Sc, MPH
Associate Professor of Medicine,
Harvard Medical School

Liver Center, Beth Israel Deaconess Medical Center
110 Francis Street, Suite 4A, Boston 02215, MA, United States.
Email: dlau@bidmc.harvard.edu.

Abstract

Hepatitis B virus (HBV) infection remains a global public problem despite the availability of effective vaccine. In the past decades, nonalcoholic fatty liver disease (NAFLD) has surpassed HBV as the most common cause of chronic liver disease worldwide. The prevalence of concomitant chronic hepatitis B (CHB) and NAFLD, thus, reaches endemic in geographic regions where both conditions are common. Patients with CHB and NAFLD are at increased risk of liver disease progression to cirrhosis and hepatocellular carcinoma. Due to complexity of the pathogenesis, accurate diagnosis of NAFLD in CHB patients can be challenging. Liver biopsy is considered the gold standard for diagnosing and determining the disease severity, but it is an invasive procedure with potential complications. There is a growing body of literatures on the application of novel noninvasive serum biomarkers and advanced radiological modalities to diagnose and evaluate NAFLD, but most have not been adequately validated especially for patients with CHB. Currently, there is no approved therapy for NAFLD though many new agents are in different phases of development. This review provides a summary of the epidemiology, clinical features, diagnosis and management of the NAFLD and highlights the unmet needs in the areas of CHB and NAFLD coexistence.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease (CLD) globally and a major cause of liver-related mortality and morbidity. It consists of a spectrum of liver disease from simple steatosis, non-alcoholic steatohepatitis (NASH), progressive fibrosis and hepatocellular carcinoma (HCC) complication [1, 2]. Histologically, patients with NASH have infiltration of inflammatory cells in the hepatic lobules, hepatocyte injury with ballooning, perisinusoidal or advanced fibrosis in addition to macrovesicular steatosis involving $\geq 5\%$ of the hepatocytes [2, 3]. NAFLD is associated with metabolic risk factors such as obesity, dyslipidemia, and type 2 diabetes mellitus [2]. Owing to the trend of sedentary lifestyle and globalization of Western diet, the prevalence of NAFLD has increased exponentially at about 114% for men and 80% for women between 2004 - 2016 [4, 5]. Hepatitis B virus (HBV) infection is another CLD of global magnitude; it is a significant cause of cirrhosis, hepatocellular carcinoma, grave morbidity and mortality. Since both chronic hepatitis B (CHB) and NAFLD are common liver conditions that can lead to end-stage liver disease (ESLD) and HCC, it is important to understand the impact of NAFLD on hepatitis B and vice-versa. Recent studies reported that concomitant presence of HBV and hepatic steatosis is associated with increased risk of disease progression to cirrhosis and hepatic and extra-hepatic malignancies [6-8]. In this review, we will discuss the epidemiology, natural history, diagnostics, including non-invasive biomarkers and radiological modalities, and management strategies for NAFLD among patients with chronic hepatitis B.

Epidemiology

Globally, the cumulative prevalence of NAFLD is estimated to be more than 1 billion of the world population [9, 10]. There has been a notable rise in NAFLD disease burden in the past decade with the highest rates in the Middle East (32%), followed by South America (31%), Asia (27%), USA (24%) and Europe (20-24%) [9-11]. (Figure 1A) In the US, the prevalence of NAFLD has increased from 15% in 2005 to approximately 25% in 2017 [12]. Similarly, an analysis of 237 studies from Asia noted that the prevalence of NAFLD has increased from 25.3% in 2005 to almost 34% in 2017 [13]. The increased prevalence of NAFLD has been attributed to the surging rates of obesity, insulin

resistance, socioeconomic and lifestyle factors. Over 95% of obese individuals undergoing bariatric surgery and >70% of patients with type 2 diabetes mellitus have NAFLD [14]. The presence of NAFLD varies in different racial and ethnic populations with the highest prevalence among Hispanics (45% to 58%), followed by non-Hispanic Whites (33%) and African Americans (24%) [15]. NAFLD has become the second most common cause of liver transplantation in the US and the most common liver disorder in the Western countries [4, 5].

It is estimated that 240 to 350 million of the world population has hepatitis B [16]. (Figure 1B) NAFLD is common in some of the HBV endemic regions such as Asia. North America is considered to have low HBV prevalence with an estimated rate of 2.2 million in the United States. That is likely an underestimation due to the influx of immigrants from high HBV endemic regions. [17]. Since NAFLD and hepatitis B are common, it is frequent for an individual to have both liver diseases [18]. According to meta-analysis by Hui et al including 17 studies, the prevalence of hepatic steatosis was about 25 to 30% among patients with CHB [19].

Clinical manifestations in patients with concurrent NAFLD and CHB

With an alarming increase in the number of patients with concomitant NAFLD and CHB [20-24], there is an urgent need to evaluate the interactions between these two diseases and the roles of hepatic steatosis (HS) and NAFLD on the prognosis of hepatitis B. There are also some controversial reports on the steatogenic effects of HBV in *in vitro* models. Wu and colleagues reported that HBV X protein (HBx) might promote hepatic lipid accumulation through up-regulating fatty acid binding protein (FABP1) [25]. Another study by Kim et al determined that hepatic lipogenesis was mediated by HBx up-regulation of sterol regulatory element binding protein 1 (SREBP-1) and peroxisome proliferator activated receptor gamma (PPAR-g) [26]. Conversely, clinical studies showed that host metabolic factors rather than viral factors are important in the development of HS in CHB [27,28]. In the aforementioned studies, high BMI, hypertriglyceridemia, increased waist circumference and insulin resistance were found to be more prevalent in CHB patients with steatosis compared to those without steatosis.

A retrospective study conducted in a military hospital in Turkey, Karacaer et al investigated the influence of HS on necroinflammation and fibrosis in 254 young male CHB patients with and without HS. They found that patients with HS had significantly more fibrosis ($p = 0.012$; $r = 0.158$) and higher histologic activity index (HAI) ($p = 0.029$; $r = 0.137$) [29]. Similar results were reported by Estakhri and colleagues; abnormal serum aminotransferases, increased fibrosis and inflammation were noted in CHB patients with concurrent NAFLD [30]. It is important to note that both the aforementioned studies showed no relationship between HBV DNA titers and severity of liver disease among those CHB patients with NAFLD [29, 30]. Charatcharoenwittaya et al identified steatohepatitis as an independent predictor of significant fibrosis (METAVIR score, $F \geq 2$) (OR, 10.0; 95% CI, 2.08-48.5) and advanced fibrosis (METAVIR score, $F \geq 3$) (OR, 3.45; 95% CI, 1.11-10.7) after adjusting for HBV DNA levels and metabolic syndrome [31]. On the contrary, some studies did not observe a correlation between HS and disease severity in CHB patients [32-35]. These discrepancies can be partially explained by the small sample size, heterogenous patient populations and retrospective nature of the study design.

In a large retrospective multiethnic North American and European cohort study, Choi et al reported that patients with CHB and NASH had higher liver-related outcomes and overall mortality than those with CHB alone [36]. They proposed a “two-hit theory” for the pathogenesis of disease progression for those with both NASH and CHB. In this theory, HBV is considered a first hit in inducing hepatocyte injury, the superimposed NASH serves as the second hit leading to progressive hepatic inflammation and fibrosis. This theory is substantiated by the observation where the lipid-laden hepatocytes induce mitochondrial and peroxisomal dysfunction that resulting in oxidative stress, toxic fatty acid metabolite production and hepatocyte damage [37]. The ongoing chronic inflammation with activated myofibroblasts derived from hepatic stellate cells can lead to increase extracellular matrix (ECM) deposition [37, 38]. Hence, CHB and NASH can synergistically cause liver disease progression.

CHB is the most important risk factor for HCC accounting for about 50% of the HCC cases globally. The HCC risk in HBV endemic areas is even higher and contributes to 70-80% of the cases [39]. Patients with NASH-related cirrhosis are also at a greater risk for HCC with a yearly cumulative incidence of 2.6% [40]. In a retrospective cohort study, Chan and colleagues in Hong Kong evaluated the HCC risk in CHB patients with concurrent NAFLD [24]. Out of 270 CHB patients, 107 had concomitant hepatic steatosis. Among them, nine patients with fatty liver (8.4%) and two patients without fatty liver (1.2%) developed HCC with a hazard ratio of 6.84 (95% CI: 1.48-3.66; P=0.014). Similar results were found in a study conducted by Lee et al. in a total of 321 CHB patients, a 3-fold increased risk of HCC was observed among those with NAFLD [22]. It is increasingly recognized that metabolic factors, the precursors of NAFLD, also contribute to HCC development in CHB [41, 42]. Yu et al evaluated 1,690 Taiwanese patients with CHB and a positive correlation was established between metabolic factors and HCC; there was a significantly increased risk of HCC for those with 3 or more metabolic risk factors compared to none (HR, 5.06; 95% CI, 2.23–11.47) [43]. Insulin resistance and obesity were recognized as 2 of the most significant risk factors for HCC in CHB. [28, 44, 45]

Current and novel serum biomarkers for NAFLD

The interplay of NAFLD and CHB is complex; both disease entities can cause elevation of serum aminotransferases. This may pose diagnostic and management challenges in patients with combined disease. Demir et al. observed that NAFLD rather than CHB may be the most common cause of elevated ALT levels among patients with HBeAg negative CHB and HBV DNA levels <2000 IU/mL [46]. It is desirable to have biomarkers that can identify NAFLD among CHB patients for timely management of both liver conditions [8]. Liver biopsy is the gold standard to diagnose NAFLD and NASH however there are several limitations including its invasive nature, cost, sampling error and interobserver variability among pathologists [47].

Serum biomarkers for diagnosing hepatic steatosis, NASH and fibrosis

A) Serum Biomarkers to detect hepatic steatosis

There are a number of non-invasive biomarker assays available to detect hepatic steatosis. They include Fatty Liver Index (FLI) [48], Hepatic Steatosis Index (HSI) [49], NAFLD Liver Fat Score [50], Steato Test [51] and NAFLD Ridge Score [52] (Table 1). HSI, NAFLD Liver Fat Score and NAFLD Ridge Score are specifically developed to detect steatosis due to NAFLD whereas FLI and Steato Test can be applied to detect steatosis due to any cause. FLI and HSI are feasible in different clinical settings for both are derived from common laboratory parameters. They have good accuracy when measured against ultrasound [48, 49]. They have not been evaluated using liver biopsy. NAFLD Liver Fat Score provides quantitative value for steatosis and has high accuracy with AUROC of 0.86-0.87; it may not be feasible for routine clinical use for serum insulin is required in its algorithm [50]. Steato Test and NAFLD Ridge Score combine multiple parameters to predict hepatic steatosis and have been used in many cross-sectional studies [51, 52]. Their ability to detect changes in liver fat content over time is limited. Steato Test has modest accuracy with AUROC of 0.79-0.80 but has good prognostic value in patients with diabetes mellitus (DM) and dyslipidemia. The presence of severe steatosis by Steato Test is associated with a 2-fold increase in all-cause mortality and cardiovascular mortality. It, however, may not be feasible in some regions due to its high cost. NAFLD Ridge Score has very good accuracy with AUROC of 0.87 and has excellent negative predictive value of 96% to exclude NAFLD; its use is limited to research setting currently.

