Gene Expression The Journal of Liver Research, Vol. 19, pp. 187–198
Printed in the USA.

1052-2166/19 $90.00 + .00
DOI: https://doi.org/10.3727/105221619X15553433838609
E-ISSN 1555-3884
www.cognizantcommunication.com

Review

Nonalcoholic Fatty Liver Disease: An Update on the Diagnosis

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Nonalcoholic fatty liver disease (NAFLD) is a common liver disease and a major cause of related complications such as cirrhosis and hepatocellular carcinoma (HCC). NAFLD progresses through the stages of simple steatosis, nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and HCC. However, NAFLD usually cannot be diagnosed in a timely manner, which is largely attributed to the asymptomatic features of NAFLD patients and the lack of an effective and accurate noninvasive screening approach. Although liver biopsy has been recognized as a gold standard for diagnosing NAFLD, this approach is not suitable for screening and monitoring NAFLD because of its high cost and invasiveness. Several noninvasive screening and diagnostic systemic assessments have been developed in recent years for NAFLD evaluation. Here we summarize the current status and methods for NAFLD diagnosis, including both noninvasive (imaging, biomarkers) and invasive (liver biopsy) assessments. We further discuss the advantages and disadvantages of these developed diagnostic approaches for NAFLD.

Key words: Nonalcoholic fatty liver disease (NAFLD); Diagnosis; Noninvasive tests; Biomarkers; Liver biopsy

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common chronic liver disease affecting 25% of the global adult population[1]. It is also worth noting that the incidence of NAFLD in adolescents has increased in recent years. The disease spectrum ranges from simple steatosis to nonalcoholic steatohepatitis (NASH), cirrhosis, and even hepatocellular carcinoma (HCC). Patients with steatosis have only a very low risk of liver-related and non-liver-related adverse outcomes, whereas the presence of NASH substantially increases the risk of advanced comorbidity, accounting for increasing liver-related mortality and liver transplantation[2]. Therefore, early intervention in NAFLD assures termination of progression and even reversal of the disease along with its advanced complications[3,4]. However, there is no accurate noninvasive approach for distinguishing NAFLD from NASH. Liver biopsy is still considered the gold standard for NAFLD diagnosis, yet it is invasive and expensive, with a risk of complications, hindering its application in the early diagnosis of NAFLD and for large-population screening. Therefore, the development of cost-effective and reproducible noninvasive methods for assessing NAFLD is essential for screening, monitoring, and treatment. This review summarizes current and emerging noninvasive and invasive approaches for the diagnosis of NAFLD. In addition, the applications and limitations of these methods are discussed.

CURRENT DIAGNOSTIC FLOW TO ASSESS NAFLD DISEASE SEVERITY

Due to the high prevalence and progressive development of NAFLD, it is crucial to screen potential patients in general populations. According to the current guidelines from the American Association for the Study of

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Liver Diseases (AASLD), National Institute for Health and Care Excellence (NICE) and European associations for the study of the liver, diabetes and obesity (EASL-EASD-EASO), systematic screening programs should be established, and patients may benefit from population screening for early assessment and lifestyle interventions[5]. For NAFLD screening, the diagnostic criteria initially require that (i) no excessive alcohol consumption and (ii) no secondary chronic liver disease be present. Nonetheless, current guidelines mainly focus on subjects with high-risk factors for NAFLD, including obesity, metabolic syndrome, type 2 diabetes, overnutrition, insulin resistance, dyslipidemia, age, sex, and ethnicity[6–9]. It should be noted that these abovementioned NAFLD risk factors may themselves represent a systemic disorder, and NAFLD may need to be separated from these pathological conditions.

When performing NAFLD screening, the natural history should be known, and the cost of sensitive tests should be appropriate. Ultrasound is preferred as the first-line examination to detect steatosis because of its low cost and availability[10]. However, ultrasonography is unable to distinguish steatosis from fibrosis and is not sensitive enough to detect steatosis with a fat content less than 20%. The controlled attenuation parameter (CAP) appears to be more sensitive than ultrasound for diagnosing steatosis, and proton magnetic resonance spectroscopy (^{1}H-MRS) is also a quantitative measure of steatosis. Laboratory biochemical testing is also an acceptable noninvasive method that can identify steatosis in a high-risk population[10]. According to the EASL and Asia-Pacific guidelines, the fatty liver index (FLI) and NAFLD liver fat score can identify liver steatosis[5,11]. When steatosis is absent but liver enzymes are elevated, noninvasive screening should be promptly performed.

