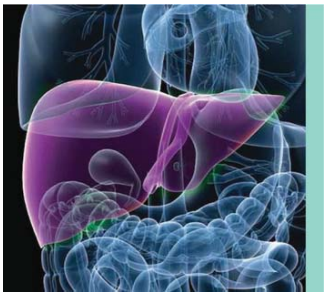

Diagnostic Challenges of Nonalcoholic Fatty Liver Disease/ Nonalcoholic Steatohepatitis

Erin Cleveland, M.D., Andrew Bandy, M.D.,† and Lisa B. VanWagner, M.D., M.Sc.**,‡

Nonalcoholic fatty liver disease (NAFLD) is a global epidemic that ranges from isolated hepatic steatosis (nonalcoholic fatty liver [NAFL]) to steatosis plus inflammation (nonalcoholic steatohepatitis [NASH]) with or without fibrosis (Fig. 1).[1] Whereas NAFL generally follows a benign course, NASH carries a significant risk for progression to fibrosis.[2] The key diagnostic challenges in NAFLD are to accurately detect NASH and to quantify the degree of fibrosis to identify those at highest risk for liver-related morbidity and mortality. Thus, when seeing a patient with possible NAFLD, the primary questions to answer are: (1) Does this patient have NAFLD? (2) Does this patient have underlying NASH? (3) Does this patient have any fibrosis? and (4) Does this patient have advanced fibrosis (stage 3 or 4)?

THE ROLE OF LIVER BIOPSY

Liver biopsy remains the gold standard for diagnosing NAFLD; however, its widespread use is limited by the risk

Abbreviations: AASLD, American Association for the Study of Liver Diseases; ALT, alanine aminotransferase; APRI, AST-to-platelet ratio index; ARFI, acoustic radiation force impulse; AST, aspartate aminotransferase; BARD, BMI, AST:ALT ratio, and diabetes status score; BMI, body mass index; CAP, controlled attenuation parameter; CK-18, cytokeratin-18; CT, computed tomography; EASL, European Association for the Study of the Liver; EASO, European Association for the Study of Obesity; ELF, enhanced liver fibrosis; FIB-4, Fibrosis-4; GGT, gamma-glutamyltransferase; HAIR, hypertension, age, insulin resistance; MR, magnetic resonance; MRE, magnetic resonance elastography; MRI-PDFF, magnetic resonance-proton density fat fraction; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value; TE, transient elastography; VCTE, vibration-controlled transient elastography.

From the *Department of Medicine, Division of Gastroenterology & Hepatology, Northwestern University, Feinberg School of Medicine, Chicago, IL, †Department of Pathology, Northwestern University, Feinberg School of Medicine, Chicago, IL, and ‡Department of Preventive Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL.

This study was supported by the National Institutes of Health, National Heart, Lung and Blood Institute (grant K23HL136891 to L.B.V.W.).

Potential conflict of interest: Nothing to report.