B) Serum Biomarkers to detect NASH

NASH is associated with worse liver disease prognosis compared to simple hepatic steatosis. It is, therefore, important to identify patients with NASH to intensify interventions and to prevent further disease progression to advanced fibrosis. The liver injury in NASH is complex and involves pathological processes such as hepatocellular apoptosis, inflammation, oxidative stress and abnormal adipokine signaling [53]. Various noninvasive serum biomarkers for NASH based on different pathophysiologic mechanisms have been developed. They include apoptosis markers (CK18 fragments [54], total cytokeratin [55] and sFAS [56], inflammatory biomarkers (CRP, TNF, IL-8, CXCL 10) [56], lipid peroxidation products (11 HETE, 9-HODE 13HODE, 13 -oxo-ODE, LA-13-HODE) [57, 58], adipocytokines and hormones (adiponectin, leptin, resistin,

visfatin, RBP4, FABP4, FGF21 [59, 60], lysosomal enzymes (cathepsin D) [61] and combined tests such as NASH Test [62] and NASH Diagnostic Panel [63] (TABLE 2). CK18 is commercially available and was noted to correlate with histological improvement in NASH. When combined with sFAS, the diagnostic accuracy of CK18 is higher but the optimal predictive cut-off value remains uncertain. The various inflammatory biomarkers have been used to correlate the inflammatory activity in NASH. The inflammatory markers are not suitable to diagnose NASH specifically for their levels are influenced by other systemic inflammatory conditions [56]. The measurement of lipid peroxidation products requires mass spectroscopy and have not been extensively validated for NASH [57, 58]. Combination of adipocytokines has been applied to validate NASH in bariatric population but need further research and validation in other patient cohorts. Their accuracy may be influenced by visceral adiposity besides hepatic steatosis [59, 60]. Due to limitations of individual markers for NASH, combined panels such as NASHTest and NASH Diagnostic Panel were formulated as proprietary algorithms to increase the predictive accuracy. NASHTest comprises of age, gender, height, weight and serum levels of triglyceride, cholesterol, α 2m, apolipoprotein AI, haptoglobin, GGT, ALT, AST and total bilirubin [62, 63]. NASH Diagnostic Panel includes diabetes mellitus, gender, BMI and serum levels of triglycerides, CK18 fragments and total CK18. The AUROC values for NASHTest and NASH Diagnostic Panel were 0.69-0.79 and 0.81 respectively in predicting NASH. Both combined panels have been tested in relatively small number of patients and in predominantly bariatric populations.

C) Serum Biomarkers to detect Fibrosis in NAFLD

Progression of NASH to fibrosis, especially \geq F2 fibrosis, is associated with increased morbidity and mortality [64]. Several non-proprietary biomarkers and panels have been evaluated for the detection of fibrosis. These include AST: ALT ratio [65], AST: Platelet Ratio Index (APRI) [66], Fibrosis 4 (Fib 4) Index [67], NAFLD Fibrosis Score [68], and BARD Score [69]. (Table 3) AST: ALT Ratio Index and APRI have low accuracy in diagnosing fibrosis. Fib 4 Index has modest accuracy with AUROC of 0.83 for detecting F3 fibrosis which is comparable to NAFLD Fibrosis Score [50, 66, 67]. NAFLD Fibrosis Score, which comprises of age, BMI, impaired fasting glucose, AST, ALT, platelet count

and albumin, has high accuracy with AUROC of 0.83. It was extensively validated and has been used as a predictor of liver decompensation and mortality in patients with NAFLD [69]. Major limitation in using NAFLD Fibrosis Score and BARD Score is the varied interpretations of BMI across the different ethnic groups [68, 69]. Several specific fibrosis markers and panels are also available. These include hyaluronic acid (HA) [70], PIIINP [71], Pro -C3 [72], Tissue Metalloproteinases 1 (TIMP1) [73, 74], Laminin [75], Enhanced liver fibrosis (ELF) [76], FibroTest [77], and FibroMeter NAFLD [78, 79]. Most of the biomarkers have high negative predictive value of >90% in ruling out fibrosis. The challenge is the inability of these makers to differentiate between different stages of fibrosis.

There are currently no official guidelines to diagnose and manage NAFLD in patients with CHB. Most of the biomarkers applied to detect steatosis, inflammation and fibrosis for NAFLD as mentioned above have not been validated for patients with underlying CHB. Moreover, many of those biomarker panels for steatosis and fibrosis include ALT and AST. The fact that ALT and AST are frequently elevated in both NAFLD and CHB, these biomarkers may not be applicable for patients with both liver diseases. Novel noninvasive serological biomarkers and panels that have been evaluated in patients with concurrent CHB and NAFLD are summarized below.

Serum biomarkers for diagnosing hepatic steatosis, NASH and fibrosis in CHB patients

A) Serum Biomarkers to detect Hepatic Steatosis in CHB patients

Routine clinical parameters to assess risks of NAFLD in CHB patients include fasting glucose, total cholesterol (TC) and triglycerides (TGs). A Hepatic Steatosis Model consists of BMI, Hemoglobin (Hb), TGs and serum uric acid was applied to CHB patients in China [77]. It has a good accuracy with AUROC of 0.84 in comparison to a threshold of 22% of steatosis on liver biopsy. Large multicenter cohort studies are required to further validate the results. Ou et al applied a Fatty Liver Test composed of diastolic blood pressure, weight, and waistline on 1,312 Asian CHB patients [78]; 618 (47%) of them had steatosis by Controlled attenuation parameter (CAP). The AUROC for detecting steatosis

was 0.79 in the training phase which was comparable to 0.82 in the validation phase. The advantage of the Fatty Liver Test is its feasibility. CAP, however, is still considered a research tool. Before it can be recommended for clinical use, it would require validation using preferably liver biopsy and histologic quantification in larger patient cohorts.

There are a number of gene polymorphisms, especially PNPLA3, that have been shown to have genetic predisposition to NAFLD. PNPLA3 gene codes for adiponutrin, a protein located in both adipocytes and liver cells. Pan et al evaluated PNPLA3 polymorphisms in Chinese CHB patients with and without biopsy proven NAFLD. Four single nucleotide polymorphisms (SNPs) of PNPLA3 (rs738409 G, rs3747206 T, rs4823173 A and rs2072906 G alleles) were found to be linked to high risk of NAFLD in CHB after adjusting for age, sex, and BMI. These genotypes were also associated with progression from steatosis to NASH and liver fibrosis. These important observations need to be validated in patients with different racial and ethnic background. PNPLA3 polymorphism was found to downregulate HCV replication in human study and mouse models [80]. The effects of these PNPLA3 SNPS on HBV pathogenesis are not well understood.

B) Serum Biomarkers to detect NASH in patients with CHB

A number of noninvasive score models were applied to identify NASH among patients with CHB. In a relatively small study with 64 CHB patients, Liang et al found that elevated serum level of CK18M30, higher CAP scores and elevated fasting plasma glucose level were all independent risk factors for NASH [81]. Subsequently, a logistic regression model combining CK-18 M30, CAP, fasting plasma glucose and HBV DNA level was established for diagnosis of NASH in CHB patients. Multicenter studies with larger cohorts are required to validate this score model for clinical utility [81].

Another active research area to identify NASH in CHB patients is lipidomic profile assessment. Yang et al applied ultra-performance liquid chromatography-tandem mass spectrometry to determine lipidomic profiles between CHB patients with and without NASH [82]. Most of the serum ceramide and neutral lipids were elevated in the NAFLD group. Serum monounsaturated Triacylglycerols (TAG) were significantly increased in

NASH subjects (OR=3.215; 95% CI 1.663-6.331 and correlated positively with histological activity ($r=0.501$, $P<0.001$). A limitation of the study is the lack of simultaneous evaluation of liver tissue. It is important to validate these results in CHB patients with mild to severe liver fibrosis.

Micro RNAs (miRNAs) are small noncoding protein transcripts which may be involved in many physiological and cellular mechanisms. Circulating levels of these miRNAs are stable, sensitive and specific to serve predictors of various pathological processes. Zhang et al applied several miRNAs to differentiate liver injury caused by hepatitis B, NASH compared to healthy control subjects [83]. There were 34 differential expressions of miRNAs in patients with CHB compared to control subjects. They identified higher levels of miRNA -122, -638, -572, -575 and lower levels of miRNA -744 in the sera of patients with CHB and NASH. In another study, Liu et al assessed a panel of miRNAs for NASH diagnosis [83]. They found that MiRNA-34a was a more accurate marker for the diagnosis of NASH compared to CK-18, FIB-4, and APRI with AUROC of 0.811, 95% CI: 0.670-0.953). These studies demonstrated that miRNAs may serve as potential biomarkers for NAFLD and HBV-associated liver injury, but they need to be carefully validated in different patient populations [83, 84].

C) Serum Biomarkers to detect Fibrosis in NAFLD and CHB patients

As none of the commonly available fibrosis biomarkers have been validated for patients with concurrent CHB and NAFLD, Dong et al. applied the Forns Index consisting of platelet count, gamma glutamyl transpeptidase (GGT), age and cholesterol levels, Fibroscan and acoustic radiation force impulse (ARFI) alone and in combination to evaluate hepatic fibrosis in CHB patients. In addition, they assessed the effects of inflammation and steatosis on the accuracy of these diagnostic methods. A total of 81 patients were included in the study with liver biopsy as the standard for comparison. They concluded that the combination of Forns index with Fibroscan or ARFI increases the accuracy of diagnosing fibrosis compared to the individual modality. Inflammation, but not steatosis, may affect the diagnostic accuracy of these methods [85]. Lemoine and colleagues reported that a novel fibrosis model utilizing Gamma Glutamyl transpeptidase

-to-platelet ratio (GPR) may be better than APRI (aspartate aminotransferase-to-platelet ratio index) in diagnosing cirrhosis [86]. This result was not substantiated by a latter study including patients with CHB and NAFLD [87]. Ozlem et al. evaluated the role of soluble urokinase plasminogen activator (uPAR) in determining severity of liver fibrosis in patients with chronic hepatitis B or C and NAFLD. Plasma levels of uPAR were significantly elevated in those with hepatitis B or C compared to healthy controls, but the uPAR levels in patients with NAFLD were similar to healthy controls. [88] It is unknown how this marker will perform among patients with both chronic viral hepatitis and NAFLD.

Imaging modalities for detection of NAFLD in CHB

Imaging modalities for detection of hepatic steatosis

Due to rising prevalence of NAFLD, it is important to develop and evaluate non-invasive methods for the diagnosis of the wide spectrum of NAFLD from steatosis to NASH and fibrosis. Liver biopsy is considered a gold standard diagnostic tool, but it is an invasive procedure with potential complications such as pain and bleeding. Imaging modalities have gained a better patient acceptance and have been used as a substitute for the detection of steatosis and fibrosis [89].

A) Abdominal Ultrasound

Ultrasound as been applied widely as a screening tool for hepatic steatosis due to its accessibility. In a study conducted by Mottin et al, the sensitivity and specificity of ultrasound in detecting hepatic steatosis were only 49% and 75% respectively using liver biopsy as a reference in a cohort of morbidly obese patients [89]. Besides the presence of obesity, ultrasound has reduced accuracy for detection of hepatic steatosis in patients with significant fibrosis or renal disease, [91]

B) Controlled Attenuation Parameter (CAP)

Controlled attenuation parameter (CAP) is a recently developed software that measures ultrasound attenuation. It is implemented on Fibroscan, an ultrasound-based vibration

controlled transient elastography device, used to assess the elasticity of the liver. Early study provided encouraging results that that CAP could detect >11%, >33%, and >66% hepatic steatosis with AUROCs of 0.91, 0.95, and 0.89 respectively. There were, however, significant overlapping CAP scores for the different degree of steatosis using the standard M-probe especially for obese individuals [92, 93]. The XL probe was later developed for obese patients. The resulting CAP scores with the individualized M probe and XL probe have similar performance in terms of AUROC and cut-off values. One must practice caution when interpreting CAP scores relative to the timing of food as CAP scores increase after meals across all stages of fibrosis [94]

Since our review focuses on NAFLD and CHB, it is quite relevant to compare the performance of CAP to ultrasound in patients with CHB. Liang et al evaluated 366 CHB patients and found CAP had better diagnostic accuracy compared to conventional ultrasound. The rate of steatosis overestimation, however, was significantly greater for CAP than for ultrasound (30.5% vs 12.4%) [95]. CAP is an attractive and efficient point-of-care assessment tool but needs to be further validated especially in patients with concomitant chronic liver disease such as CHB.

