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Theel, W.; Boxma-de Klerk, B.M.; Dirksmeier-Harinck, F.; Rossum, E.F.C. van; Kanhai, D.A.; Apers, J.; ... ; Cabezas, M.C.

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Evaluation of nonalcoholic fatty liver disease (NAFLD) in severe obesity using noninvasive tests and imaging techniques

Willy Theel[1,2] | Bianca M. Boxma-de Klerk[3] | Femme Dirksmeier-Harinck[4] | Elisabeth F. C. van Rossum[2,5] | Danny A. Kanhai[6] | Jan Apers[7] | Bas M. van Dalen[8] | Robert J. de Knegt[9] | Adriaan G. Holleboom[10] | Maarten E. Tushuizen[11] | Diederick E. Grobbee[12,13] | Janneke Wiebolt[1,2] | Manuel Castro Cabezas[1,5,13]

[1]Department of Internal Medicine, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[2]Obesity Center CGG, Rotterdam, The Netherlands

[3]Department of Statistics and Education, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[4]Department of Gastroenterology and Hepatology, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[5]Department of Internal Medicine, Division of Endocrinology, Erasmus University Medical Center, Rotterdam, The Netherlands

[6]Department of Pediatrics, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[7]Department of Bariatric Surgery, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[8]Department of Cardiology, Franciscus Gasthuis & Vlietland, Rotterdam, The Netherlands

[9]Department of Gastroenterology and Hepatology, Erasmus Medical Centre, Rotterdam, The Netherlands

[10]Department of Vascular Medicine, Amsterdam UMC, Amsterdam, The Netherlands

[11]Department of Gastroenterology and Hepatology, Leiden UMC, Leiden, The Netherlands

[12]Julius Centre for Health Science and Primary Care, University Medical Center Utrecht, Utrecht, The Netherlands

[13]Julius Clinical, Zeist, The Netherlands

Correspondence
Willy Theel, MSc., Franciscus Gasthuis & Vlietland, Dept. of Internal Medicine, Centre of Endocrinology, Diabetes and Vascular Medicine, Kleiweg 500, 3045 PM Rotterdam, The Netherlands.
Email: w.theel@franciscus.nl

Summary

The prevalence of nonalcoholic fatty liver disease (NAFLD) and the more severe and inflammatory type, nonalcoholic steatohepatitis (NASH), is increasing rapidly. Especially in high-risk patients, that is those with obesity, metabolic syndrome, and type 2 diabetes mellitus, the prevalence of NAFLD can be as high as 80% while NASH may be present in 20% of these subjects. With the worldwide increase of obesity, it is most likely that these numbers will rise. Since advanced stages of NAFLD and NASH are strongly associated with morbidity and mortality—in particular, cardiovascular disease, liver cirrhosis, and hepatocellular