AASLD guidelines recommend that verifying the presence of steatosis is less relevant because inflammation and fibrosis are the real pathologies representing NAFLD progression[12]. As current laboratory measures and imaging technologies cannot diagnose steatohepatitis, the diagnostic strategy for disease is always a combination of lab scoring systems and imaging (Fig. 1). The EASL and AASLD recommend that ultrasound examination and liver enzyme determination be conducted for all cases with metabolic risk factors. Patients in high-risk groups with approved steatosis and high liver enzyme levels should be further diagnosed by specialist referral. Scoring systems for fibrosis markers such as NFS, FIB-4, the enhanced liver fibrosis (ELF) score, or the FibroTest should be employed to evaluate the presence of advanced fibrosis. Liver biopsy is suggested to be performed on subjects with fibrosis identified by a scoring system. According to EASL guidelines, patients with NASH or fibrosis should be monitored with annual tests and advised to reduce weight to prevent fibrosis progression[13,14]. Individuals with cirrhosis should be followed up and examined every 6 months.

DIAGNOSIS OF STEATOSIS

NAFLD is defined as >5% hepatocyte steatosis in the liver (defined by histology or imaging techniques), and the disease has a wide spectrum[12]. Patients with simple steatosis always have a low risk of progression. Hence, early diagnosis of NAFLD is crucial for providing timely and effective strategies for preventing disease progression. However, the role of liver biopsy in early screening is limited because it is an invasive procedure. To address this, there has been a rapid development of noninvasive methods. The disadvantages and advantages of noninvasive methods for diagnosing simple steatosis are discussed below.

Serum Biomarker Panels

FLI. The FLI was proposed by Giorgio Bedogni and colleagues in 2006 based on 224 subjects with NAFLD and 287 individuals without suspected liver disease. Four predictors (waist circumference, triglycerides, BMI, and GGT) were found to be tightly correlated with fatty liver[15]. The formula and cutoff points are shown in Table 1. The sensitivity and specificity for fatty liver are 61% and 86%, respectively, when the cutoff is 60 or greater. This equation is popular in clinical practice because of its accuracy and simplicity. An external validation study found that FLI is also associated with cardiovascular diseases, cancer-related mortality, and metabolic syndrome such as type 2 diabetes, though its ability to assess steatosis remains unclear[16–19].

SteatoTest. The SteatoTest is a panel of biochemical markers for predicting the degree of steatosis. In 2005, Thierry Poynard and colleagues reported that 12 predictors were closely related to the severity of steatosis in 884 subjects[20]. A meta-analysis also found that the accuracy of the SteatoTest in evaluating moderate to severe steatosis is reasonable for clinical application[21]. Regardless, the formula has not been disclosed, and no classical prospective or large-population study has evaluated its accuracy and effectiveness.

NAFLD Liver Fat Score. In 2009, Kotronen and his group developed the NAFLD liver fat score, which can detect the presence of liver fat precisely and easily[22]. MRS was used to quantify steatosis. One study indicated that the score tends to identify type-2 diabetes mellitus (T2DM) patients who have NAFLD[23]. However, this score is not correlated with hepatic histology and lacks external validation. Consequently, this method is not frequently used in clinical practice.