C) *Magnetic Resonance technology*

Magnetic resonance (MR)-based techniques such as magnetic resonance elastography (MRE) and proton density fat fraction (PDFF) have been shown to accurately diagnose steatosis and fibrosis. MRE utilizes propagating mechanical shear waves to assess the stiffness of the tissue. Waves propagate faster in stiffer tissues as compared to softer tissues. The speed of propagation is reflected on wavelength as increased stiffness of tissue associated with longer wavelengths. Imaging of these waves are performed with an MRI sequence and processed to generate elastograms [96]. Proton density fat fraction (PDFF) is another non-invasive tool which utilize triglyceride-specific signal intensity and offers a sensitive approach for steatosis detection. It has been developed to specifically assess fat over the entire liver [97]. It determines the ratio of the density of mobile protons from the triglycerides and the total density of protons from mobile triglycerides and mobile water. It has been validated in multiple studies with different racial cohorts of NAFLD

patients. In those studies, MRI-PDFF outperformed CAP in terms of AUROC and accuracy across all grades of steatosis [98, 99, 100]. In a cross-sectional study of 100 patients, Park et al systematically showed that MRI-PDFF was more accurate than CAP in diagnosing all grades of steatosis with liver biopsy as the reference standard [99] (Table 4). MRI-PDFF, however, is not suitable for routine clinical setting due to its high costs and requires specific facility infrastructure.

Imaging modalities for detection of NASH

Different imaging modalities has been tried and tested for the diagnosis of steatohepatitis. Studies using transient elastography such as Fibroscan and magnetic resonance technology to assess NASH have shown a wide range of AUROCS and the optimal cut-off was affected by the degree of fibrosis. In a study by Imajo et al, magnetic resonance imaging had a higher capability of diagnosing NASH as compared to transient elastography (TE) with AUROC 0.91 vs 0.82 [100]. Recently, Naganawa et al studied the role of non-contrast enhanced CT in the diagnosis of NASH and found that in patients without fibrosis, it has high sensitivity (100%) and specificity (92%) in diagnosing NASH with AUROC value of 0.93-0.94. For patients with high hyaluronic acid and hepatic fibrosis, the sensitivity (42%) and specificity (31%) was much reduced, however [101]. Eddowes et al, evaluated the role of Multiparametric MRI (Liver Multiscan) to differentiate simple steatosis (SS) from NASH. The results were suboptimal with AUROC for SS at 0.69 (0.50-0.88) and AUROC for NASH at 0.74 (0.59 – 0.89) [102]. All these studies have shown promising results but considering limitations especially in the presence of variable amount of hepatic fibrosis. Liver biopsy remains the gold standard for diagnosing NASH [103].

Imaging modalities to assess fibrosis

Two types of imaging techniques have been applied. First is ultrasound-based technology which measures the speed of the shear waves provoked in the liver tissue. Based on the generation and detection of these shear waves, different ultrasound-based elastography techniques have been invented including transient elastography, supersonic shear wave elastography, and acoustic radiation force impulse (ARFI) elastography. The second

imaging technique is magnetic resonance-based which utilizes a magnetic resonance scanner to detect the difference in MR frequency between the protons in fat and water.

A) Ultrasound based elastography modalities:

Transient elastography (TE), measured by Fibroscan has emerged as one of the most commonly used technology to access hepatic fibrosis. Several meta-analyses have shown that it has an accuracy of 88%-89% and 93%-94% for diagnosing advanced fibrosis and cirrhosis respectively [104, 105, 106, 107, 108]. The Vibration-controlled transient elastography (VCTE) used in the Fibroscan device provides readily available results with convenient examination and clinical settings. Currently, transient elastography is recommended for the assessment of fibrosis in NAFLD [109]. 2-Dimensional Shear Wave Elastography (2D-SWE) is another ultrasound-based technology with promising results. In one meta-analysis, it showed a diagnostic accuracy of 93% and 92% for advanced fibrosis and cirrhosis in NAFLD patients [110]. ARFI measures a beam that passes over a standardized region of the liver [111]. ARFI had poor diagnostic performance in patients with a BMI greater than 35 kg/m² [112]. Both 2D-SWE and ARFI techniques need further prospective studies to validate their performance in NAFLD patients and are not recommended currently in management guidelines.

B) Magnetic Resonance based elastography modalities

A meta-analysis by Singh et al reported that magnetic resonance elastography (MRE) has a very high accuracy for detecting advanced fibrosis and cirrhosis regardless of BMI and etiology [113]. This makes MRE a very appropriate modality if we want to access disease progression and treatment response in patients with chronic liver diseases. In a cross-sectional study of 100 patients, MRE was more accurate in assessing the degree of fibrosis in patients with NAFLD as compared to TE (Fibroscan). Receiver operating characteristic curves (ROC) were used to assess the performance of MRE compared to Fibroscan in diagnosis of fibrosis with liver biopsy as reference. MRE was found to be more accurate in detecting any fibrosis versus no fibrosis compared to Fibroscan ($p=0.01$). There was no significant difference between MRE and Fibroscan for accurate diagnosis of in between various stages of fibrosis [99] (Table 5).

Management of patients with HBV and NAFLD

To date, there is no U.S. Food and Drug Administration (FDA)-approved treatment available for NAFLD. The current management options aim at lifestyle modifications with a goal of weight reduction. Medications including anti-diabetics are being investigated and repurposed for NAFLD. There are many novel pharmacological agents in clinical trials with the goal to reduce inflammation and fibrosis in NASH. The American Association for the Study of liver Disease (AASLD) currently recommends pharmacological treatment only for the patients with biopsy confirmed NASH and fibrosis. [2]

Lifestyle Modifications for NAFLD

Although there have been many breakthroughs in understanding epidemiology and pathophysiology of NAFLD, weight loss remains the cornerstone treatment for NAFLD [94]. The AASLD suggests a weight loss goal of 3-5% of total body weight for improvement in steatosis. In addition, 7-10% weight reduction is required to improve fibrosis and other histological features of NASH [2, 114]. Weight loss can lead to remission of NAFLD in patients with BMI <25%. [115].

Generally, low calorie diets are recommended for patients with NAFLD/NASH [94]. Nguyen, V., & George, J. recommend hypo-caloric diet (1200-1500 kcal/day in normal weight or 500-1000 kcal/day in overweight populations) with the aim of achieving 5-7% reduction in baseline weight over a year for NAFLD management; this resulted in histological improvement of steatosis and steatohepatitis [116, 117].

Intake of simple carbohydrates is associated with NASH development. Fructose being a simple carbohydrate is associated with increased hepatic fibrosis due to its rapid metabolism in the liver leading to a decrease in hepatic ATP level and hepatic oxidative damage [118, 119]. Polyunsaturated fatty acids (PUFAs) such as n-3 PUFA have been reported to reduce systemic inflammation and oxidation [120, 121]. The WELCOME study demonstrated a mild reduction in liver fat with omega-3 fatty acids used at a dose of 4 g/day for 15 to 18 months but did not result in an improvement in fibrosis scores [122].

It is important to note that macronutrient specific diets have not been as efficacious as total caloric restriction for NAFLD. Many dietary regimens such as the Mediterranean Diet and Dietary Approach to Stop Hypertension (DASH) have been proposed to control NAFLD. A case control study carried out by Hekmatdoost et al. showed that adherence to DASH diet is negatively associated with risk of NAFLD development [123]. The European Association for the study of the Liver (EASL), European Association for the study of Diabetes (EASD) and European Association for the study of Obesity (EASO) recommends Mediterranean diet for the treatment of NAFLD [124]. Mediterranean diet consists of whole grain cereals with low- or medium glycemic index ($GI \leq 55$), polyunsaturated and monounsaturated fatty acids (PUFA and MUFA) from olive oil, phytochemicals and antioxidants and moderate amount of wine has been shown to have a positive impact in NAFLD through early satiety and reduction in hepatic steatosis and inflammation [125,126]. Houghton et al. reported that exercise, independent of weight loss, confers beneficial effects in patients with biopsy-proven NASH. Both aerobic and resistance exercises have been implicated to have equal benefits by decreasing intra-hepatic triglyceride and increasing insulin sensitivity. [127]

The amount of weight loss required to improve histological features of NASH is difficult to achieve and maintained by lifestyle interventions [128]. A weight loss of more than 1.6 kilograms (kgs) per week can cause a paradoxical increase in the state of inflammation within the liver [129]. An additive benefit when cognitive-behavioral therapy in conjunction with weight loss has been reported [130].

For selective patients, bariatric surgery leads to improve co-morbidities, decrease cardiovascular related deaths and all-cause mortality in addition to weight loss [131]. In a prospective study, laparoscopic sleeve gastrectomy was associated with improvement of NASH, hepatocyte ballooning, steatosis, lobular inflammation and even fibrosis at one year after surgery [132]. Similar benefits such as weight reduction, improved ALT, GGT, steatosis, hepatocyte ballooning, lobular inflammation, and fibrosis at 1 year post bariatric surgery were observed in another study [133]. AASLD does not recommend bariatric

surgery for the treatment of NASH alone. It may be considered in eligible patients with morbid obesity and NASH [2]. More prospective randomized, controlled trials are required to further evaluate the long-term effects of bariatric surgeries. Coffee consumption can slow disease progression and has been showed to have a protective effect in patients with NAFLD [134, 135]. Alcohol consumption should be avoided in these patients, especially those with concurrent obesity as it increases the risk of hepatocellular carcinoma (HCC) [136].

Therapeutic agents in development for NASH

Pharmacological management of NAFLD is recommended for subgroups of patients with progressive NASH, early-stage NASH with high risk of fibrosis (age >50 years, metabolic syndrome, diabetes, increased ALT) or active NASH with necroinflammatory activity [2]. The AASLD, EASL, EASD and EASO recommended the use of pioglitazone and vitamin E for the treatment of biopsy-proven NASH in patients with or without type II diabetes mellitus, keeping in consideration the side effects of pioglitazone and Vitamin E [2, 124]. In a randomized control trial comparing the efficacy of Vitamin E, Vitamin E and pioglitazone and placebo for patients with type 2 diabetes mellitus and NASH did not show any improvement with Vitamin E alone or placebo. Combination therapy with Vitamin E and Pioglitazone reduced the NAS score by >2 points and resolution of NASH in 33% of patients [137, 138]. Treatment with Vitamin E carries few but serious side-effects including hemorrhagic stroke and prostate cancer [139,140].

Current recommendations from the AASLD and EASL recommend vitamin E to be used at a dose of 800 IU daily as a short-term option for nondiabetic adults with biopsy-proven NASH [141, 142].

The therapeutic use of ursodeoxycholic acid (UDCA) in NAFLD/NASH has been ascribed to its cytoprotective effects through modulation of mitochondrial pathways to reduce apoptosis [143]. There are conflicting results to its beneficial outcomes despite early promise. Pentoxifylline, a TNF- α inhibitor, increases hepatic glutathione synthesis and decreases the production of free oxygen radicals. Owing to its antioxidant effects, it has shown to be hepatoprotective resulting in improvement in liver histology [144].

Antidiabetic drugs such as Glucagon like peptide 1 (GLP-1) agonists have been found to be effective in NASH. In a randomized, double-blinded placebo controlled LEAN trial, 1.8 mg Liraglutide for forty-eight weeks achieved resolution of NASH in 39% of patients compared to 9% in the placebo-treated group ($p=0.019$). Fibrosis progression was noted in only 9% of the Liraglutide treated patients compared to 36% in the placebo group ($p=0.04$) [145]. In a prospective non-randomized, non-placebo-controlled, six months therapy with 1.2 mg liraglutide in patients with uncontrolled type II diabetes improved hepatic fat content as measured by Hepatic-Magnetic Resonance Spectroscopy (^1H -MRS). The reduction in steatosis was highly correlated with weight reduction in these patients ($r=0.490$, $p<0.0001$). In contrast, patients with similar baseline characteristics who were managed with insulin therapy had no change in BMI and liver fat content [146]. Despite the promising results from recent trials, more randomized controlled trials are needed for liraglutide to be recommended for NASH. Several trials have evaluated the use of metformin in patients with biopsy-proven NAFLD. Although it initially decreased liver enzymes and improved insulin sensitivity, it did not result in histological improvement. [147, 148, 149, 150].