Abbreviations: **ALT**, Alanine aminotransferase; **APRI**, AST to platelet ratio index; **ARFI**, Acoustic radiation force impulse imaging; **ASK1**, Apoptosis signal-regulating kinase 1; **AST**, Aspartate transaminase; **BMI**, Body mass index; **CAP**, Controlled attenuation parameter; **circRNAs**, circular RNAs; **CK-18**, Cytokeratin-18; **CRP**, C-reactive protein; **CVD**, Cardiovascular disease; **E**, Elasticity; **EASD**, European Association for the Study of Diabetes; **EASL**, European Association for the Study of the Liver; **EASO**, European Association for the Study of Obesity; **ECM**, Extracellular matrix; **ELF test**, Enhanced liver fibrosis test; **FAST score**, Fibroscan-AST score; **FFAs**, Free fatty acids; **FGF12**, Fibroblast growth factor 12; **FGFR-1**, Fibroblast growth factor receptor 1; **FIB4**, Fibrosis 4 algorithm; **FLI**, Fatty liver index; **GGT**, Gamma glutamyltransferase; **GMCS**, Granulocyte-macrophage colony-stimulating factor; **HA**, Hyaluronic acid; **HCC**, Hepatocellular carcinoma; **HSC**, Hepatic stellate cell; **IκKβ**, Inhibitor of nuclear factor kappa-B kinase subunit beta; **IL**, Interleukin; **IQR/M**, Interquartile range/median ratio; **IR**, Insulin resistance; **JNK**, c-Jun N-terminal kinase; **lncRNAs**, Long-noncoding RNAs; **LSEC**, Liver sinusoidal endothelial cell; **MAFLD**, Metabolic dysfunction-associated fatty liver disease; **MCP-1**, Monocyte chemoattractant protein 1; **MetS**, Metabolic syndrome; **miRNAs**, microRNAs; **MRE**, Magnetic resonance elastography; **MRI-PDDF**, Magnetic resonance imaging-proton density fat fraction; **mRNAs**, Messenger RNAs; **MRS**, Magnetic resonance spectrometry; **NAFLD**, Nonalcoholic fatty diver disease; **NAFLD-LFS**, NAFLD liver fat score; **NASH**, Nonalcoholic steatohepatitis; **NF-κB**, Nuclear factor kappa-light-chain-enhancer of activated B-cells; **NFS**, NAFLD fibrosis score; **NILTs**, Noninvasive liver tests; **P3NP**, Serum procollagen III amino-terminal peptide; **PAI-1**, Plasminogen activator inhibitor-1; **RBP4**, Retinol binding protein 4; **SWE**, Shear wave elastography; **T2DM**, Type 2 diabetes mellitus; **TE**, Transient elastography; **TIMP1**, Tissue inhibitor of metalloprotease-1; **TLR4**, Toll-like receptor 4; **TNFα**, Tumor necrosis factor alpha; **TPO**, Hormone thrombopoietin; **US**, Ultrasound imaging; **VCTE**, Vibration-controlled transient elastography.

carcinoma—it is of great importance to identify subjects at risk. A great variety of noninvasive tests has been published to diagnose NAFLD and NASH, especially using blood- and imaging-based tests. Liver biopsy remains the gold standard for NAFLD/NASH. This review aims to summarize the different mechanisms leading to NASH and liver fibrosis, the different noninvasive liver tests to diagnose and evaluate patients with severe obesity.

KEYWORDS
morbid obesity, NASH, noninvasive diagnosis

1 | INTRODUCTION: EPIDEMIOLOGY, RISKS, AND COMPLICATIONS IN NAFLD

In the last decades, the prevalence of obesity has risen rapidly in most parts of the world, closely associated with comorbidities such as type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, sleep apnea syndrome, nonalcoholic fatty liver disease (NAFLD) and its more severe form nonalcoholic steatohepatitis (NASH).[1] The silent obesity pandemic also affects 5% of children and young adults in the world,[2] and the increasing prevalence of subjects with obesity closely parallels the increasing NAFLD/NASH occurrence in these same groups.[3] The prevalence of overweight and obesity differs significantly between countries. While in Europe, more than half (52.7%) of the population is suffering from overweight (BMI 25–29.9 kg/m^2 and) and almost 17% from obesity (BMI ≥ 30 kg/m^2),[4] the situation in the United States is far more alarming with 73.6% of the population having overweight and 42.4% obesity.[5] A similar situation can be described for NAFLD/NASH. Multiple studies estimate that NAFLD is present in 17%–33% of the world population making this number one liver disease in many countries.[6–8] In patients with severe obesity (BMI ≥ 40 kg/m^2), NAFLD prevalence may be as high as 90%.[9–16] Most of the patients with NAFLD will maintain a mild form of this condition, but a significant percentage (∼20%) may progress to NASH.[17–20]