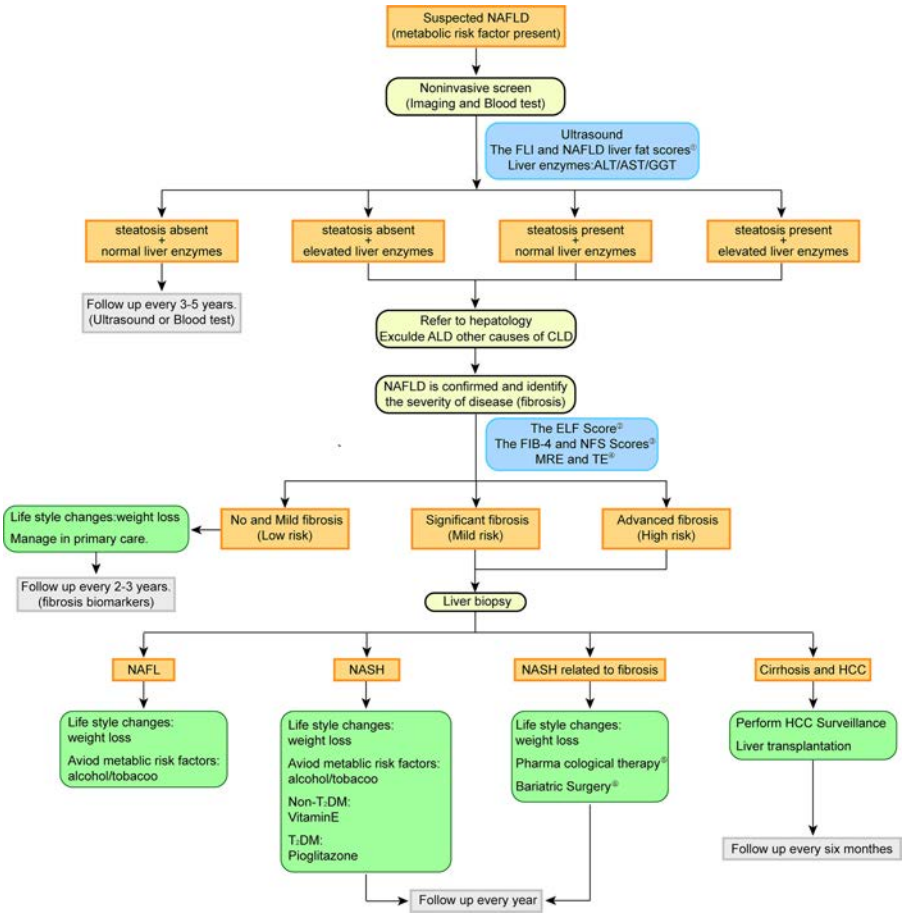

Figure 1. Flow chart for diagnosis and management of suspected NAFLD patients. ① The European associations for the study of the liver, diabetes and obesity (EASL-EASD-EASO) and Asia-Pacific guidelines. ② The National Institute for Health and Care Excellence (NICE) guidelines. ③ The American Association for the Study of Liver Diseases (AASLD) and EASL-EASD-EASO guidelines. ④ The AASLD guidelines. ⑤ The Asia-Pacific, AASLD, and Asia-Pacific guidelines. ⑥ The EASL-EASD-EASO, AASLD, and Asia-Pacific guidelines.

Lipid Accumulation Product (LAP). The LAP was established in 2009 by Giorgio Bedogni and colleagues, using a population from Northern Italy and individuals who also participated in the study for the development of the FLI[24]. The results indicated that the LAP is associated with the severity of steatosis, depending on sex. Nonetheless, additional external validation of LAP for NAFLD diagnosis is still needed for clinical practice.

Hepatic Steatosis Index (HSI). The HIS was developed from a cross-sectional study of 10,000 subjects participating in a health checkup in 2010[25]. The logistic regression model revealed that the ALT-to-AST ratio, BMI, and diabetes were strongly associated with NAFLD. NAFLD is excluded when the score is less than 30, and NAFLD is identified when the score is greater than 36. However, independent validation is limited to date, and a relationship with pathological features has not been confirmed[26]. The accuracy of diagnosis is also restricted by the operator's level of expertise.

Imaging Diagnostic Methods

Ultrasonography (US). US is widely used in clinical practice due to its convenience and cost effectiveness, but its diagnostic capacity is limited. Overall, the sensitivity of this method decreases when the fatty content is less than 30%, though the sensitivity is greater than 90% if the fat content is higher than 30%[27,28]. Regardless, the convenience of US makes it acceptable as the primary means of screening NAFLD patients in clinical practice.

Computerized Tomography (CT). Although the diagnostic accuracy of CT is much more precise than US for grading moderate to severe steatosis, its capability is also limited for mild steatosis. Moreover, the radiation exposure limits its use as a screening or early diagnostic tool[29].

Table 1. Nonalcoholic Fatty Liver Disease (NAFLD) Scoring Systems

Scoring System	Components/Formula	Critical Value
Steatosis		
SteatoTest		
	Age, gender, BMI, ALT, TBL, GGT, TG, FTG, cholesterol, 2-MG, apolipoproteinA1, haptoglobin	0.3001
FLI	TG, BMI, GGT, WC	30 & 60
NAFLD Liver Fat Score	Metabolic syndrome, type 2 diabetes, FS-insulin, AST, ALT	−0.640
LAP	Male, female, TC	4.28
HSI	ALT, AST, BMI, DM, male, female	30.0 & 36.0
NASH		
The NASH test	a-MG, haptoglobin, apolipoproteinA1, TBIL, GGT, ALT, AST, TC, cholesterol, age, gender, height, weight	Not applicable
NAFIC	Ferritin, fasting insulin, type IV collagen 7S	4
Fibrosis		
FibroTest	Age, a2-macroglobulin, TBIL, GGT, apolipoprotein A1	0.3 & 0.7
The NAFLD fibrosis score	Age, BMI, IFG, diabetes, AST, ALT, platelet, albumin	−1.455 & 0.676
ELF	PIIINP, HA, TIMP1	7.7
BARD Score	BMI, AST, ALT, Diabetes	2–4
AST:ALT ratio	AST, ALT	0.8 & 1
Fibro meter	Glucose, AST, ALT, Ferritin, Platelet, Body weight, Age	0.611 & 0.715
Hepascore	Age, Sex, 2-macroglobulin, HA, bilirubin, GGT	0.37, 0.44, & 0.7
APRI	ALT, platelet	0.98
FIB-4	Age, AST, ALT, platelet count	1.45 & 3.25