Sitagliptin, a dipeptidyl peptidase 4 (DPP-4) inhibitors had conflicting results regarding improvement in NASH. In an open, randomized controlled clinical trial, 46 patients with NASH were advised to have lifestyle modifications and 23 patients were given sitagliptin 100 mg once daily for 1 year. Paired biopsies of forty patients who completed the trial showed that sitagliptin was associated with improvement in steatosis and NAS compared to control group. [151]. Prospective study with larger patient sample size is required to confirm the benefits.

Sodium glucose transport protein 2 (SGLT2) inhibitors are used in the treatment of type II diabetes mellitus and were evaluated in NAFLD [152, 153]. Empagliflozin, at a dose of 25 mg/day for 24 weeks was used in an open-label pilot study involving 9 diabetic patients with biopsy-proven NASH. Histological features were unchanged or improved in all but one patient who had evidence of worsening of hepatocellular ballooning [154]. Large, randomized placebo-controlled trials including histological endpoints by biopsies are

warranted to learn the definitive benefits of SGLT2 inhibitors in patients with NASH with or without diabetes.

Farnesoid X receptor (FXR), a receptor found in the nucleus of liver cells and intestine, is a key regulator of bile acid metabolic pathways. [155]. FXR increases the expression of small heterodimer protein (SHP) 1 which is inhibitory in nature and down-regulates sterol regulatory element-binding protein (SREBP)1c. SREBP1c is a regulator of genes' expression involved in the de novo lipogenesis; fatty acid synthetase, stearoyl coenzyme A desaturase and acetyl Coenzyme A carboxylase [155]. In a randomized, multicenter, placebo-controlled phase II FLINT (Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, NASH trial), 25 mg/day of obeticholic acid (OCA) for 72 weeks was associated with improvement in ≥ 2 points decrease in NAS without worsening of fibrosis in 45% of the patients compared to 23% of those in placebo group (relative risk = 1.9, $p=0.0002$) [156]. However, decrease in HDL was noted with obeticholic acid treatment. In addition, pruritus was noted in 23% of the treated patients compared to 6% in the placebo arm ($p<0.0001$). The FDA currently has a black box warning regarding the use of OCA in patients with primary biliary cholangitis (PBC) and Child-Pugh Class B or C or decompensated cirrhosis. When dosed incorrectly, OCA can lead to hepatic decompensation and liver failure in these patients. A randomized, placebo-control, double-blind phase II trial showed that Cilofexor, a non-steroidal FXR agonist, was associated with improvement in NASH. A greater than 30% reduction in hepatic steatosis by MRI-PDFF was observed in 39% of patients receiving 100 mg of cilofexor ($p=0.011$ vs placebo) compared to 14% of patients taking 30 mg ($p=0.87$ vs placebo) [157]. The benefit and risk balance of this class of medication need to be carefully evaluated.

The liver plays a central role in lipid metabolism. Cardiovascular causes are the leading cause of death in patients with NASH. A few lipid lowering agents have found application in the treatment of NASH. Statins have been widely studied and used as therapeutic agents in NASH. Despite initial concerns of drug-induced hepatotoxicity, their safety in patients with NAFLD has now been well established [158]. Saroglitazar magnesium, a first in class dual PPAR α/γ agonist, was approved in India in March 2020 for treatment of

NASH [159]. Several trials are underway in the United States. The EVIDENCES II, a phase 3, multi-center, placebo-controlled trial of 102 patients reported saroglitazar 4 mg was associated with significant improvement in hepatocyte ballooning, steatosis, and lobular inflammation [160]. Currently, Saroglitazar is being further evaluated in a clinical trial with 3 different dosing regimens at multiple centers in the United States. Full results are highly awaited [161].

Ezetimibe is an inhibitor of intestinal cholesterol absorption. Its use was evaluated in the MOZART trial which compared ezetimibe 10 mg given for 24 weeks to placebo in 50 patients with biopsy-proven NASH. Although an improvement in liver fat measured by magnetic resonance imaging-derived proton density-fat fraction [MRI-PDFF] was seen with ezetimibe, it was not superior to placebo and did not affect histological features [162].

The discovery of the implication of cytokines such as transforming growth factor-beta 1 (TGF- β 1), platelet-derived growth factor, and angiotensin II in the pathogenesis of NAFLD led to a study evaluating the use of angiotensin II receptor blockers have been explored as a potential treatment option. Losartan at a dose 50 mg/day significantly reduced blood markers of hepatic fibrosis and ALT levels. Repeat biopsies at 48 weeks of therapy showed an improvement in inflammation in 5 patients and a reduction in fibrosis in 4 patients [163]. Further large-scale trials are required to substantiate these beneficial effects.

Effects of NAFLD on Response to antiviral treatment for chronic hepatitis B

There have been conflicting results regarding the response to antiviral treatment among patients with chronic hepatitis B and NAFLD. Some studies reported no statistical difference in virologic response in patients with or without NAFLD treated with either pegylated interferon alpha-2a or nucleotide analogues [164,165]. On the other hand, in a retrospective study on 334 CHB patients treated with entecavir or tenofovir, Kim et al. concluded HBeAg loss was higher in patients without hepatic steatosis as measured by CAP ($p=0.022$) [166]. The difference in virological response was thought to be due to a reduction in the contact area between drug and hepatocytes leading to a decrease in drug

bioavailability. Further detailed investigation and prospective studies are needed to address these controversial findings.

Summary and Future research directions

Chronic hepatitis B and NAFLD are both common causes of chronic liver disease and their coexistence has become an endemic health problem. The pathogenesis of concomitant HBV and NAFLD is complex and require dedicated research efforts to understand the impact of each entity to the overall liver injury and carcinogenesis. While there are large number of novel serum biomarkers and radiological methods are available or in development for NAFLD, many are not accessible in resource-limited clinical settings and have not been carefully validated in ethnically-diverge patient populations. While it is critically important to have noninvasive tests to identify the presence of NAFLD among patients with hepatitis B, the accuracy and reliability of the NAFLD diagnostic modalities need to be independently evaluated in patients with another liver condition such as chronic hepatitis B. Similarly, there are a number of therapeutic challenges in patients with coexisting NAFLD and HBV infection. The AALSD and EASL guidelines provide specific treatment recommendations for hepatitis B based on ALT levels among others. ALT elevation can be contributed from both NAFLD and HBV infection, no specific recommendations for HBV treatment in the setting of concurrent NAFLD and hepatitis B, however. There are some controversial observations that NAFLD may influence the virological response of HBV antiviral therapy. That could have significant clinical implications and deserve careful confirmation. Equally important, the efficacy and side-effects of the therapeutic agents for NAFLD need to be studied and validated among patients with both hepatitis B and NAFLD. As stated, there are many unmet medical needs with this new endemic of concomitant HBV and NAFLD; there are also new technologies and novel approaches to equip us to solve these challenges and to provide timely effective management to these patients.



Figure 1A. Regional prevalence of NAFLD diagnosed by radiological imaging. Pubmed and MEDLINE database from 1989 to 2015. Pooled prevalence was calculated by weighting the individual study estimates by inverse of their variation. [11, 20]

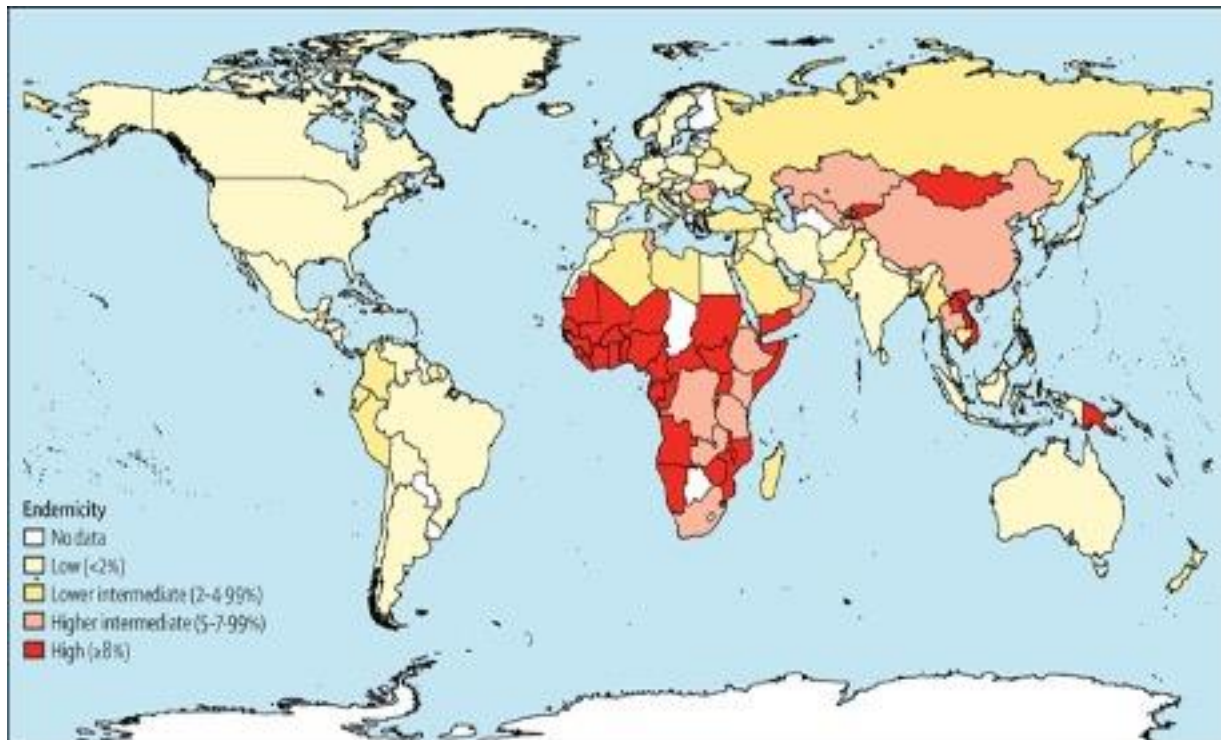


Figure 1B. Global Prevalence of Chronic Hepatitis B. Regional HBsAg prevalence estimates were produced from country specific estimates. 95% CI were obtained from country-specific variances of the prevalence estimates from logistic regression model. (Copyright permission taken from Elsevier: Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *The Lancet*. 2015 Oct 17;386(10003):1546-55.) [21]

Table 1 Serum Biomarkers For Hepatic steatosis

Serum Biomarkers	Description	Accuracy	Feasibility	Limitations
Fatty Liver Index (FLI)	BMI, WC, TG, GGT	Moderate AUROC:0.84	High	Suboptimal reference standard- ultrasound is operator dependent, insensitive to mild steatosis
Hepatic Steatosis Index	AST: ALT ratio, BMI, Female sex and DM	Moderate AUROC: 0.81	High (common parameters included)	Same as above
NAFLD Liver Fat Score	MetS, Type 2 Dm, fasting insulin, ALT and AST:ALT ratio	Very good AUROC: 0.86-0.87	Intermediate	Inclusion of serum insulin –not a routine test
Steato Test	Six components of fibrotest, Acti Test plus BMI, cholesterol. TG, Glucose adjusted for age and sex	Moderate AUROC: 0.79-0.80	Intermediate	High Cost not available in all regions and clinical settings
NAFLD Ridge Score	ALT, HDL-C, TG, HbA1C, WBC, HTN	Good AUROC: 0.87	Limited to research setting	Low PPV (69%)