For the diagnosis of NAFLD, alcohol use cannot exceed a daily intake of 30 g for men and 20 g for women.[21] NAFLD is a risk factor for and a precursor of metabolic syndrome (MetS) with a bidirectional link with a number of metabolic abnormalities.[9–11,22–24] Hence, the term "*metabolic dysfunction-associated fatty liver disease*" (MAFLD) has been proposed.[25,26] Major liver-related complications of NAFLD include the development of NASH, liver cirrhosis, and hepatocellular carcinoma (HCC).[6,7,22,23] NASH has been defined as the inflammatory form of NAFLD with one of the two additional characteristic histological features: lobular inflammation (with the presence of neutrophil infiltration) and hepatocyte ballooning (reflecting injury of the hepatocytes).[27] The severity of NASH is determined by the presence and stage of fibrosis,[28] which is a key determinant of the liver-specific disease outcome and overall mortality.[29–32] Patients with NASH often have multiple cardiovascular risk factors which generally determine the main cause of death.[7,33] An association between NAFLD and increased mortality due to cardiovascular disease (CVD) has been shown.[22,31,34–37] However, a debate is still ongoing whether NAFLD can be considered as an independent risk factor for CVD or whether the increased risk can be explained by the sum of the risk factors associated to NAFLD like insulin resistance (IR), dyslipidemia, obesity, and hypertension.[38,39]

NASH-associated liver cirrhosis is responsible for an increase of the liver transplant waiting list registration in the United States of 114% in men and 80% in women.[40] The economic burden is considerable since it has been estimated that medical care costs related to NASH are in the billions.[20] While there is no registered pharmacological treatment for NASH yet, bariatric surgery in these subjects could be very cost effective.[41] Liver fibrosis decreases significantly in the majority of patients after bariatric surgery, but a recent systematic review and meta-analysis showed that 75% of patients with liver fibrosis before bariatric surgery show persistent fibrosis after the procedure.[42] Weight loss, which is considered as therapeutic goal for patients with NAFLD/NASH, is therefore no guarantee for complete remission of liver fibrosis. Weight loss after surgery or non-pharmacological (i.e., lifestyle) interventions is also often not sustainable.[43,44] Consequently, progression of liver fibrosis to cirrhosis may occur in these patients. This raises the issue of control and evaluation of these patients in the follow-up phase after bariatric surgery in order to detect complications at an early stage. We will review the mechanisms leading to NASH and liver fibrosis and the different noninvasive tests available to evaluate this condition in patients with severe obesity.

2 | CELLULAR MECHANISMS UNDERLYING NAFLD/NASH IN OBESITY

2.1 | Molecular basis of steatosis in NAFLD

An accumulation of fat in hepatocytes (i.e., >5%) defines the term steatosis.[45] Hepatic fat accumulation has different origins and can be triggered by factors such as peripheral IR, obesity, gut barrier dysfunction, and unhealthy dietary habits.[46] These factors also interact together since we know that adipose tissue is nowadays considered to be a full-fledged organ with different functions. Adipose tissue is considered to be an endocrine organ producing

pro-inflammatory hormones called adipokines (i.a. leptin, resistin, TNFα, and IL6) and also anti-inflammatory adipokines (i.e., adiponectin)[47] (Figure 1). The enlargement of adipose tissue leads to higher levels of leptin whereas adiponectin is decreased in people with obesity.[48–53] Leptin has an antisteatotic activity at the early stages of NAFLD, but it may have adverse effects in the long term by stimulating hepatic inflammation and fibrosis.[54,55]

Fat is usually stored in adipocytes; however, an excessive fat storage makes them dysfunctional and resistant for insulin.[56] Therefore, IR plays a central role in the development of NAFLD/NASH in obesity.[57–60] Furthermore, IR induces adipocyte lipolysis which leads to high serum concentrations of free fatty acids (FFAs) and enhances uptake by the liver, thereby converting hepatocytes into fat storing cells.[61] These fatty acids impair the lysosomal permeability of hepatocytes stimulating the expression and release of tumor necrosis factor α (TNFα), inhibiting insulin receptor activity and enhancing IR.[62] Therefore, control of IR is one of the major targets in clinical practice in order to prevent disease progression.