BMI, body mass index; T2DM, type 2 diabetes mellitus; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, -glutamyl transpeptidase; DM, diabetes mellitus; TIMP1, tissue inhibitor of metalloproteinase 1; PIIINP, procollagen III amino terminal peptide; TC, triglycerides; WC, waist circumference; HA, hyaluronic acid; TBIL, total bilirubin.

CAP. The CAP is an ultrasound-based technique for quantifying steatosis. The CAP results are presented as 100–400 db/m, and it can detect >10% steatosis with an AUROC of 0.91[30]. Although the results can be unreliable[31], Asia-Pacific guidelines still recommend the CAP as a useful tool for identifying steatosis and screening NAFLD patients due to its low cost and convenience. However, adequate training and appropriate cutoffs of grading are required.

Magnetic Resonance Imaging (MRI) and MRS. Through T1 and T2 measures, MRI has accuracy in detecting hepatic steatosis and fibrosis, but the high cost limits its availability as a screening tool[32]. MRS is an MR-based technique for assessing the fat content in the liver, with the capacity to detect and grade steatosis. The study by Szczepaniak et al.[33] revealed that MRS can detect 5.56% fat content in the liver, based on an analysis of 2,349 subjects. Thus, MRS may become the gold standard for steatosis. However, the complex algorithm and requirement for professional operators restricts MRS application in routine clinical practice[34].

MRI-Estimated Proton Density Fat Fraction (MRI-PDFF). The MRI-PDFF is an MRI method that differs from other imaging techniques. Traditional MRI is inaccurate for quantifying steatosis and is affected by T1 bias and T2 decay. In addition, MRS cannot detect all liver fat, and it is limited in clinical practice due to its operational complexity. Conversely, MRI-PDFF directly measures fat content in the liver using imaging-based biomarkers and can detect all liver fat. Furthermore, MRI-PDFF-based NAFLD diagnosis is not affected by age, sex, BMI, or the etiology of liver disease. However, the cost of MRI-PDFF is high, and the inspection time is long. The accuracy of diagnosis of MRI-PDFF is not precise in patients with implantable devices, acute inflammation, or iron overload, resulting in limited clinical use[35].

DIAGNOSIS OF NASH

NASH is a pattern of liver injury that is associated with increased rates of disease progression and liver transplantation compared with those of NAFL[36]. Thus, distinguishing simple steatosis from NASH is very important in decreasing disease progression and monitoring disease dynamics. NASH diagnosis requires a pattern of histopathological features, including steatosis, inflammation, and hepatocyte ballooning. Despite the rapid development of reliable noninvasive tests for diagnosing NASH, liver biopsy is still the gold standard.

Biomarkers

For noninvasive NASH diagnosis and evaluation, several serum biomarkers have been extensively examined

and discussed, but the consequence is unsatisfactory. The circulating levels of cytokeratin 18 (CK18) fragments, including M30 and M65[37], are associated with hepatocyte apoptosis and have modest accuracy for diagnosing NASH. The sensitivity and specificity of CK18-based NAFLD diagnosis is 66% and 82%, respectively[11]. Inflammatory markers such as ferritin, TNF- , and hs-CRP are reported to be associated with the presence and severity of NAFLD[38–40]. However, neither CK18 nor inflammatory markers are specific for NASH diagnosis[41–44]. Oxidative stress markers such as oxidized low-density lipoprotein, malondialdehyde, and thiobarbituric acid play an important role in assessing NASH pathogenesis and progression[45,46]. However, their accuracy for predicting NASH is insufficient, and the results are conflicting. Furthermore, plasma oxidative stress markers cannot reflect the level of hepatic oxidation. Insulin resistance (IR)-related elevation of glucose and insulin levels have been observed in NASH subjects[47,48], whereas IR is more strongly associated with metabolic syndrome and T2DM than NASH[49]. Thus, the ability of IR to contribute toward diagnosing the entire spectra of the disease is insufficient[50–53]. Cathepsin D (CatD), a lysosomal enzyme, participates in the development of hepatic inflammation and increases in the serum of NASH subjects[54]. A study including a total of 127 adults found that serum CatD was associated with NASH, especially after bariatric surgery[54]. Another study found that the diagnostic accuracy of CatD for NASH was 84%[55]. However, due to the small sample size, a multicenter and randomized controlled study is needed.