Table 2. Serum Biomarkers For NASH

Serum Biomarkers	Description	Accuracy/advantages	Limitations
Apoptosis markers	CK18fragments, Total cytokeratin, sFAS	-CK18 most validated, available - commercially, correlate with histological improvement	-Less accurate alone -Optimal cut offs uncertain -Poor sensitivity
Inflammatory markers	CRP, TNF, IL8, CXCL10	-Poor -Commercial assays available -correlate with inflammatory activity in NASH	-influenced by systemic inflammation, not validated as diagnostic markers
Lipid oxidation products	11-HETE, 9 HODE, 13 HODE, 13-oxo-ODE, LA-13-HODE, 11,12-diHETrE	- Good to excellent accuracy in small studies	-Need further validation -Require mass spectroscopy
Adipocytokines and hormones	Adiponectin, leptin, resistin, visfatin RBP4, FBP4, FGF21	<ul style="list-style-type: none"> • Majority are commercialized assays • FGF21 dynamic to changes in NAFLD over time 	-Mostly validated in bariatric population -Limited accuracy in isolation
Lysosomal enzymes	Cathepsin D	-Can track changes in NAFLD over time -commercial assay available	-limited validation -varied interpretation in children and adults
Combined panels	NASH test NASH diagnostic panel	-Moderate to high accuracy -Reliable -Available commercially	-high cost -validation only in bariatric population and unknown if d -unknown whether dynamic to changes in hi

Table 3. Noninvasive Biomarkers for Hepatic Fibrosis

Serum biomarkers	Description	Accuracy	Limitations
AST: ALT Ratio	AST and ALT	AUROC: 0.66-0.74 for F3 fibrosis Sensitivity (Sn): 40%, Specificity (Sp): 80%)	Low sensitivity Non reproducible as ALT may change due to the presence of NAFLD or HBV infection.
AST: Platelet Ratio Index	AST and platelet count	AUROC 0.74 for F3 fibrosis Sn: 65%; Sp:72%	Low accuracy
Fibrosis -4 Index	Age, AST, ALT and platelet count	AUROC0.80 for F3 fi fibrosis Sn:65%, sp:97%; by dual cut offs	Low Accuracy
NAFLD Fibrosis Score	Age, BMI, impaired fasting glucose, and /or DM, AST, ALT, platelet count and albumin	AUROC 0.75-0.82 for F3 fibrosis Sn 73%-82%; Sp: 96-98% by dual cut offs)	Interpretation of BMI might differ across different ethnic groups Use of ALT may confer variable results
BARD Score	AST, ALT, BMI and diabetes	AUROC 0.69-0.81 for F3 fibrosis Sn: 62%; Sp: 66%	Interpretation of BMI might differ across different ethnic groups

Table 4: Comparison of MRI-PDFF and CAP for Diagnosis of Steatosis [99]

Grades of Steatosis		AUROC (CI)	P values
Grade 1-3 versus Grade 0	MRI - PDFF	0.99 (0.98 – 1.00)	<0.01
	CAP	0.85 (0.75 – 0.96)	
Grade 2-3 versus Grade 0-1	MRI- PDFF	0.90 (0.82 – 0.97)	<0.01
	CAP	0.70 (0.58 – 0.82)	
Grade 3 versus Grade 0-2	MRI- PDFF	0.92 (0.84 – 0.99)	0.02
	CAP	0.73 (0.58 – 0.89)	

Table 5: Comparison of MRE and TE for Diagnosis of Fibrosis [99]

Stages of Fibrosis		AUROC (CI)	P values
Stage 1-4 versus Stage 0	MRE	0.82 (0.74 – 0.91)	0.01
	*Fibroscan	0.67 (0.56 – 0.68)	
Stage 2-4 versus Stage 0-1	MRE	0.89 (0.83 – 0.86)	0.46
	Fibroscan	0.86 (0.77 – 0.95)	
Stage 3-4 versus Stage 0-2	MRE	0.87 (0.78 – 0.96)	0.19
	Fibroscan	0.80 (0.67 – 0.93)	
Stage 4 versus Stage 0-3	MRE	0.87 (0.71 – 1.00)	0.05
	Fibroscan	0.65 (0.45 – 0.94)	

* Transient elastography (TE)

References:

1. Sanyal AJ. Past, present, and future perspectives in nonalcoholic fatty liver disease. *Nature Reviews Gastroenterology & Hepatology*. 2019 Jun;16(6):377-86.
2. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, Brunt EM, Sanyal AJ. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. *Hepatology*. 2018 Jan 1;67(1):328-57.
3. Sheka AC, Adeyi O, Thompson J, Hameed B, Crawford PA, Ikramuddin S. Nonalcoholic steatohepatitis: a review. *Jama*. 2020 Mar 24;323(12):1175-83.
4. Wong RJ, Aguilar M, Cheung R, Perumpail RB, Harrison SA, Younossi ZM, Ahmed A. Nonalcoholic steatohepatitis is the second leading etiology of liver disease among adults awaiting liver transplantation in the United States. *Gastroenterology*. 2015 Mar 1;148(3):547-55.
5. Nouredin M, Vipani A, Bresee C, Todo T, Kim IK, Alkhouri N, Setiawan VW, Tran T, Ayoub WS, Lu SC, Klein AS. NASH leading cause of liver transplant in women: updated analysis of indications for liver transplant and ethnic and gender variances. *American Journal of Gastroenterology*. 2018 Nov 1;113(11):1649-59.
6. Wong GH, Chan HY, Yu Z, Chan AH, Choi PL, Chim AL, Chan HY, Tse CH, Wong VS. Coincidental metabolic syndrome increases the risk of liver fibrosis progression in patients with chronic hepatitis B—a prospective cohort study with paired transient elastography examinations. *Alimentary pharmacology & therapeutics*. 2014 Apr;39(8):883-93.
7. Peleg N, Issachar A, Arbib OS, Cohen-Naftaly M, Braun M, Leshno M, Barsheshet A, Shlomai A. Liver steatosis is a strong predictor of mortality and cancer in chronic hepatitis B regardless of viral load. *JHEP Reports*. 2019 May 1;1(1):9-16.
8. Wong GL, Wong VW, Choi PC, Chan AW, Chim AM, Yiu KK, Chan HY, Chan FK, Sung JJ, Chan HL. Metabolic syndrome increases the risk of liver cirrhosis in chronic hepatitis B. *Gut*. 2009 Jan 1;58(1):111-7.
9. Loomba R, Sanyal AJ. The global NAFLD epidemic. *Nature reviews Gastroenterology & hepatology*. 2013 Nov;10(11):686-90.
10. Younossi Z, Anstee QM, Marietti M, Hardy T, Henry L, Eslam M, George J, Bugianesi E. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. *Nature reviews Gastroenterology & hepatology*. 2018 Jan;15(1):11.
11. 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, and outcomes. *Hepatology*. 2016 Jul;64(1):73-84.
12. Perumpail BJ, Khan MA, Yoo ER, Cholanteril G, Kim D, Ahmed A. Clinical epidemiology and disease burden of nonalcoholic fatty liver disease. *World journal of gastroenterology*. 2017 Dec 21;23(47):8263.
13. Seto WK, Yuen MF. Nonalcoholic fatty liver disease in Asia: emerging perspectives. *Journal of gastroenterology*. 2017 Feb 1;52(2):164-74.

14. Huang T, Behary J, Zekry A. Non-alcoholic fatty liver disease (NAFLD): a review of epidemiology, risk factors, diagnosis and management. *Internal medicine journal*. 2019 Nov 24.
15. Browning JD, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology*. 2004 Dec;40(6):1387-95.
16. MacLachlan JH, Cowie BC. Hepatitis B virus epidemiology. *Cold Spring Harbor perspectives in medicine*. 2015 May 1;5(5):a021410.
17. Jefferies M, Rauff B, Rashid H, Lam T, Rafiq S. Update on global epidemiology of viral hepatitis and preventive strategies. *World journal of clinical cases*. 2018 Nov 6;6(13):589.
18. Pais R, Rusu E, Ratzu V. The impact of obesity and metabolic syndrome on chronic hepatitis B and drug-induced liver disease. *Clinics in liver disease*. 2014 Feb 1;18(1):165-78.
19. Hui RW, Seto WK, Cheung KS, Mak LY, Liu KS, Fung J, Wong DH, Lai CL, Yuen MF. Inverse relationship between hepatic steatosis and hepatitis B viremia: results of a large case-control study. *Journal of viral hepatitis*. 2018 Jan;25(1):97-104.
20. Adams LA, Roberts SK, Strasser SI, Mahady SE, Powell E, Estes C, Razavi H, George J. Nonalcoholic fatty liver disease burden: Australia, 2019–2030. *Journal of Gastroenterology and Hepatology*. 2020 Feb 11.
21. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013. *The Lancet*. 2015 Oct 17;386(10003):1546-55.
22. Lee YB, Ha Y, Chon YE, Kim MN, Lee JH, Park H, Kim KI, Kim SH, Rim KS, Hwang SG. Association between hepatic steatosis and the development of hepatocellular carcinoma in patients with chronic hepatitis B. *Clinical and molecular hepatology*. 2019 Mar;25(1):52.
23. Contos MJ, Sanyal AJ. The clinicopathologic spectrum and management of nonalcoholic fatty liver disease. *Advances in anatomic pathology*. 2002 Jan 1;9(1):37-51.
24. Chan AW, Wong GL, Chan HY, Tong JH, Yu YH, Choi PC, Chan HL, To KF, Wong VW. Concurrent fatty liver increases risk of hepatocellular carcinoma among patients with chronic hepatitis B. *Journal of gastroenterology and hepatology*. 2017 Mar;32(3):667-76.
25. Wu YL, Peng XE, Zhu YB, Yan XL, Chen WN, Lin X. Hepatitis B virus X protein induces hepatic steatosis by enhancing the expression of liver fatty acid binding protein. *Journal of virology*. 2016 Feb 15;90(4):1729-40.
26. Kim KH, Shin HJ, Kim K, Choi HM, Rhee SH, Moon HB, Kim HH, Yang US, Yu DY, Cheong J. Hepatitis B virus X protein induces hepatic steatosis via transcriptional activation of SREBP1 and PPAR γ . *Gastroenterology*. 2007 May 1;132(5):1955-67.
27. Ben AS, Ksiaa M, Souguir A, Issaoui D, Sriha B, Ajmi S. Hepatic steatosis in chronic hepatitis B: prevalence, risk factors, and impact on fibrosis and therapeutic response. *La Tunisie medicale*. 2013 Jul;91(7):431-4.