Received 18 January 2018; accepted 12 March 2018

View this article online at wileyonlinelibrary.com

© 2018 by the American Association for the Study of Liver Diseases

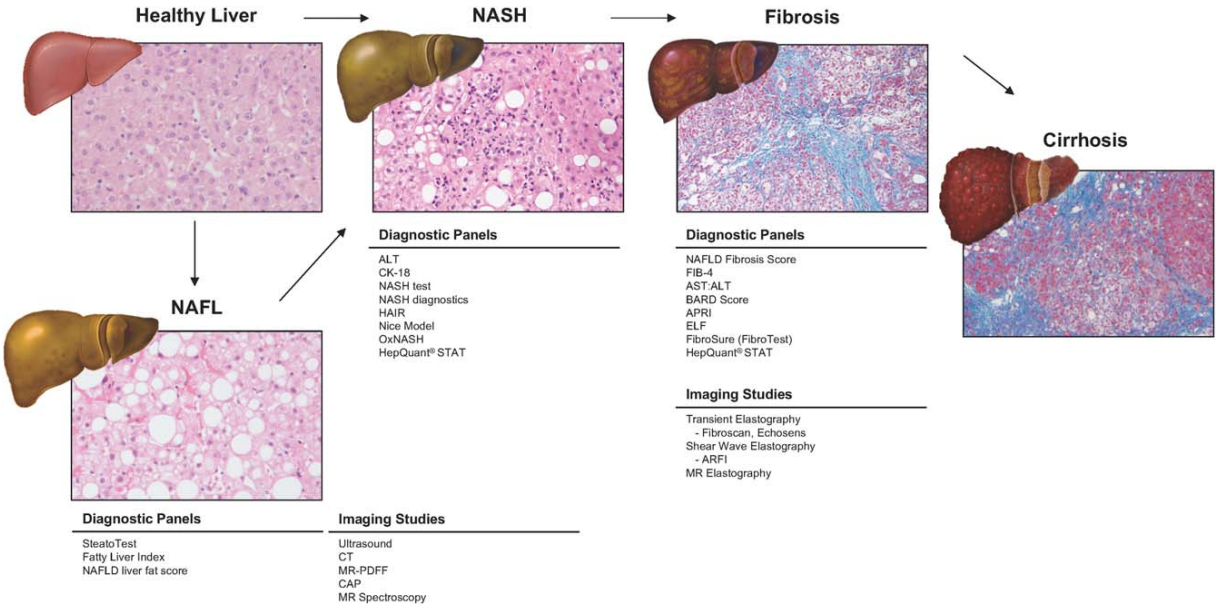

FIG 1 Serum biomarkers and imaging modalities across the NAFLD spectrum.
Abbreviations: APRI, AST-to-platelet ratio index; ARFI, acoustic radiation force impulse; BARD, BMI, AST:ALT ratio, and diabetes status score; CK-18, cytokeratin-18; HAIR, hypertension, age, insulin resistance.

associated with an invasive procedure, cost, and sampling error.[3] Thus, noninvasive diagnostic modalities allow for risk stratification of patients with NAFLD to select those who would benefit most from liver biopsy, while potentially avoiding this invasive procedure in others.

Does This Patient Have Nonalcoholic Fatty Liver Disease?

Not all hepatic steatosis, defined as fat >5% to 10% of the liver parenchyma, is NAFLD.[4] It is important to rule out other causes of hepatic steatosis, particularly alcohol (Table 1). NAFLD is typically associated with the features of the metabolic syndrome, which includes central adiposity, hypertension, dyslipidemia, and insulin resistance.[4,5] Thus, presence of one or more metabolic risk factors should raise clinical suspicion for NAFLD.

Hepatic steatosis is commonly detected incidentally on imaging such as ultrasound or computed tomography (CT). Notably, these modalities have poor sensitivity, detect fat only when 20% to 33% of the liver parenchyma is involved, and cannot accurately quantify the amount of hepatic fat present. Newer modalities such as magnetic resonance (MR) imaging–based spectroscopy, MR-proton density fat fraction (MR-PDFF), and transient elastography (TE)-based controlled attenuation parameter (CAP) are more sensitive and allow for relatively accurate quantification of hepatic steatosis (Table 2).[6] However, each of these imaging modalities has strengths and limitations that must be considered before implementation

TABLE 1. CAUSES OF HEPATIC STEATOSIS

Macrovesicular	Microvesicular
NAFLD	Reye's syndrome
Alcoholic liver disease	Acute fatty liver of pregnancy
Hepatitis C, genotype 3	HELLP syndrome
Medications	Medications
Amiodarone	Antiretroviral medications
Corticosteroids	Valproate
Methotrexate	Inherited metabolic disorders
Tamoxifen	Lysosomal acid lipase deficiency
Wilson's disease	Lecithin-cholesterol acyltransferase deficiency
Hemochromatosis	
Starvation	
Parenteral nutrition	
Lipodystrophy	
Abetalipoproteinemia	

Adapted with permission from *Hepatology*.[4] Copyright 2018, American Association for the Study of Liver Diseases.