Recently, the development of genetic biomarkers has grown exponentially. Because such markers are present from birth, they are useful for screening programs, in identifying patients with genetic risk of NAFLD. Additionally, knowledge of genetic biomarkers may help in better understanding of the pathogenesis of NAFLD. In a large screening program, three genetic markers, rs738409, rs58542926, and rs780094, in the genes PNPLA3, TM6SF2, and GCKR, respectively, were proposed for predicting the presence and progression of NAFLD, although their ability to differentiate NASH from simple steatosis was not proven[56,57].

Other markers, such as microRNAs originating from the affected tissue, might be useful for monitoring the dynamic changes in liver injury[58]. Additionally, cell-free DNA, which has proven to be associated with liver fibrosis, might be a marker for predicting NASH, though further studies are needed before cell-free DNA can be used in routine clinical testing[59]. FGF21, which is expressed preferentially in the liver, is related to glucose and lipid metabolism[60]. Studies have indicated that plasma FGF21 is increased in patients with NAFLD and has modest accuracy in diagnosing NASH (sensitivity of 62% and specificity of 78%)[61]. -Ketoglutarate is an important metabolite produced by the tricarboxylic acid cycle that is related to metabolic disorders. Rodriguez-Gallego et al. conducted a case-control study and found that serum -ketoglutarate levels were higher in obese patients than in normal individuals. An -ketoglutarate level AUROC of 0.9 to 0.96 showed specificity and sensitivity of 0.93 and 0.8[62] for obesity, respectively. External validation studies suggest that -ketoglutarate may be a potential factor for evaluating NAFLD[63].

Combination of Biomarkers

Because the diagnostic efficiency of a single marker is low, some studies have combined two or more biomarkers to improve diagnostic accuracy. For example, the combination of CK18 and FAS or the combination of CK18[64] and FGF21[65] has been used for diagnosing NASH, with better predictability[66]. However, the sample size in these studies is small and requires additional validation.

Serum Biomarker Panels

The NASH test model is designed as a tool for differentiating NASH from simple steatosis. Despite a diagnostic accuracy of 0.9–1, a systematic review found that the sensitivity and specificity are 22.9% and 95.3%[67], respectively. In 2011, Yoshio Sumida and his group reported the NAFIC algorithm for predicting NASH in a study in Japan enrolling 177 patients diagnosed with NAFLD[68]. This scoring system remains to be confirmed in a larger population.

LIVER BIOPSY

Liver biopsy remains the gold standard for identifying the histological features and detecting the severity of NAFLD and for determining diagnostic strategies for treatment[69]. As other noninvasive measures are unable to distinguish simple steatosis from NASH, the role of liver biopsy in diagnosing and managing NAFLD patients is crucial. However, the limitations of liver biopsy should be noted. For instance, it can increase mortality from 0.009% to 0.14%[21], and pain is unavoidable. In addition, percutaneous puncture possibly can lead to intraperitoneal hemorrhage[70]. Furthermore, the size of the biopsy sample is only 1/50,000 of the whole liver tissue, and sampling error may occur, leading to an incorrect diagnosis. Histological scoring systems and indices have been developed to establish a definitive and effective diagnostic system based on histological features of NAFLD (i.e., steatosis, inflammation, liver cell injury (ballooning), and fibrosis). The criteria of each scoring system are shown in Table 2.