28. Pais R, Rusu E, Zilisteanu D, Circiumaru A, Micu L, Voiculescu M, Poynard T, Ratzu V. Prevalence of steatosis and insulin resistance in patients with chronic hepatitis B compared with chronic hepatitis C and non-alcoholic fatty liver disease. *European journal of internal medicine*. 2015 Jan 1;26(1):30-6.
29. Karacaer Z, Okur G, Cermik H, Altun D. Is there an influence of hepatic steatosis on fibrosis and necroinflammation in young patients with chronic viral hepatitis B?. *Postgraduate medicine*. 2016 Oct 2;128(7):697-700.
30. Arezoo E, Ali Akbari S, Sahar Naz N, Marym R, Naser R, Seyed Mohammad T, Reza M, Ghodrat M. The effect of NAFLD (non-alcoholic fatty liver disease) on long-term outcome of chronic hepatitis B in Iranian patients. *Open Journal of Gastroenterology*. 2012 Feb 15;2012.
31. Charatcharoenwittaya P, Pongpaibul A, Kaosombatwattana U, Bhanthumkomol P, Bandidniyamanon W, Pausawasdi N, Tanwandee T. The prevalence of steatohepatitis in chronic hepatitis B patients and its impact on disease severity and treatment response. *Liver International*. 2017 Apr;37(4):542-51.
32. Chen Y, Fan C, Chen Y, Liu H, Wang S, Dong P, Li L, Ding H. Effect of hepatic steatosis on the progression of chronic hepatitis B: A prospective cohort and in vitro study. *Oncotarget*. 2017 Aug 29;8(35):58601.
33. Elloumi H, Hefaieth R, Gargouri D, Chedly-Debbiche A, Khayat O, Bel NH, Kharrat J, Ghorbel A. Hepatic steatosis in chronic hepatitis B: contributing factors and effect on fibrosis. *La Tunisie medicale*. 2008 Nov;86(11):1000-3.
34. Shi JP, Fan JG, Wu R, Gao XQ, Zhang L, Wang H, Farrell GC. Prevalence and risk factors of hepatic steatosis and its impact on liver injury in Chinese patients with chronic hepatitis B infection. *Journal of gastroenterology and hepatology*. 2008 Sep;23(9):1419-25.
35. Lesmana LA, Lesmana CR, Pakasi LS, Krisnuhoni E. Prevalence of hepatic steatosis in chronic hepatitis B patients and its association with disease severity. *Acta Med Indones*. 2012 Jan 1;44(1):35-9.
36. Choi HS, Brouwer WP, Zanjir WM, de Man RA, Feld JJ, Hansen BE, Janssen HL, Patel K. Nonalcoholic steatohepatitis is associated with liver-related outcomes and all-cause mortality in chronic hepatitis B. *Hepatology*. 2020 Feb;71(2):539-48.
37. Schuppan D, Surabattula R, Wang XY. Determinants of fibrosis progression and regression in NASH. *Journal of hepatology*. 2018 Feb 1;68(2):238-50.
38. Koyama Y, Brenner DA. Liver inflammation and fibrosis. *The Journal of clinical investigation*. 2017 Jan 3;127(1):55-64.
39. Chan HL, Tse CH, Mo F, Koh J, Wong VW, Wong GL, Lam Chan S, Yeo W, Sung JJ, Mok TS. High viral load and hepatitis B virus subgenotype cc are associated with increased risk of hepatocellular carcinoma. *Journal of Clinical Oncology*. 2008 Jan 10;26(2):177-82.
40. Ascha MS, Hanounieh IA, Lopez R, Tamimi TA, Feldstein AF, Zein NN. The incidence and risk factors of hepatocellular carcinoma in patients with nonalcoholic steatohepatitis. *Hepatology*. 2010 Jun;51(6):1972-8.

41. Zhao Y, Wang H, Gu X, Ji J, Gao C. Association between metabolic abnormalities and HBV related hepatocellular carcinoma in Chinese: a cross-sectional study. *Nutrition journal*. 2011 Dec;10(1):49.
42. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, Vilgrain V, Bedossa P, Belghiti J. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. *Hepatology*. 2009 Mar;49(3):851-9.
43. Yu MW, Lin CL, Liu CJ, Yang SH, Tseng YL, Wu CF. Influence of metabolic risk factors on risk of hepatocellular carcinoma and liver-related death in men with chronic hepatitis B: a large cohort study. *Gastroenterology*. 2017 Oct 1;153(4):1006-17.
44. Caldwell SH, Crespo DM, Kang HS, Al-Osaimi AM. Obesity and hepatocellular carcinoma. *Gastroenterology*. 2004 Nov 1;127(5):S97-103.
45. Davila JA, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut*. 2005 Apr 1;54(4):533-9.
46. Demir K, Akyuz F, Ozdil S, Aksoy N, Kaymakoglu S, Poturoglu S, Akyüz Ü, Besik F, Boztas G, Mungan Z, Cevikbas U. What is the reason of elevated alanine aminotransferase level in HBeAg negative patients with low viremia: NAFLD or chronic hepatitis?. *Annals of hepatology*. 2007;6(2):92-6.
47. Sumida Y, Nakajima A, Itoh Y. Limitations of liver biopsy and non-invasive diagnostic tests for the diagnosis of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World journal of gastroenterology: WJG*. 2014 Jan 14;20(2):475.
48. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC gastroenterology*. 2006 Dec 1;6(1):33.
49. Lee JH, Kim D, Kim HJ, Lee CH, Yang JI, Kim W, Kim YJ, Yoon JH, Cho SH, Sung MW, Lee HS. Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease. *Digestive and Liver Disease*. 2010 Jul 1;42(7):503-8.
50. Kotronen A, Peltonen M, Hakkarainen A, Sevastianova K, Bergholm R, Johansson LM, Lundbom N, Rissanen A, Ridderstråle M, Groop L, Orho-Melandar M. Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors. *Gastroenterology*. 2009 Sep 1;137(3):865-72.
51. Poynard T, Ratzu V, Naveau S, Thabut D, Charlotte F, Messous D, Capron D, Abella A, Massard J, Ngo Y, Munteanu M. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comparative hepatology*. 2005 Dec 1;4(1):10.
52. Yip TF, Ma AJ, Wong VS, Tse YK, Chan HY, Yuen PC, Wong GH. Laboratory parameter-based machine learning model for excluding non-alcoholic fatty liver disease (NAFLD) in the general population. *Alimentary pharmacology & therapeutics*. 2017 Aug;46(4):447-56.

53. Wong VW, Chitturi S, Wong GL, Yu J, Chan HL, Farrell GC. Pathogenesis and novel treatment options for non-alcoholic steatohepatitis. *The Lancet Gastroenterology & Hepatology*. 2016 Sep 1;1(1):56-67.
54. Eguchi A, Wree A, Feldstein AE. Biomarkers of liver cell death. *Journal of hepatology*. 2014 May 1;60(5):1063-74.
55. Vuppalanchi R, Jain AK, Deppe R, Yates K, Comerford M, Masuoka HC, Neuschwander-Tetri BA, Loomba R, Brunt EM, Kleiner DE, Molleston JP. Relationship between changes in serum levels of keratin 18 and changes in liver histology in children and adults with nonalcoholic fatty liver disease. *Clinical Gastroenterology and Hepatology*. 2014 Dec 1;12(12):2121-30.
56. Puri P, Wiest MM, Cheung O, Mirshahi F, Sargeant C, Min HK, Contos MJ, Sterling RK, Fuchs M, Zhou H, Watkins SM. The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology*. 2009 Dec;50(6):1827-38.
57. Goh GB, Issa D, Lopez R, Dasarathy S, Dasarathy J, Sargent R, Hawkins C, Pai RK, Yerian L, Khiyami A, Pagadala MR. The development of a non-invasive model to predict the presence of non-alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease. *Journal of gastroenterology and hepatology*. 2016 May;31(5):995-1000.
58. Feldstein AE, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, Zhang R, McIntyre TM, Hazen SL. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Journal of lipid research*. 2010 Oct 1;51(10):3046-54.
59. Jarrar MH, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Alimentary pharmacology & therapeutics*. 2008 Mar;27(5):412-21.
60. Polyzos SA, Kountouras J, Mantzoros CS. Adipokines in nonalcoholic fatty liver disease. *Metabolism*. 2016 Aug 1;65(8):1062-79.
61. Selvakumar PK, Kabbany MN, Lopez R, Tozzi G, Alisi A, Alkhouri N, Nobili V. Reduced lysosomal acid lipase activity—A potential role in the pathogenesis of non alcoholic fatty liver disease in pediatric patients. *Digestive and Liver Disease*. 2016 Aug 1;48(8):909-13.
62. Poynard T, Ratzu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, Massard J, Bonyhay L, Tahiri M, Thabut D, Cadranel JF. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease. *BMC gastroenterology*. 2006 Dec 1;6(1):34.
63. Younossi ZM, Jarrar M, Nugent C, Randhawa M, Afendy M, Stepanova M, Rafiq N, Goodman Z, Chandhoke V, Baranova A. A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH). *Obesity surgery*. 2008 Nov 1;18(11):1430-7.
64. Kwok R, Tse YK, Wong GH, Ha Y, Lee AU, Ngu MC, Chan HY, Wong VS. Systematic review with meta-analysis: non-invasive assessment of non-alcoholic fatty liver disease—the role of transient elastography and plasma cytokeratin-18 fragments. *Alimentary pharmacology & therapeutics*. 2014 Feb;39(3):254-69.

65. Lu Q, Liu X, Liu S, Xie C, Liu Y, Ma C. The Relationship Between AST/ALT Ratio and Metabolic Syndrome in Han Young Adults-AST/ALT Ratio and Metabolic Syndrome. *Recent Advances in Cardiovascular Risk Factors*.(12). 2012 Mar 21:247-55.
66. Shaheen AA, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: A systematic review. *Hepatology*. 2007 Sep;46(3):912-21.
67. Sumida Y, Yoneda M, Hyogo H, Itoh Y, Ono M, Fujii H, Eguchi Y, Suzuki Y, Aoki N, Kanemasa K, Fujita K. Validation of the FIB4 index in a Japanese nonalcoholic fatty liver disease population. *BMC gastroenterology*. 2012 Dec 1;12(1):2.
68. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007 Apr;45(4):846-54.
69. Ruffillo G, Fassio E, Alvarez E, Landeira G, Longo C, Domínguez N, Gualano G. Comparison of NAFLD fibrosis score and BARD score in predicting fibrosis in nonalcoholic fatty liver disease. *Journal of hepatology*. 2011 Jan 1;54(1):160-3.
70. Suzuki A, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe hepatic fibrosis in patients with non-alcoholic fatty liver disease. *Liver International*. 2005 Aug;25(4):779-86.
71. Monarca A, Petrini C, Perolini S, Pozzi F, Adelasco L, Natangelo R, Croce G. Procollagen-type III peptide serum concentrations in alcoholic and non-alcoholic liver disease. *Ricerca in clinica e in laboratorio*. 1985 Apr 1;15(2):167-71.
72. Nielsen MJ, Nedergaard AF, Sun S, Veidal SS, Larsen L, Zheng Q, Suetta C, Henriksen K, Christiansen C, Karsdal MA, Leeming DJ. The neo-epitope specific PRO-C3 ELISA measures true formation of type III collagen associated with liver and muscle parameters. *American journal of translational research*. 2013;5(3):303.
73. Hemmann S, Graf J, Roderfeld M, Roeb E. Expression of MMPs and TIMPs in liver fibrosis—a systematic review with special emphasis on anti-fibrotic strategies. *Journal of hepatology*. 2007 May 1;46(5):955-75.
74. Abdelaziz R, Elbasel M, Esmat S, Essam K, Abdelaaty S. Tissue Inhibitors of metalloproteinase-1 and 2 and obesity related non-alcoholic fatty liver disease: is there a relationship?. *Digestion*. 2015;92(3):130-7.
75. Dos Santos VN, Leite-Mór MM, Kondo M, Martins JR, Nader H, Lanzoni VP, Parise ER. Serum laminin, type IV collagen and hyaluronan as fibrosis markers in non-alcoholic fatty liver disease. *Brazilian Journal of Medical and Biological Research*. 2005 May;38(5):747-53.
76. Nobili V, Parkes J, Bottazzo G, Marcellini M, Cross R, Newman D, Vizzutti F, Pinzani M, Rosenberg WM. Performance of ELF serum markers in predicting fibrosis stage in pediatric non-alcoholic fatty liver disease. *Gastroenterology*. 2009 Jan 1;136(1):160-7.