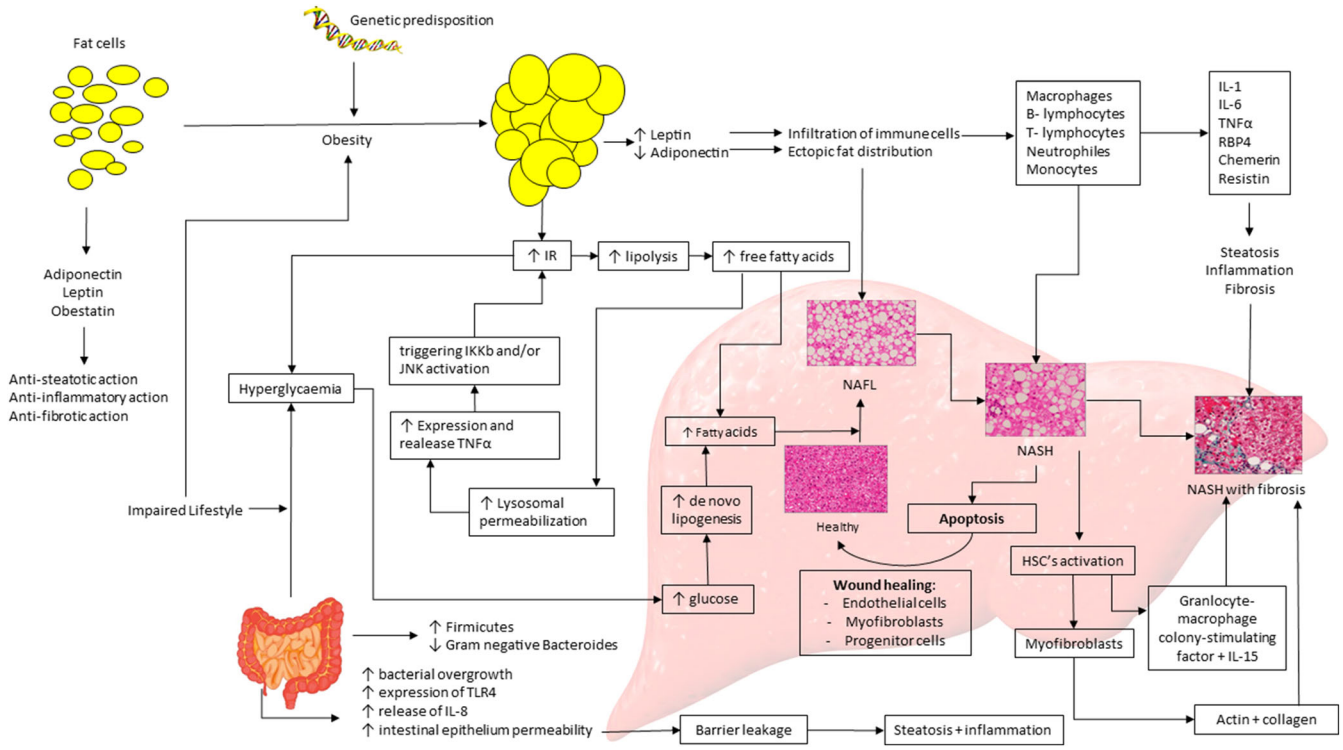

FIGURE 1 Physiopathology of NASH in obesity. NAFL: nonalcoholic fatty liver; NASH: nonalcoholic steatohepatitis; IL-1: Interleukin-1; IL-6: Interleukin-6; IL-15: Interleukin-15; TNF-α: Tumor necrosis factor α; RBP4: Retinol binding protein 4; ↑: Increased; ↓: Decreased; JNK; c-Jun N-terminal kinases; IKKb: IκB kinase β; TLR4: Toll-like receptor 4. The enlargement of adipose tissue leads to higher level of leptin and lower level of adiponectin in people with obesity. Fat is usually stored in adipocytes which become dysfunctional and resistant for insulin contributing to ectopic fat accumulation of TG-derived toxic metabolites. Hypoadiponectemia also induces ectopic fat distribution including the hepatic cells which acquire an adipocyte-like-function. The storage of fat in the form of triglycerides results first in simple steatosis. When hepatocytes cannot dispose the excess of free fatty acids anymore, inflammatory pathways are activated; cellular dysfunction occur causing lipo-apoptosis. The increase of adipose tissue also initiates the infiltration of immune cells. The most common cells are macrophages, B-lymphocytes, T-lymphocytes, and neutrophils which produce different cytokines. Adiponectin and obestatin have a favorable effect defending the liver integrity, whereas TNFα, IL-6, RBPP4, chemerin, or resistin promotes steatosis, inflammation and/or fibrosis. Leptin, which has an antisteatotic action at the early stage of NAFLD, becomes an unfavorable factor at a later stage stimulating hepatic inflammation and fibrosis. The storage of fat in the liver is also emphasized with the insulin resistance of the patient. This situation leads to hyperglycemia and lipolysis resulting in increased fatty acids in the liver. These fatty acids affect the lysosomal permeability which stimulates the expression and release of TNFα. Through the IKKβ and/or JNK pathways, the inhibition of insulin receptor signaling occurs and maintains the insulin resistance. The hepatocyte injury, also called ballooning, and inflammation, with or without fibrosis, then becomes visible: The initiation of NASH development. In order to restore the liver integrity, wound-healing cells are engaged. Endothelial cells, myofibroblasts, and progenitor cells are recruited after the death or apoptosis of the hepatocytes to recover. The myofibroblasts are hepatocytes stellate cells (HSCs) which