Brunt Score (1999) and Dixon Score (2000)

In 1999, Dr. Brunt and colleagues put forward a proposal for identifying the severity of histological lesions

Table 2. Brunt Grading System

Grade	Steatosis	Inflammation	Liver Cell Injury Ballooning	Fibrosis
1	<33%	Acinar: <2 foci Portal: No or mild	Minimal	Zone 3: perisinusoidal/pericellular fibrosis; focally or extensively present
2	33%–66%	Acinar: 2–4 foci Portal: moderate	Present	Zone 3: perisinusoidal/pericellular fibrosis with focal or extensive periportal fibrosis
3	>66%	Acinar: >4 foci Portal: severe	Marked	Zone 3: perisinusoidal/pericellular fibrosis and portal fibrosis with focal or extensive bridging fibrosis
4	–	–	–	Cirrhosis

Grade 1 (mild): Steatosis (predominantly macrovesicular) involving up to 66% of biopsy; may see occasional ballooned zone 3 hepatocytes; scattered rate intra-acinar pmn's six intra-acinar lymphocytes; no or mild portal chronic inflammation. Grade 2 (moderate): Steatosis of any degree; ballooning of hepatocytes (predominantly zone 3) obvious; intra-acinar pmn's noted, may be associated with zone 3 pericellular fibrosis; portal and intra-acinar chronic inflammation noted, mild to moderate. Grade 3 (severe): Panacinar steatosis; ballooning and disarray obvious, predominantly in zone 3; intra-acinar inflammation noted as scattered pmn's, pms's associated with ballooned hepatocytes six mild chronic inflammation; portal chronic inflammation mild or moderate, not marked.

(Table 2) by analyzing 10 histological variables[71]. Steatosis, ballooning, and acinar and portal inflammation are included to evaluate the stage and grade of severity of NASH (mild, moderate, and severe). As the fibrosis score includes five stages independently (0–4), the system is only able to determine the severity of NASH. Moreover, it cannot be used for detection in children or to assess the entire spectrum of disease. In 2000, Dixon and colleagues reported a system in which the criteria for diagnosing NASH were based on steatosis and two of three zone 3-centric factors, including necroinflammation, hepatocyte ballooning, and pericellular fibrosis with or without portal inflammation (Table 3)[72]. However, the patients in this study were severely obese, and the samples were thus not highly representative.

NAS Score (2005)

In 2005, the pathology committee of the NASH Clinical Research Network designed a system of histological features[73]. Among 14 histological features, four were selected for semiquantitative analysis: steatosis (0–3), lobular inflammation (0–2), hepatocellular ballooning (0–2), and fibrosis (0–4) (Table 4). NASH is diagnosed at a NAS score of 5, whereas a score of less than 4 excludes NASH. This approach can be used for diagnosis in both children and adults. However, the score cannot be used to assess the entire spectra of the disease, but is always used in clinical trials.

SAF Score (2012)

The semiquantitative SAF score evaluates steatosis (S, S0–S3), activity (A, A0–A4), and fibrosis (F, F0–F4) (Table 5). Grades 0–3 of steatosis represent 5% to 33%, 33% to 66%, and over 66% steatosis, respectively. The grade of activity is identified by lobular inflammation and ballooning scores. This score can be used by pathologists to diagnose NASH and identify the full spectra of the nonalcoholic fatty liver disease[74,75].

DIAGNOSIS OF FIBROSIS

The stage of fibrosis is the most important factor that correlates with liver-related mortality[76]. In a retrospective study that included 619 NAFLD patients with a median follow-up of 12.6 years, fibrosis stage was found to be the most important factor correlating with patient mortality. Advanced fibrosis and cirrhosis are often end points

Table 3. Dixon Grading System

Grade	Steatosis	Inflammation	Fibrosis
0	No steatosis	No hepatocyte injury or inflammation	Normal connective tissue
1	<5%	Sparse zone 3 inflammation	Focal pericellular fibrosis in zone 3
2	5%–25%	Mild focal zone3 hepatocyte injury/ inflammation	Perivenular and pericellular fibrosis confined to zone 2 and 3 with or without portal/periportal fibrosis
3	25%–75%	Noticeable zone3 hepatocyte injury/ inflammation	Bridging or extensive fibrosis with architectural distortion; no obvious cirrhosis
4	>75%	Severe zone 3 hepatocyte injury/ inflammation	Cirrhosis

Table 4. NAS Grading System

Grade	Steatosis	Inflammation	Ballooning	Fibrosis
0	<5%	No foci	None	None
1	5%–33%	<2 foci	Few	Perisinusoidal or periportal; 1A: Mild, zone 3, perisinusoidal; 1B: Moderate, zone 3, perisinusoidal; 1C: Portal/periportal
2	>33%–66%	2–4 foci	Many	Perisinusoidal and portal/periportal
3	>66%	>4 foci	–	Bridging fibrosis
4	–	–	–	Cirrhosis

Nonalcoholic fatty liver disease activity score (NAS): Steatosis + inflammation + ballooning (0–8) NAS < 4: non-NASH; NAS = 4: borderline; NAS > 4: NASH.

for clinical trials. Numerous studies have investigated methods for diagnosing fibrosis stages with imaging and noninvasive tests.