77. Munteanu M, Tiniakos D, Anstee Q, Charlotte F, Marchesini G, Bugianesi E, Trauner M, Romero Gomez M, Oliveira C, Day C, Dufour JF. Diagnostic performance of FibroTest, SteatoTest and ActiTest in patients with NAFLD using the SAF score as histological reference. *Alimentary pharmacology & therapeutics*. 2016 Oct;44(8):877-89.
78. Boursier J, de Ledinghen V, Leroy V, Anty R, Francque S, Salmon D, Lannes A, Bertrais S, Oberti F, Fouchard-Hubert I, Calès P. A stepwise algorithm using an at-a-glance first-line test for the non-invasive diagnosis of advanced liver fibrosis and cirrhosis. *Journal of hepatology*. 2017 Jun 1;66(6):1158-65.
79. Loong TC, Wei JL, Leung JC, Wong GL, Shu SS, Chim AM, Chan AW, Choi PC, Tse YK, Chan HL, Wong VW. Application of the combined FibroMeter vibration-controlled transient elastography algorithm in Chinese patients with non-alcoholic fatty liver disease. *Journal of gastroenterology and hepatology*. 2017 Jul;32(7):1363-9.
80. Pan Q, Zhang RN, Wang YQ, Zheng RD, Mi YQ, Liu WB, Shen F, Chen GY, Lu JF, Zhu CY, Zhang SY. Linked PNPLA3 polymorphisms confer susceptibility to nonalcoholic steatohepatitis and decreased viral load in chronic hepatitis B. *World Journal of Gastroenterology: WJG*. 2015 Jul 28;21(28):8605.
81. Liang J, Liu F, Wang F, Han T, Jing L, Ma Z, Gao Y. A noninvasive score model for prediction of NASH in patients with chronic hepatitis B and nonalcoholic fatty liver disease. *BioMed research international*. 2017 Mar 2;2017.
82. Yang RX, Hu CX, Sun WL, Pan Q, Shen F, Yang Z, Su Q, Xu GW, Fan JG. Serum monounsaturated triacylglycerol predicts steatohepatitis in patients with non-alcoholic fatty liver disease and chronic hepatitis B. *Scientific reports*. 2017 Sep 5;7(1):1-1.
83. Zhang H, Li QY, Guo ZZ, Guan Y, Du J, Lu YY, Hu YY, Liu P, Huang S, Su SB. Serum levels of microRNAs can specifically predict liver injury of chronic hepatitis B. *World journal of gastroenterology: WJG*. 2012 Oct 7;18(37):5188.
84. Liu XL, Pan Q, Zhang RN, Shen F, Yan SY, Sun C, Xu ZJ, Chen YW, Fan JG. Disease-specific miR-34a as diagnostic marker of non-alcoholic steatohepatitis in a Chinese population. *World journal of gastroenterology*. 2016 Nov 28;22(44):9844.
85. Dong, D.R., Hao, M.N., Li, C., Peng, Z., Liu, X., Wang, G.P. and Ma, A.L., 2015. Acoustic radiation force impulse elastography, FibroScan®, Forns' index and their combination in the assessment of liver fibrosis in patients with chronic hepatitis B, and the impact of inflammatory activity and steatosis on these diagnostic methods. *Molecular medicine reports*, 11(6), pp.4174-4182.
86. The gamma-glutamyl transpeptidase to platelet ratio (GPR) predicts significant liver fibrosis and cirrhosis in patients with chronic HBV infection in West Africa by Lemoine et al. *Gut*. 2016 May 1;65(5):882-4.
87. Li Q, Lu C, Li W, Huang Y, Chen L. The gamma-glutamyl transpeptidase to platelet ratio for non-invasive assessment of liver fibrosis in patients with chronic hepatitis B and non-alcoholic fatty liver disease. *Oncotarget*. 2017 Apr 25;8(17):28641.
88. Akdoğan Ö, Yücel AA, Sargin ZG, Sönmez C, Yilmaz GE, Özenirler S. Evaluation of Plasma Urokinase-Type Plasminogen Activator Receptor (UPAR)

- in Patients With Chronic Hepatitis B, C and Non-Alcoholic Fatty Liver Disease (NAFLD) as Serological Fibrosis Marker. *Journal of clinical and experimental hepatology*. 2019 Jan 1;9(1):29-33.
89. Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population-based study including 4275 biopsies. *Liver International*. 2008 May;28(5):705-12.
 90. Mishra P, Younossi ZM. Abdominal ultrasound for diagnosis of nonalcoholic fatty liver disease (NAFLD).
 91. Mottin CC, Moretto M, Padoin AV, Swarowsky AM, Toneto MG, Glock L, Repetto G. The role of ultrasound in the diagnosis of hepatic steatosis in morbidly obese patients. *Obesity surgery*. 2004 May 1;14(5):635-7.
 92. Sasso, M., Beaugrand, M., De Ledinghen, V., Douvin, C., Marcellin, P., Poupon, R., Sandrin, L. and Miette, V., 2010. Controlled attenuation parameter (CAP): a novel VCTE™ guided ultrasonic attenuation measurement for the evaluation of hepatic steatosis: preliminary study and validation in a cohort of patients with chronic liver disease from various causes. *Ultrasound in medicine & biology*, 36(11), pp.1825-1835.
 93. Karlas T, Petroff D, Sasso M, Fan JG, Mi YQ, de Lédighen V, Kumar M, Lupsor-Platon M, Han KH, Cardoso AC, Ferraioli G. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *Journal of hepatology*. 2017 May 1;66(5):1022-30.
 94. Kjærgaard M, Thiele M, Jansen C, Madsen BS, Gørtzen J, Strassburg C, Trebicka J, Krag A. High risk of misinterpreting liver and spleen stiffness using 2D shear-wave and transient elastography after a moderate or high calorie meal. *PloS one*. 2017;12(4).
 95. 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. *Digestive and Liver Disease*. 2017 Aug 1;49(8):910-7.
 96. Venkatesh SK, Yin M, Ehman RL. Magnetic resonance elastography of liver: technique, analysis, and clinical applications. *Journal of magnetic resonance imaging*. 2013 Mar;37(3):544-55.
 97. Caussy C, Reeder SB, Sirlin CB, Loomba R. Noninvasive, quantitative assessment of liver fat by MRI-PDFF as an endpoint in NASH trials. *Hepatology*. 2018 Aug 1;68(2):763-72.
 98. Runge JH, Smits LP, Verheij J, Depla A, Kuiken SD, Baak BC, Nederveen AJ, Beuers U, Stoker J. MR spectroscopy-derived proton density fat fraction is superior to controlled attenuation parameter for detecting and grading hepatic steatosis. *Radiology*. 2018 Feb;286(2):547-56.
 99. Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, Hooker J, Sy E, Savides MT, Alquiraish MH, Valasek MA. Magnetic resonance elastography vs transient elastography in detection of fibrosis and noninvasive measurement of steatosis in patients with biopsy-proven nonalcoholic fatty liver disease. *Gastroenterology*. 2017 Feb 1;152(3):598-607.
 100. Imajo K, Kessoku T, Honda Y, Tomeno W, Ogawa Y, Mawatari H, Fujita K, Yoneda M, Taguri M, Hyogo H, Sumida Y. Magnetic resonance imaging more

- accurately classifies steatosis and fibrosis in patients with nonalcoholic fatty liver disease than transient elastography. *Gastroenterology*. 2016 Mar 1;150(3):626-37.
101. Naganawa S, Enooku K, Tateishi R, Akai H, Yasaka K, Shibahara J, Ushiku T, Abe O, Ohtomo K, Kiryu S. Imaging prediction of nonalcoholic steatohepatitis using computed tomography texture analysis. *European radiology*. 2018 Jul 1;28(7):3050-8.
 102. Eddowes PJ, McDonald N, Davies N, Semple SI, Kendall TJ, Hodson J, Newsome PN, Flinham RB, Wesolowski R, Blake L, Duarte RV. Utility and cost evaluation of multiparametric magnetic resonance imaging for the assessment of non-alcoholic fatty liver disease. *Alimentary pharmacology & therapeutics*. 2018 Mar;47(5):631-44.
 103. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. 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 Jun;55(6):2005-23.
 104. Ajmera V, Loomba R. Can elastography differentiate isolated fatty liver from nonalcoholic steatohepatitis?. In *Seminars in liver disease* 2018 Feb (Vol. 38, No. 01, pp. 014-020). Thieme Medical Publishers.
 105. Friedrich-Rust M, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology*. 2008 Apr 1;134(4):960-74
 106. Stebbing J, Farouk L, Panos G, Anderson M, Jiao LR, Mandalia S, Bower M, Gazzard B, Nelson M. A meta-analysis of transient elastography for the detection of hepatic fibrosis. *Journal of clinical gastroenterology*. 2010 Mar 1;44(3):214-9.
 107. Talwalkar JA, Kurtz DM, Schoenleber SJ, West CP, Montori VM. Ultrasound-based transient elastography for the detection of hepatic fibrosis: systematic review and meta-analysis. *Clinical gastroenterology and hepatology*. 2007 Oct 1;5(10):1214-20.
 108. Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. *Nature reviews Gastroenterology & hepatology*. 2016 Jul;13(7):402.
 109. European Association for the Study of The Liver, European Association for the Study of Diabetes (EASD). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Obesity facts*. 2016;9(2):65-90.
 110. Herrmann E, de Lédinghen V, Cassinotto C, Chu WC, Leung VY, Ferraioli G, Filice C, Castera L, Vilgrain V, Ronot M, Dumortier J. Assessment of biopsy-proven liver fibrosis by two-dimensional shear wave elastography: An individual patient data-based meta-analysis. *Hepatology*. 2018 Jan;67(1):260-72.
 111. Palmeri ML, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *Journal of hepatology*. 2011 Sep 1;55(3):666-72.
 112. Karlas T, Dietrich A, Peter V, Wittekind C, Lichtinghagen R, Garnov N, Linder N, Schaudinn A, Busse H, Prettin C, Keim V. Evaluation of transient

- elastography, acoustic radiation force impulse imaging (ARFI), and enhanced liver function (ELF) score for detection of fibrosis in morbidly obese patients. *PLoS One*. 2015;10(11).
113. Singh S, Venkatesh SK, Wang Z, Miller FH, Motosugi U, Low RN, Hassanein T, Asbach P, Godfrey EM, Yin M, Chen J. Diagnostic performance of magnetic resonance elastography in staging liver fibrosis: a systematic review and meta-analysis of individual participant data. *Clinical Gastroenterology and Hepatology*. 2015 Mar 1;13(3):440-51.
 114. Younossi ZM, Loomba R, Rinella ME, Bugianesi E, Marchesini G, Neuschwander-Tetri BA, Serfaty L, Negro F, Caldwell SH, Ratziu V, Corey KE. Current and future therapeutic regimens for nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. *Hepatology*. 2018 Jul 1;68(1):361-71.
 115. Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, Friedman SL, Diago M, Romero-Gomez M. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology*. 2015 Aug 1;149(2):367-78.
 116. Wong VW, Wong GL, Chan RS, Shu SS, Cheung BH, Li LS, Chim AM, Chan CK, Leung JK, Chu WC, Woo J. Beneficial effects of lifestyle intervention in non-obese patients with non-alcoholic fatty liver disease. *Journal of hepatology*. 2018 Dec 1;69(6):1349-56.
 117. Nguyen V, George J. Nonalcoholic fatty liver disease management: dietary and lifestyle modifications. In *Seminars in liver disease* 2015 Aug (Vol. 35, No. 03, pp. 318-337). Thieme Medical Publishers.
 118. Mardinoglu A, Wu H, Bjornson E, Zhang C, Hakkarainen A, Räsänen SM, Lee S, Mancina RM, Bergentall M, Pietiläinen KH, Söderlund S. An integrated understanding of the rapid metabolic benefits of a carbohydrate-restricted diet on hepatic steatosis in humans. *Cell metabolism*. 2018 Mar 6;27(3):559-71.
 119. Basaranoglu M, Basaranoglu G, Bugianesi E. Carbohydrate intake and nonalcoholic fatty liver disease: fructose as a weapon of mass destruction. *Hepatobiliary surgery and nutrition*. 2015 Apr;4(2):109.
 120. Li YH, Yang LH, Sha KH, Liu TG, Zhang LG, Liu XX. Efficacy of polyunsaturated fatty acid therapy on patients with nonalcoholic steatohepatitis. *World Journal of Gastroenterology: WJG*. 2015 Jun 14;21(22):7008.
 121. Yang J, Fernández-Galilea M, Martínez-Fernández L, González-Muniesa P, Pérez-Chávez A, Martínez JA, Moreno-Aliaga MJ. Oxidative stress and non-alcoholic fatty liver disease: effects of omega-3 fatty acid supplementation. *Nutrients*. 2019 Apr;11(4):872.
 122. Scorletti E, Bhatia L, McCormick KG, Clough GF, Nash K, Hodson L, et al. Effects of purified eicosapentaenoic and docosahexaenoic acids in nonalcoholic fatty liver disease: results from the Welcome* study. *Hepatology*. 2014;60(4):1211-21.
 123. Hekmatdoost A, Shamsipour A, Meibodi M, Gheibizadeh N, Eslamparast T, Poustchi H. Adherence to the dietary approaches to stop hypertension (DASH) and risk of nonalcoholic fatty liver disease. *International journal of food sciences and nutrition*. 2016 Nov 16;67(8):1024-9.