TABLE 2. NONINVASIVE METHODS FOR DETECTING HEPATIC STEATOSIS AND ASSOCIATED TEST CHARACTERISTICS

Test	Components	Low Cutoff	High Cutoff	Sensitivity/ Specificity, %	PPV/NPV, %
SteatoTest	Age, sex, BMI, fasting glucose, cholesterol, triglycerides, ALT, bilirubin, GGT, haptoglobin, α_2-macroglobulin, apolipoprotein A1	<0.30	>0.72	90/90	63/93
NAFLD liver fat score	Metabolic syndrome, diabetes, fasting insulin, AST, AST/ALT	<−1.413	>1.257	95/95	
Fatty liver index	BMI, waist circumference, triglycerides, GGT	<30	>60	87/86*	
Ultrasonography				66-94/66-97	
Ultrasound Fatty Liver Indicator (US-FLI)	Liver brighter than kidney; liver brightness graded as mild/moderate (2 points) or severe (3 points). One extra point for each of the following: 1) Posterior attenuation of ultrasound beam, 2) Vessel blurring; 3) difficult visualization of gallbladder wall, 4) difficult visualization of diaphragm, 5) areas of focal sparing		Score ≥ 2	46/unknown	unknown/94
Unenhanced CT scan	Three available measures: liver parenchyma attenuation, liver to spleen attenuation difference, and liver to spleen attenuation ratio			85/100	
TE-CAP			>261	72/86	98/23
MRI-PDFF			>3.71	96/100	100/70

Abbreviations: GGT, gamma-glutamyltransferase; NPV, negative predictive value; PPV, positive predictive value.
^predictor of absence of significant hepatic steatosis.
$^{+}$predictor of presence of significant hepatic steatosis.
*Comparator group: liver ultrasound.
Data are from Machado and Cortez-Pinto,[17] Hernaez et al.,[18] and Chen et al.[12]

in the general population (Table 3).[4] There are also several panels that have been proposed to diagnose hepatic steatosis, many of which have been used in population-based studies aimed at estimating the epidemiology and natural history of NAFLD (Table 2).[6]

Does This Patient Have Underlying Nonalcoholic Steatohepatitis?

Reliable noninvasive methods to detect NASH remain limited. Commonly investigated methods can be grouped into two broad categories: serum biomarkers and predictive models. Serum aminotransferases, which are often used in clinical practice as a surrogate for inflammation, have poor predictive value for NASH.[4,5] Serum alanine aminotransferase (ALT) greater than two times the upper limit of normal (>70 U/L) has only 50% sensitivity and 61% specificity for NASH.[7] In addition, patients with NAFLD can have normal ALT levels, particularly as the disease progresses.[4] Therefore, although elevated aminotransferases should raise suspicion for NASH, normal levels should not be used to exclude NASH.[4,5]

Although serum biomarkers are not currently available for clinical use, many are under investigation (Table 4). These biomarkers broadly reflect the pathways involved in NASH development, including hepatocyte apoptosis, oxidative stress, and inflammation. Several diagnostic panels, such as the NashTest,[8] use a combination of biomarkers and clinical factors to predict NASH (Table 4). Given the lack of reliable noninvasive tests, physicians must use clinical factors to risk-stratify patients prior to liver biopsy. The presence of one or more features of the metabolic syndrome in a patient with NAFLD warrants referral to a specialist for consideration of liver biopsy.[4,5]

Does This Patient Have Any Fibrosis?

Hepatic fibrosis is the primary predictor of liver-related mortality in NAFLD. Furthermore, this relationship is