Serum Biomarker Panels

Aspartate Aminotransferase-to-Platelet Ratio Index (APRI). The APRI was first proposed as a diagnostic tool for HCV patients[77]. Later, the APRI was validated in a study with 111 NAFLD patients, in which the APRIs were found to be high in patients with advanced fibrosis[78]. However, APRI is more useful for predicting, but not identifying, advanced fibrosis and may constitute a simple tool for excluding advanced fibrosis, though it is less useful in clinical practice.

NAFLD Fibrosis Score. The NAFLD fibrosis score is regarded as the most accurate way to address severity of fibrosis. Several external validations have confirmed that NFS has good accuracy in ruling in or ruling out advanced fibrosis[79,80]. A cutoff value lower than −1.455 excludes advanced fibrosis (sensitivity 51% and specificity 98%)[81]. Nevertheless, the limitation of this score is its low sensitivity. The diagnostic accuracy of the FibroMeter is similar to that of the NAFLD fibrosis score. This method was initially reported in a study of 235 NAFLD patients and consists of seven factors[82]. A cutoff 0.611 and 0.715 had good accuracy for including and excluding advanced fibrosis. In addition, the NAFLD fibrosis score has better accuracy in diagnosing significant fibrosis than in diagnosing advanced fibrosis.

ELF Score. The ELF score was assessed by the European Liver Fibrosis group in 2008 for identifying the fibrosis stage, with AUCs of 0.9, 0.82, and 0.76 for advanced fibrosis, significant fibrosis, and no fibrosis, respectively. Other independent validations indicated that the ELF score has good accuracy for identifying advanced fibrosis but is less sensitive for early fibrosis stages[83,84]. The ELF score was also verified as being tightly associated with liver-related morbidity.

FIB-4 Algorithm. The FIB-4 algorithm consists of four variables: age, AST, ALT, and platelet count. The algorithm was first established in HCV and HIV patients[85] and was later confirmed in NAFLD patients. Based on the FIB-4 algorithm, scores less than 1.45 can rule out advanced fibrosis[86], while scores greater than 3.25 are likely to indicate advanced fibrosis. In a study of 634 patients, the FIB-4 algorithm exhibited low specificity in individuals who were older than 65 years, suggesting that age might affect the accuracy of this diagnosis[87].

BARD Score and Hepascore. The BARD score can be regarded as a tool for identifying fibrosis[88]. Studies in Japanese and Polish populations found its applicability and reliability in verifying advanced fibrosis satisfactory[89]. The FibroTest was proposed by Ratziu and colleagues, who tested blood samples from 267 patients

Table 5. SAF Scoring System

Grade	Steatosis	Inflammation	Ballooning	Fibrosis
0	<5%	No	Normal hepatocytes	None
1	5%–33%	2 foci	Presence of clusters of hepatocytes with a rounded shape and pale cytoplasm usually reticulated	Perisinusoidal or periportal; 1A: Mild, zone 3, perisinusoidal; 1B: Moderate, zone 3, perisinusoidal; 1C: portal/periportal
2	>33%–66%	>2 foci	Same as grade 1 with some enlarged hepatocytes, at least 2-fold that of normal cells	Perisinusoidal and portal/periportal
3	>66%	–	–	Bridging fibrosis
4	–	–	–	Cirrhosis

The SAF score: S0AanyFany: NO NAFLD; S 1A = 1Fany: NAFL; S 1A 2Fany: NASH.

with NAFLD and compared these results with liver biopsies, reporting that the FibroTest is likely to diagnose advanced fibrosis. A cutoff of 0.3 is likely to indicate advanced fibrosis with an under 90% NPV, while a cutoff of 0.7 only had a 73% PPV[90]. The Hepascore, derived from a study of 242 patients, is composed of six variables. The Hepascore shows greater accuracy in identifying and predicting fibrosis than BARD and APRI[91]. An AST/ALT ratio greater than 1 might predict bridging fibrosis/cirrhosis, and an AST/ALT ratio less than 1 can rule out advanced fibrosis[92]. Although the accuracy of these biomarkers and panels is not sufficiently precise, they are popular in clinical practice and have the ability to exclude advanced fibrosis. In general, these biomarkers and panels can be used as the first step to determine whether a patient should undergo a liver biopsy.