124. . European Association for the Study of The Liver, European Association for the Study of Diabetes (EASD. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *Obesity facts*. 2016;9(2):65-90.
125. Soleimani D, Ranjbar G, Rezvani R, Goshayeshi L, Razmpour F, Nematy M. Dietary patterns in relation to hepatic fibrosis among patients with nonalcoholic fatty liver disease. *Diabetes, metabolic syndrome and obesity: targets and therapy*. 2019;12:315
126. Godos J, Federico A, Dallio M, Scazzina F. Mediterranean diet and nonalcoholic fatty liver disease: Molecular mechanisms of protection. *International journal of food sciences and nutrition*. 2017 Jan 2;68(1):18-27.
127. Houghton D, Thoma C, Hallsworth K, Cassidy S, Hardy T, Burt AD, Tiniakos D, Hollingsworth KG, Taylor R, Day CP, McPherson S. Exercise reduces liver lipids and visceral adiposity in patients with nonalcoholic steatohepatitis in a randomized controlled trial. *Clinical Gastroenterology and Hepatology*. 2017 Jan 1;15(1):96-102.
128. Musso G, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia*. 2012 Apr 1;55(4):885-904.
129. Suarez M, Boque N, Del Bas JM, Mayneris-Perxachs J, Arola L, Caimari A. Mediterranean Diet and Multi-Ingredient-Based Interventions for the Management of Non-Alcoholic Fatty Liver Disease. *Nutrients*. 2017;9(10).
130. Moscatiello S, Di Luzio R, Bugianesi E, Suppini A, Hickman IJ, Di Domizio S, et al. Cognitive-behavioral treatment of nonalcoholic Fatty liver disease: a propensity score-adjusted observational study. *Obesity (Silver Spring)*. 2011;19(4):763-70.
131. Aminian A, Zajichek A, Arterburn DE, Wolski KE, Brethauer SA, Schauer PR, Kattan MW, Nissen SE. Association of metabolic surgery with major adverse cardiovascular outcomes in patients with type 2 diabetes and obesity. *Jama*. 2019 Oct 1;322(13):1271-82.
132. Salman MA, Salman AA, Abdelsalam A, Atallah M, Shaaban HE, El-Mikkawy A, Omar MG, Elshenoufy M. Laparoscopic sleeve gastrectomy on the horizon as a promising treatment modality for NAFLD. *Obesity Surgery*. 2020 Jan 1;30(1):87-95.
133. Lassailly G, Caiazzo R, Buob D, Pigeyre M, Verkindt H, Labreuche J, Raverdy V, Leteurtre E, Dharancy S, Louvet A, Romon M. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. *Gastroenterology*. 2015 Aug 1;149(2):379-88.
134. Hino A, Adachi H, Enomoto M, Furuki K, Shigetoh Y, Ohtsuka M, et al. Habitual coffee but not green tea consumption is inversely associated with metabolic syndrome: an epidemiological study in a general Japanese population. *Diabetes Res Clin Pract*. 2007;76(3):383-9.
135. Catalano D, Martines GF, Tonzuso A, Pirri C, Trovato FM, Trovato GM. Protective role of coffee in non-alcoholic fatty liver disease (NAFLD). *Dig Dis Sci*. 2010;55(11):3200-6.

136. Bellentani S, Saccoccio G, Masutti F, Croce LS, Brandi G, Sasso F, et al. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med.* 2000;132(2):112-7.
137. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, Neuschwander-Tetri BA, Lavine JE, Tonascia J, Unalp A, Van Natta M. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. *New England Journal of Medicine.* 2010 May 6;362(18):1675-85.
138. Bril F, Biernacki DM, Kalavalapalli S, Lomonaco R, Subbarayan SK, Lai J, Tio F, Suman A, Orsak BK, Hecht J, Cusi K. Role of vitamin E for nonalcoholic steatohepatitis in patients with type 2 diabetes: a randomized controlled trial. *Diabetes Care.* 2019 Aug 1;42(8):1481-8.
139. Schurks M, Glynn RJ, Rist PM, Tzourio C, Kurth T. Effects of vitamin E on stroke subtypes: meta-analysis of randomised controlled trials. *BMJ.* 2010;341:c5702.
140. Klein EA, Thompson IM, Jr., Tangen CM, Crowley JJ, Lucia MS, Goodman PJ, et al. Vitamin E and the risk of prostate cancer: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA.* 2011;306(14):1549-56.
141. Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: Practice guidance from the American Association for the Study of Liver Diseases. *Hepatology.* 2018;67(1):328-57.
142. European Association for the Study of the L, European Association for the Study of D, European Association for the Study of O. EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016;64(6):1388-402.
143. Mueller M, Castro RE, Thorell A, Marschall HU, Auer N, Herac M, Rodrigues CM, Trauner M. Ursodeoxycholic acid: Effects on hepatic unfolded protein response, apoptosis and oxidative stress in morbidly obese patients. *Liver International.* 2018 Mar;38(3):523-31.
144. Zein CO, Lopez R, Fu X, Kirwan JP, Yerian LM, McCullough AJ, et al. Pentoxifylline decreases oxidized lipid products in nonalcoholic steatohepatitis: new evidence on the potential therapeutic mechanism. *Hepatology.* 2012;56(4):1291-9.
145. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, Hazlehurst JM, Guo K, Abouda G, Aldersley MA, Stocken D. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *The Lancet.* 2016 Feb 13;387(10019):679-90.
146. Petit JM, Cercueil JP, Loffroy R, Denimal D, Bouillet B, Fourmont C, Chevallier O, Duvillard L, Verges B. Effect of liraglutide therapy on liver fat content in patients with inadequately controlled type 2 diabetes: the Lira-NAFLD study. *The Journal of Clinical Endocrinology & Metabolism.* 2017 Feb 1;102(2):407-15.
147. Nair S, Diehl AM, Wiseman M, Farr GH, Jr., Perrillo RP. Metformin in the treatment of non-alcoholic steatohepatitis: a pilot open label trial. *Aliment Pharmacol Ther.* 2004;20(1):23-8.

148. Haukeland JW, Konopski Z, Eggesbo HB, von Volkmann HL, Raschpichler G, Bjoro K, et al. Metformin in patients with non-alcoholic fatty liver disease: a randomized, controlled trial. *Scand J Gastroenterol*. 2009;44(7):853-60.
149. Uygun A, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, et al. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Aliment Pharmacol Ther*. 2004;19(5):537-44.
150. Shields WW, Thompson KE, Grice GA, Harrison SA, Coyle WJ. The Effect of Metformin and Standard Therapy versus Standard Therapy alone in Nondiabetic Patients with Insulin Resistance and Nonalcoholic Steatohepatitis (NASH): A Pilot Trial. *Therap Adv Gastroenterol*. 2009;2(3):157-63.
151. Alam S, Ghosh J, Mustafa G, Kamal M, Ahmad N. Effect of sitagliptin on hepatic histological activity and fibrosis of nonalcoholic steatohepatitis patients: a 1-year randomized control trial. *Hepatic medicine: evidence and research*. 2018;10:23
152. Kuchay MS, Krishan S, Mishra SK, Farooqui KJ, Singh MK, Wasir JS, Bansal B, Kaur P, Jevalikar G, Gill HK, Choudhary NS. Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: a randomized controlled trial (E-LIFT Trial). *Diabetes care*. 2018 Aug 1;41(8):1801-8.
153. Gastaldelli A, Repetto E, Guja C, Hardy E, Han J, Jabbour SA, Ferrannini E. Exenatide and dapagliflozin combination improves markers of liver steatosis and fibrosis in patients with type 2 diabetes. *Diabetes, Obesity and Metabolism*. 2020 Mar;22(3):393-403.
154. Lai LL, Vethakkan SR, Nik Mustapha NR, Mahadeva S, Chan WK. Empagliflozin for the Treatment of Nonalcoholic Steatohepatitis in Patients with Type 2 Diabetes Mellitus. *Dig Dis Sci*. 2020;65(2):623-31.
155. Adorini L, Pruzanski M, Shapiro D. Farnesoid X receptor targeting to treat nonalcoholic steatohepatitis. *Drug discovery today*. 2012 Sep 1;17(17-18):988-97.
156. Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, Chalasani N, Dasarathy S, Diehl AM, Hameed B, Kowdley KV. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. *The Lancet*. 2015 Mar 14;385(9972):956-65.
157. Patel K, Harrison SA, Elkashab M, Trotter JF, Herring R, Rojter S, Kayali Z, Wong VW, Greenbloom S, Jayakumar S, Shiffman ML. Cilofexor, a Nonsteroidal FXR Agonist, in Non-Cirrhotic Patients with Nonalcoholic Steatohepatitis: A Phase 2 Randomized Controlled Trial. *Hepatology*. 2020 Mar 1.
158. Sigler MA, Congdon L, Edwards KL. An Evidence-Based Review of Statin Use in Patients With Nonalcoholic Fatty Liver Disease. *Clin Med Insights Gastroenterol*. 2018;11:1179552218787502.
159. Choudhary NS, Kumar N, Duseja A. Peroxisome Proliferator-Activated Receptors and Their Agonists in Nonalcoholic Fatty Liver Disease. *J Clin Exp Hepatol*. 2019;9(6):731-9.
160. Sarin SK, Sharma M, Koradia P, Duseja A, Bhatia S, Dixit V, et al. A prospective, multi-center, double-blind, randomized trial of saroglitazar 4 mg compared to

- placebo in patients with non-alcoholic steatohepatitis. *Hepatol Int.* 2020;14:S1-470.
161. Feetham L, Gawrieh S. The Liver Meeting 2019: saroglitazar magnesium in NAFLD or NASH. *Lancet Gastroenterol.* 2020;5(2):P112.
 162. Loomba R, Sirlin CB, Ang B, Bettencourt R, Jain R, Salotti J, et al. Ezetimibe for the treatment of nonalcoholic steatohepatitis: assessment by novel magnetic resonance imaging and magnetic resonance elastography in a randomized trial (MOZART trial). *Hepatology.* 2015;61(4):1239-50.
 163. Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, et al. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. *Hepatology.* 2004;40(5):1222-5.
 164. Ateş F, Yalınz M, Alan S. Impact of liver steatosis on response to pegylated interferon therapy in patients with chronic hepatitis B. *World journal of gastroenterology: WJG.* 2011 Oct 28;17(40):4517.
 165. Ceylan B, Arslan F, Batirel A, Fincanci M, Yardimci C, Fersan E, Pasaoglu E, Yilmaz M, Mert A. Impact of fatty liver on hepatitis B virus replication and virologic response to tenofovir and entecavir. *Turk J Gastroenterol.* 2016 Jan 1;27(1):42-6.
 166. Kim DS, Jeon MY, Lee HW, Kim BK, Park JY, Kim DY, Ahn SH, Han KH, Kim SU. Influence of hepatic steatosis on the outcomes of patients with chronic hepatitis B treated with entecavir and tenofovir. *Clinical and molecular hepatology.* 2019 Sep;25(3):283