TABLE 3. STRENGTHS AND LIMITATIONS OF COMMONLY USED IMAGING MODALITIES IN NONALCOHOLIC FATTY LIVER DISEASE

Imaging Modality	Used to Assess	Strengths	Limitations
Ultrasound	Steatosis	Good for detection of moderate-to-severe steatosis	Poor sensitivity and negative predictive value
		Widely available	Unable to detect mild steatosis
		Low cost	Not quantitative
		Safe	Fibrosis and steatosis have similar appearance
			Operator dependent
			Accuracy influenced by BMI
CT	Steatosis	Good for detection of moderate-to-severe steatosis	Poor sensitivity
		Better specificity than ultrasound	Unable to detect mild steatosis
		Provides additional anatomic information	Ionizing radiation exposure
			Limited by variable amounts of iron
MR imaging	Steatosis	Better sensitivity and specificity than ultrasound	Limited by high iron burden
CAP	Steatosis	Quantitative	Limited clinical experience
		More sensitive than conventional ultrasound	
MR Spectroscopy	Steatosis	Quantitative	Limited availability
		Sensitive	High cost
			Less accurate with nonhomogeneous fat distribution
TE	Fibrosis	Correlates with stage of fibrosis	Accuracy reduced in obesity
		Point-of-care test	Severe steatosis may lead to false positives
			Operator dependent
			Accuracy influenced by BMI
Acoustic radiation force impulse	Fibrosis	Similar sensitivity/specificity as TE	Higher failure rates than TE
			Operator dependent
			Accuracy influenced by BMI
MR elastography	Fibrosis	Most accurate test for determining fibrosis stage	Limited availability
		Accuracy not affected by BMI, degree of steatosis	High cost

Adapted with permission from *Journal of Hepatology*.[5] Copyright 2016, European Association for the Study of the Liver.

stage dependent, and higher fibrosis stage is associated with higher liver-related mortality.[9] Identifying patients with early-stage fibrosis is key to implementing risk-reduction strategies to prevent disease progression. Unfortunately, most diagnostic tests currently in use are best suited to detect advanced fibrosis. Even the most accurate imaging studies available are relatively insensitive for stage 1 fibrosis (Table 5).

Does This Patient Have Advanced Fibrosis (Stage 3 or 4)?

Several clinical prediction rules, serum biomarkers, and imaging techniques are available to detect advanced hepatic fibrosis (Table 6). Clinical prediction rules, including the NAFLD Fibrosis Score, the Fibrosis-4 (FIB-4) index, and the aspartate aminotransferase (AST):ALT ratio, have the advantage of using readily available, cost-effective laboratory tests and have recently been shown to correlate with mortality in NAFLD.[10] These scoring systems are best suited to rule out the presence of advanced fibrosis with negative predictive values >90%.[6] Comparatively, the positive predictive value is modest, ranging from 55% to 79%.[6] Thus, values greater than the upper cutoff require liver biopsy for confirmation of fibrosis, whereas a score less than the cutoff is likely sufficient to rule out advanced fibrosis and may reduce the need for liver biopsy by ~75%.[6]

Several serum biomarkers and panels directly measure by-products of fibrosis formation as a surrogate for hepatic fibrosis. One clinically available complex predictive model is the enhanced liver fibrosis (ELF) panel, which is composed of several individual biomarkers and

TABLE 4. BIOMARKERS AND COMPLEX SCORES FOR DETECTING STEATOHEPATITIS

Test	Components	Cutoff	Sensitivity, %	Specificity, %	PPV, %	NPV, %
ALT[7]		>70	50	60.7		
		53-71	72.2-50	50.6-60.7		
CK-18[14]		>216	77	65		
		>287	65	92		
NashTest	Age, sex, height, weight, cholesterol, triglycerides, AST, ALT, bilirubin, haptoglobin α_2-macroglobulin, apolipoprotein A1	Undisclosed	33	94	66	81
NASH diagnostics	CK-18, adiponectin, resistin	>0.2772	95	70	60	97
		>0.3499	77	87	74	89
HAIR	Hypertension, insulin resistance, elevated ALT (>40)	≥2 parameters	80	89		
The Nice Model	Metabolic syndrome, ALT, CK-18	>0.14	84	86	44	98
		>0.83	16	99	90	91
oxNASH[15]	Age, BMI, AST, 13-hydroxyl-octadecadenoic acids,					
Ballooning	linoleic acid	>55.2	79	65	67	77
Inflammation		>54.6	78	67	72	74
HepQuant STAT[16]	Serum concentration of tetra-deuterated cholic acid 60 minutes after oral administration	>0.50 μMol	94	76	71	95