have been activated. They contribute to the liver fibrogenesis producing actin and different types of collagens. They also induce the granulocyte-macrophage colony-stimulating factor and IL-15 which may prolong the neutrophils survival and enhance the liver damage and fibrosis. One of the causes of obesity is an impaired lifestyle. In the situation of a high fat diet, a change in the gut microbiota occurs. The ratio firmicutes/bacteroides increase. This type of diet induces a loosening of intestinal tights junctions, increases endotoxin levels, and activates of TLR pathways. Eventually, NASH patients show a higher TLR4 expression, an augmentation of IL-8 release and a greater intestinal epithelium permeability. The consequence is a barrier leakage which stimulate the steatosis and inflammation

2.2 | Molecular basis of inflammation and cell damage by hepatic steatosis

Intracellular fat accumulation also generates lipid peroxides and reactive oxygen species resulting in lipotoxicity and mitochondrial dysfunction, pro-inflammatory changes, cell damage, and finally apoptosis.[63–67] What initially appears to be simple steatosis, in fact, activates inflammatory pathways and causes cellular dysfunction until lipo-apoptosis occurs.[56] The accumulation of fatty acids and their metabolites activates the c-Jun N-terminal kinase (JNK) and the inhibitor of nuclear factor kappa-B kinase subunit beta (IκKβ),[68] promoting chronic inflammation and the development of hepatic IR.[69] The JNK pathway activates the inflammatory response and regulates hepatocyte apoptosis by the apoptosis signal-regulating kinase 1 (ASK1). IκKβ activates the nuclear factor kappa-light-chain-enhancer of activated B-cells (NF-κB) during inflammation[70,71] which stimulates the production of inflammatory cytokines like TNFα and IL-6 by these cells.[72,73]

Activation of these pathways leads to accumulation of immune cells (macrophages, B-lymphocytes, T-lymphocytes, and neutrophils) inducing a pro-inflammatory cascade of interleukins and cytokines (e.g., IL-1, IL-6, and TNF-α) in the liver.[74] Adiponectin[49] and obestatin[75] have a favorable effect protecting the liver integrity,[76] whereas TNFα, IL-6, RBP4, chemerin, and resistin promote steatosis, inflammation, and/or fibrosis.[74] The imbalance created between pro- and anti-inflammatory cytokines plays a crucial role in the generation of NASH and fibrosis.[77]