Imaging

Transient Elastography (TE). By detecting liver elasticity, TE can efficiently identify and grade the fibrosis stage and has good diagnostic accuracy for advanced fibrosis and cirrhosis in patients with hepatitis C virus[93], hepatitis B virus[94], alcoholic liver disease[95], and NAFLD[96]. The cutoff of 7.9 kPa indicates that patient requires liver biopsy. Although obesity has a marked impact on the test results, this disadvantage can be overcome with the use of the XL probe. Therefore, TE is regarded as a convenient and effective method for quantifying steatosis in the liver and ruling out advanced fibrosis. Unskilled operators and high waist circumference are important factors of failure for this approach[97].

Acoustic Radiation Force Pulse Imaging (ARFI). ARFI is also a type of US technology for detecting fibrosis that evaluates the elasticity of liver tissue. The accuracy of diagnosis in liver fibrosis is similar to that of TE, but ARFI is not limited by obesity[98]. ARFI is inexpensive and has good sensitivity for evaluating advanced fibrosis and cirrhosis. However, while a significant correlation was found between TE and histological liver fibrosis, the correlation between ARFI and histology for liver fibrosis was found to be statistically insignificant. ARFI is not broadly promoted in clinical practice and only a few related studies have been reported.

3D Shear Wave Elastography (3D-SWE) and Point Shear Wave Elastography (pSWE). The 3D SWE and pSWE are both used in US imaging to detect fibrosis in the liver. The 3D SWE has advantages in diagnosing F2 fibrosis[99,100], whereas pSWE is better at detecting advanced fibrosis and cirrhosis than 3D SWE. The results of pSWE are affected by iron overload or acute inflammation. Regardless, 3D-SWE and pSWE are not widely used in clinical practice, and further studies are needed to confirm their utility[101].

Magnetic Resonance Elastography (MRE). MRE identifies the fibrosis stage by imaging the propagation of acoustic shear waves in the liver. In a retrospective clinical study including 1,377 patients, MRE detected all stages of fibrosis, including mild to moderate fibrosis, and had a low technical failure rate of less than 5.6% (77 of 1,377 cases)[102]. Although the guidelines of AASLD recommend MRE as a useful tool for detecting advanced fibrosis, its high cost and overall scarce use limit its application[103,104].

Combinations of Imaging and Biomarker Panels. Strategies combining imaging techniques (TE) and serum biomarkers (NFS and FIB-4) have shown good diagnostic accuracy in an Italian study (321 patients), with combinations of NFS and TE having a marked ability to rule out advanced fibrosis[105]. EASL guidelines also recommend that diagnostic algorithms be used to predict patients at risk of advanced fibrosis and those needing a liver biopsy. However, further studies are still required for further validation.

CONCLUSIONS AND PROSPECTIVE

NAFLD shows a strong association with epidemic metabolic syndrome, and NASH promotes the progression to cirrhosis and even liver failure. The prevalence of NAFLD will remain a serious social health problem in decades to come. Therefore, raising awareness for early screening for NAFLD is important for detecting NAFLD and for effective interventions. A timely therapeutic intervention would effectively prevent the progression from NAFLD to liver failure and would attenuate the occurrence of metabolic disorders. However, the gold standard for NAFLD evaluation—liver biopsy—is largely limited in its use for large-scale screening and early diagnosis because it is an invasive procedure. Encouragingly, noninvasive approaches for early NAFLD diagnosis (e.g., serum biomarkers, scoring systems, and imaging) have emerged in recent years but, despite accuracy and sensitivity in grading mild to severe NAFLD, are still less than satisfactory. Developing new serum indicators and imaging techniques might improve the diagnostic accuracy, and big data analysis would contribute to the establishment of these promising approaches. Thus, developing cost-effective, convenient, and precise noninvasive strategies with high sensitivity and accuracy for NAFLD diagnosis would not only be beneficial in monitoring disease progression but also promote assessment of novel and innovative NAFLD interventions.

ACKNOWLEDGMENTS: This work was supported by grants from the National Science Fund for Distinguished Young Scholars (No. 81425005; H.L.), the Key Project of the National Natural Science Foundation (No. 81330005 and 81630011; H.L.), the Major Research Plan of the National Natural Science Foundation of China (No. 1729303 and No.91639304;

H.L.), the Creative Groups Project of Hubei Province (No. 2016CFA010; H.L.), the National Key R&D Program of China No.2016YFF0101504; Z.-G.S.), the National Natural Science Foundation of China (No. 81770053; Z.-G.S.), The National Science Foundation of China (81870171 and 81570271 to J.C.), and the Hunan Distinguished Young Scholars (2017RS3015 to J.C.).

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