Abbreviations: HAIR, hypertension, increased ALT and insulin resistance; NPV, negative predictive value; PPV, positive predictive value.
*Multiple cutoffs have been studied for several of these tests. These are shown in separate rows with their corresponding test characteristics.
Data are from Machado and Cortez-Pinto.[17]

has been shown to predict mortality in chronic liver disease.[11] Similar to the clinical prediction rules, serum biomarkers/panels have good sensitivity to rule out advanced fibrosis, but they are less accurate at detecting early fibrosis with large ranges of indeterminate scores.

Several imaging modalities have been developed that allow for quantification of hepatic fibrosis with a higher degree of accuracy than serological tests. Both vibration-controlled TE (VCTE) and MR elastography (MRE) use liver stiffness as a surrogate marker for fibrosis. VCTE, known commercially as FibroScan, is a point-of-care test that can be used in a clinic setting to predict advanced fibrosis with fairly high accuracy.[6] VCTE is an appealing screening tool given its ease of use; however, its accuracy is significantly reduced in obese patients.[6] MRE, whose accuracy is not as dependent on body mass index (BMI), has been shown in some studies to have better accuracy than VCTE in both obese[12] and nonobese[13] patients with NAFLD; however, its use is limited by high cost and limited availability.

CLINICAL APPLICATION

Both the American Association for the Study of Liver Diseases (AASLD) and the European Association for

TABLE 5. TRANSIENT ELASTOGRAPHY VERSUS MAGNETIC RESONANCE ELASTOGRAPHY FOR THE DIAGNOSIS OF FIBROSIS IN NAFLD/NASH

	TE					MRE				
Fibrosis Stage	Cutoff (kPa)	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Cutoff (kPa)	Sensitivity, %	Specificity, %	PPV, %	NPV, %
≥1 versus 0	6.10	67	65	69	62	2.65	77	79	81	74
≥2 versus 0-1	6.90	79	85	70	90	2.86	79	82	66	90
≥3 versus 0-2	7.30	78	78	45	94	2.99	78	80	48	94
≥4 versus 0-3	6.90	63	66	15	95	3.35	75	81	27	97

Abbreviations: NPV, negative predictive value; PPV, positive predictive value.
Data are from Park et al.[13]

TABLE 6. COMPLEX SCORES FOR DETECTING ADVANCED FIBROSIS (STAGE ≥ 2) IN NONALCOHOLIC FATTY LIVER DISEASE/NONALCOHOLIC STEATOHEPATITIS

Test	Components	Low Cutoff	High Cutoff	Sensitivity, %	Specificity, %	PPV, %	NPV, %	AUROC (CI)
NAFLD fibrosis score	Age, diabetes, BMI, AST, ALT, platelets, albumin	<−1.455		78	58	30	92	0.81
			>0.676	33	98	79	86	(0.71-0.91)
FIB-4	Age, AST, platelets	<1.30		85	65	36	95	0.86
			>3.25	26	98	75	85	(0.78-0.94)
AST:ALT	AST:ALT	<0.8		74	78	44	93	0.83
		1.0	>1.0	52	90	55	89	(0.74-0.91)
BARD score	BMI, AST:ALT, diabetes	2	2	89	44	27	95	0.77
								(0.68-0.87)
APRI	AST:platelets	1	1	27	89	37	84	0.67
								(0.54-0.8)
ELF score	TIMP-1, PIIINP, HA	0.375		89	96	80	98	0.87
			>0.462	78	98	87	96	(0.67-1.0)
FibroSure* (FibroTest)	Age, sex, bilirubin, GGT, haptoglobin, α_2-macroglobulin, apolipoprotein A1	30		77	77	54	90	0.81
			70	15	98	73	76	(0.74-0.86)

Abbreviations: APRI, AST-to-platelet ratio index; AUROC, area under the receiver operating characteristic; BARD, BMI, AST:ALT ratio, and diabetes status score; CI, confidence interval; GGT, gamma-glutamyltransferase; HA, hyaluronic acid; NPV, negative predictive value; PIIINP, N-terminal propeptide of type III procollagen; PPV, positive predictive value; TIMP-1, tissue inhibitor of metalloproteinases 1.