Gut microbiota can also induce inflammation. Diet plays a crucial role in the composition of the microbiome, and the Western diet offers a good breeding ground for micro-organisms like the Firmicutes species.[78] In the case of obesity, the gut microbiota changes and the ratio Firmicutes/Bacteroides are higher in comparison with normal-weight subjects.[79] This alteration may induce inflammation by increasing endotoxin levels, and it may cause disruption of intestinal tight junctions through the induction of toll-like receptor 4 (TLR4) pathways.[80] In NASH, a higher prevalence of bacterial overgrowth, a higher TLR4 expression, an augmentation of IL-8 release, and a better intestinal epithelium permeability have been reported.[81] Ultimately, barrier leakage may expose the liver to other bacteria and bacterial products that will induce pro-inflammatory signaling pathways in the liver eventually promoting the development of NASH.[82]

Altogether, these mechanisms lead to hepatocyte injury resulting in ballooning of these cells which together with inflammatory infiltration and steatosis results in the characteristic histology of NASH.[28]

2.3 | Molecular basis of fibrosis in NAFLD

In order to restore the liver integrity, wound-healing cells play an important role. Endothelial cells, myofibroblasts, and progenitor cells are recruited after apoptosis of the hepatocytes.[74] The myofibroblasts are in fact activated hepatic stellate cells (HSCs). Normally, fenestrated liver sinusoidal endothelial cells (LSECs) actively repress their activation but this is not the case after injury.[83,84] Moreover, LSECs may promote fibrosis via CXCR4 (an alpha-chemokine receptor) and the fibroblast growth factor receptor 1 (FGFR-1) on their surface.[85] This activation is enhanced when liver macrophages (i.e., Kupffer cells) release cytokines in the case of liver injury and/or inflammation.[86] Kupffer cells may also support HSCs migration by secreting CCL2, CCL3, CCL4, CCL5, CCL7 and CCL8.[87,88] Myofibroblasts remodel the liver extracellular matrix (ECM) by producing actin and replacing collagens IV and VI in the space of Disse with collagens I, III, and fibronectin[89–92] to provide mechanical stability in a damaged liver. They also induce the granulocyte-macrophage colony-stimulating factor (GMCS) and IL-15 which may prolong the neutrophil survival and enhance liver damage and fibrosis.[93] In the situation of a damaged liver, thrombocytopenia is a common hematological complication due to portal hypertension and also due to a reduced production of hormone thrombopoietin (TPO).[94–96] Interestingly, thrombocytopenia itself may also stimulate fibrosis. Indeed, platelets and platelet-derived extracts normally hinder the conversion of HSCs into myofibroblasts and consequently prevent fibrosis.[97]

2.4 | NAFLD in the pediatric population

NAFLD is not only a problem of adulthood but also even children show an increasing prevalence of fatty liver disease. In the United States, an increase of NAFLD in children between 12 and 19 years has been reported rising from 3.9% between 1988 and 1994 to 10.7% between 2007 and 2010.[98] Similarly to the situation in adults, this increase parallels closely the rise of obesity seen in children.[82] In children with obesity the prevalence of NAFLD may range from 22.5% to 44%.[99]

Different theories have been proposed to explain the development of NASH. The most popular theory is the "multiple-hits" model.[69,100,101] This model implies that different factors need to be operative leading to inflammation and fibrosis (Figure 1). An unhealthy maternal lifestyle including obesity and an early exposure to a hyperlipidemic environment during critical developmental periods may represent the "first hit" for the fetal liver and initiate liver histopathogenesis.[102,103] This perspective converges with the developmental origins of disease hypothesis which claims that early exposure to an adverse situation leads to risk of later life disease.[104,105]

The physiopathology of NAFLD in children is similar to adults. The same risk factors (i.e., those of the MetS) are related to the development of NAFLD in children.[106] When compared with nondiabetic subjects, type 2 diabetes raises the risk of NAFLD by twofold, while the presence of NAFLD increases the risk of type 2 diabetes by twofold to fivefold.[107] IR and type 2 diabetes in children have been linked to more advanced forms of NAFLD, such as NASH, or with fibrosis.[106,108] IR is partly responsible of the lipolysis in adipocytes leading to higher levels of circulating fatty acids. The disbalance between hepatic fatty acids acquisition and removal (through hepatic oxidation and VLDL secretion) is responsible for fat accumulation in

hepatocytes which causes defects in insulin signaling pathways as explained earlier.[109,110]

3 | NONINVASIVE LIVER TESTS (NILTS)

The progression of NAFLD is characterized by three overlapping processes: (a) steatosis, (b) inflammation, and (c) fibrosis. While all three are based on histological evaluation,[111] many noninvasive tests have been described reflecting several of these conditions. Both serum markers and imaging procedures have been validated for this purpose. Constantinescu et al. recently published an excellent overview of the ultrasound-based techniques to estimate the degree of liver steatosis and fibrosis.[112]

3.1 | Biomarkers for the diagnosis and evaluation of NAFLD/NASH

Serum alanine aminotransferase (ALT), aspartate transaminase (AST), and gamma glutamyltransferase (GGT) levels have been the most widely used markers to screen for liver pathology.[113] Unfortunately, they are not specific for the diagnosis of NASH and show no correlation with inflammation or fibrosis.[114,115] Serum transaminases are often elevated in different clinical settings, but they may also be within normal limits in severe stages of NAFLD.[116,117] Indeed, no relation has been demonstrated between the ALT levels and the possible histological liver changes in adults.[118,119] However, different algorithms have been described including ALT and AST which can be of clinical use to diagnose liver steatosis and fibrosis. Laboratory measurements of liver enzymes or inflammatory markers like C-reactive protein (CRP) are neither specific nor sensitive for NASH.[117,120–122] CRP has been associated with obesity but not with NAFLD/NASH.[122] Some authors have proposed that CRP may be helpful to distinguish severe NASH from NAFLD,[123–125] but this has been disputed by others.[126–128] Finally, a low platelet count may reflect hepatic fibrosis.[129]

For the initial workup and screening purposes, serum biomarkers have been recommended. The fatty liver index (FLI), the SteatoTest, and the NAFLD Liver Fat Score (NAFLD-LFS) have been proposed to identify patients with hepatic steatosis[111] (Table 1). The SteatoTest and NAFLD-LFS have been validated with histology. Validation in obesity was done in a limited number of subjects as shown in Table 1.[130–135] The best diagnostic accuracy in obesity was shown for the NAFLD-LFS. All three tests are of limited clinical value since a large proportion of subjects will fall in an intermediate range (FLI) or in a gradual score of steatosis (SteatoTest and NAFLD-LFS).

The histological score for fibrosis is based on the 5-point scale proposed by Brunt et al. and modified by Kleiner et al.[136] This classification uses stage 0 (F0) indicating the absence of fibrosis, stage 1 (F1) for perisinusoidal or portal fibrosis, stage 2 (F2) for perisinusoidal and portal/periportal fibrosis, stage 3 (F3) for septal or bridging fibrosis, and stage 4 (F4) for cirrhosis. Clinically significant fibrosis and advanced/severe fibrosis stand for F2 and F3, respectively.

Guidelines recommend the NAFLD fibrosis score (NFS) and the fibrosis 4 algorithm (FIB-4) for the identification of cases at high risk for advanced fibrosis/cirrhosis.[111] Recently, other algorithms have been proposed like the Fibrotest, the ADAPT score, the FIB-C3 score, the enhanced liver fibrosis (ELF) test, or the AST to platelet ratio index (APRI) for this purpose.

All these algorithms have been validated in subjects with obesity. The best performing was the ELF score followed by the FIB-4 score. However, the former was evaluated in a very limited number of subjects with obesity. All these tests have been validated with histology. Most of these tests use regular clinical parameters, except the ELF test.[137–148]

3.2 | Imaging modalities for the detection and evaluation of NAFLD/NASH

Conventional ultrasound imaging (USA) is frequently used to establish liver steatosis. This technique has been included in the guidelines[149] and is widely available at relatively low cost. One of the main disadvantages is the fact that the United States only provides qualitative information on liver steatosis, but it is not informative in relation to inflammation and fibrosis. This accounts for both adults and children.[150] Moreover, ultrasound is not able to detect mild hepatic steatosis (<30%)[151] and it has a lower sensitivity (39%) for patients with obesity compared with lean subjects (49.1%).[152–154] Furthermore, the United States has a significant interobserver variability.[155]