*To provide a more comprehensive evaluation of liver injury in patients with NAFLD, NASH FibroSURE (LabCorp) combines FibroTest (for assessment of fibrosis), SteatoTest (BioPredictive; for assessment of steatosis), and NashTest (for assessment of NASH).

^predictor of absence of significant hepatic fibrosis.

$^{+}$predictor of presence of significant hepatic fibrosis.

Data are from McPherson et al.[19] and Machado and Cortez-Pinto.[17]

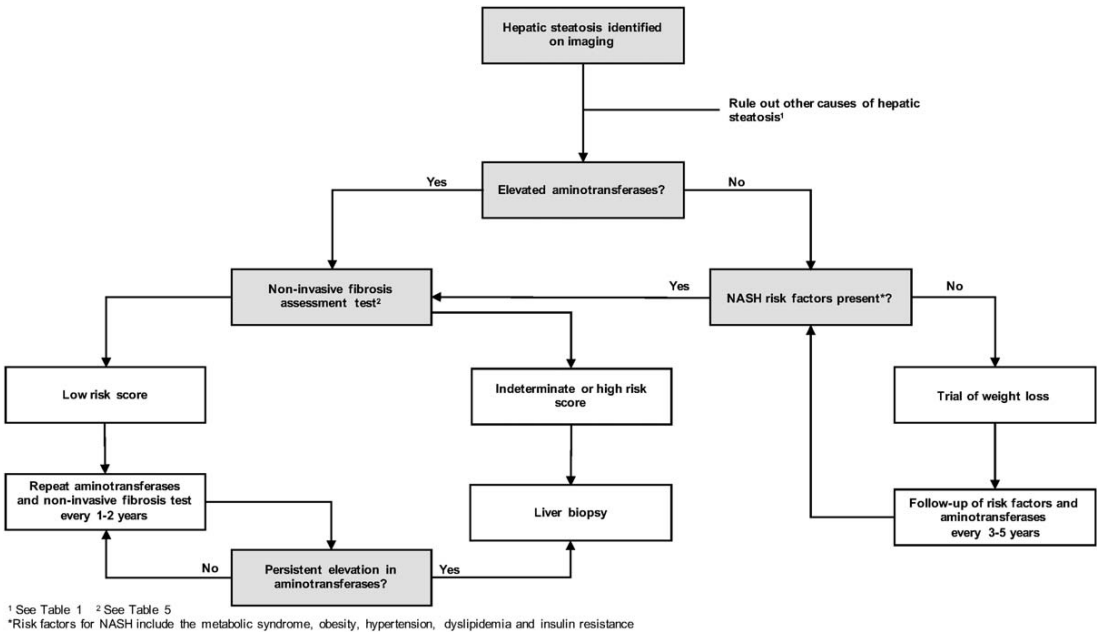

FIG 2 Diagnostic flow chart to assess and monitor disease severity in the presence of suspected NAFLD and metabolic risk factors based on the most recent AASLD and EASL-EASD-European Association for the Study of Obesity (EASO) Clinical Practice Guidelines for the diagnosis and management of NAFLD.
[1]See Table 1.[4] [2]See Table 6.[5] *Risk factors for NASH include the metabolic syndrome, obesity, hypertension, dyslipidemia, and insulin resistance.

the Study of the Liver (EASL) have published practice guidelines that can assist clinicians in integrating noninvasive methods with clinical factors to make decisions on the utility of liver biopsy (Fig. 2).[4,5] Regardless of the noninvasive method used for risk stratification, it is important to remember that NAFLD is a dynamic disease, and thus ongoing risk assessment for liver disease progression over time is of paramount importance.

In summary, the current imperfect gold standard for the diagnosis of NAFLD/NASH is liver biopsy. A number of serum markers, imaging modalities, and clinical prediction rules are available as noninvasive alternatives to liver biopsy, but most have substantial limitations in clinical practice. To date, MRI-PDFF and MRE seem to be the most accurate modalities for detecting hepatic steatosis and fibrosis, respectively.[6] However, widespread use of these modalities is limited by cost and availability in clinical practice. TE is a more widely available tool for fibrosis assessment and offers accuracy close to that of MRE. Noninvasive detection of NASH and accurate determination of fibrosis stage remain key diagnostic challenges in need of further investigation.

CORRESPONDENCE

Lisa B. VanWagner, M.D., M.Sc., Department of Medicine, Divisions of Gastroenterology & Hepatology and Preventive Medicine, Northwestern University, Feinberg School of Medicine, 676 N St Clair St, Suite 1400, Chicago, IL 60611. E-mail: lvw@northwestern.edu

REFERENCES

1) 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;64:73-84.

2) Argo CK, Northup PG, Al-Osaimi AM, Caldwell SH. Systematic review of risk factors for fibrosis progression in non-alcoholic steatohepatitis. J Hepatol 2009;51:371-379.

3) Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology 2005;128:1898-1906.

4) Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, Harrison SA, 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;67:328-357.

5) EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. J Hepatol 2016;64:1388-1402.

6) Younossi ZM, Loomba R, Anstee QM, Rinella ME, Bugianesi E, Marchesini G, et al. Diagnostic modalities for non-alcoholic fatty liver disease (NAFLD), non-alcoholic steatohepatitis (NASH) and associated fibrosis. Hepatology; doi: 10.1002/hep.29721.

7) Verma S, Jensen D, Hart J, Mohanty SR. Predictive value of ALT levels for non-alcoholic steatohepatitis (NASH) and advanced fibrosis in non-alcoholic fatty liver disease (NAFLD). Liver Int 2013;33:1398-1405.

8) Poynard T, Ratziu V, Charlotte F, Messous D, Munteanu M, Imbert-Bismut F, et al.; LIDO Study Group; CYTOL Study Group. Diagnostic value of biochemical markers (NashTest) for the prediction of non alcoholo steato hepatitis in patients with non-alcoholic fatty liver disease. BMC Gastroenterol 2006;6:34.

9) Ekstedt M, Hagstrom H, Nasr P, Fredrikson M, Stal P, Kechagias S, Hultcrantz R. Fibrosis stage is the strongest predictor for disease-specific mortality in NAFLD after up to 33 years of follow-up. Hepatology 2015;61:1547-1554.

10) Unalp-Arida A, Ruhl CE. Liver fibrosis scores predict liver disease mortality in the United States population. Hepatology 2017;66:84-95.

11) Parkes J, Roderick P, Harris S, Day C, Mutimer D, Collier J, et al. Enhanced liver fibrosis test can predict clinical outcomes in patients with chronic liver disease. Gut 2010;59:1245-1251.

12) Chen J, Yin M, Talwalkar JA, Oudry J, Glaser KJ, Smyrk TC, et al. Diagnostic performance of MR elastography and vibration-controlled transient elastography in the detection of hepatic fibrosis in patients with severe to morbid obesity. Radiology 2017;283:418-428.

13) Park CC, Nguyen P, Hernandez C, Bettencourt R, Ramirez K, Fortney L, et al. 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;152:598-607.e592.

14) Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 2009;50:1072-1078.

15) Feldstein AE, Lopez R, Tamimi TA, Yerian L, Chung YM, Berk M, et al. Mass spectrometric profiling of oxidized lipid products in human nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. J Lipid Res 2010;51:3046-3054.

16) Helmke S, Marr J, Lauriski S, Cookson M, Desanto J, Trotter J, Everson GT. Oral abstracts (abstracts 1–263): diagnosing NASH and assessing NASH disease severity with HepQuant-STAT, a simple quantitative liver function test. Hepatology 2017;66:1-148.

17) Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. J Hepatol 2013;58:1007-1019.

18) Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082-1090.

19) McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010;59:1265-1269.