Magnetic resonance imaging-proton density fat fraction (MRI-PDDF) is at present considered the best imaging technique providing both qualitative and quantitative information without sampling error as may occur with liver biopsy and transient elastography (TE).[154] MRI-PDFF determines the concentration of triglycerides in organs such as the liver.[156] MRI-PDDF combines two methodologies: the complex-based fitting and the magnitude-based fitting techniques. The fat and water signal fractions are updated after a multistep nonlinear fitting procedure. The update is based on magnitude signal equations with a multipeak fat spectral model.[157] Earlier, a comparable method called proton **magnetic resonance spectrometry** (MRS) was developed to determine the lipid volume fractions which seems to be a reliable procedure consistent with computed tomography.[158] However, MRI-PDDF has the best accuracy (i.e., 99%) outperforming all other NILTs.[159] Major drawbacks are the significant costs and limited availability.

Magnetic resonance elastography (MRE) uses low-frequency mechanical waves in organs. A phase contrast MRI technique models the propagating wave and processes the information to create qualitative results defining the mechanical properties. In this way, the liver stiffness, and therefore elasticity, is evaluated.[160] In children, MRE showed a good correlation with histology for the evaluation of liver steatosis.[161] In subjects with obesity, MRE may be of added value to MRI-PDDF, for the detection of fibrosis with 93% accuracy.[162]

TABLE 1 Algorithms for the evaluation of liver steatosis and liver fibrosis

	Components	Formula (when available)	Diagnostic accuracy in the general population/obesity	References	Number of subjects evaluated for validation In the general population/obesity
Steatosis					
Fatty liver index (FLI)	Fasting TG GGT BMI Waist circumference	$= e^{y}/(1+e^{y}) \times 100$ Where $y = 0.953 \times \ln[TG\ (mg/dl)] + 0.139 \times BMI\ (kg/m^2) + 0.718 \times \ln[GGT\ (U/L)] + 0.053 \times$ waist circumference (cm) – 15.745	84% /79%	Bedogni et al.[130] Cuthbertson et al.[131]	228/168
SteatoTest	TG GGT BMI Serum cholesterol Total bilirubin Serum glucose α-2-macroglobulin apolipoprotein AI Haptoglobin ALT	NA	80% /81%	Poynard et al.[132] Lassailly et al.[133]	884/288
NAFLD liver fat score (NAFLD-LFS)	the presence or absence of: MetS T2DM ALT AST Fasting serum insulin	$= -2.89 + 1.18 \times$ MetS (yes:1, No:0) $+ 0.45 \times$ T2DM (Yes:2, No:0) $+ 0.15 \times$ insulin (mU/L) $+ 0.04 \times$ AST (U/L) – $0.94 \times$ AST/ALT	80% /87%	Fedchuk et al.[134] Kotronen et al.[135]	324/470

TABLE 1 (Continued)

	Components	Formula (when available)	Diagnostic accuracy in the general population/obesity	References	Number of subjects evaluated for validation In the general population/obesity
Fibrosis					
NAFLD fibrosis score (NFS)	Age IFG BMI Platelet count Albumin AST/ALT ratio	$= -1.675 + 0.037 \times$ Age (years) $+ 0.094 \times$ BMI (kg/m^2) $+ 1.13 \times$ IFG (yes = 1, no = 0) $+ 0.99 \times$ AST/ALT ratio – platelet ($\times 10^9$/L) – $0.66 \times$ albumin (g/dl)	82% /87%	Angulo et al.[137] Drolz et al.[138]	733/368
FIB-4	Age AST ALT Platelet count	= (Age × AST) / (Platelet count × √ALT)	NA/89%	Sterling et al.[139] Drolz et al.[138]	832/368
Fibrotest	Haptoglobin α2M Apolipoprotein AI Bilirubin GGT	$= 1/(1 + e^{-z})$ Where $z = 4.467 \times \log_{10}$ [α2M (g/L)] – $1.357 \times \log_{10}$ [haptoglobulin (g/L)] $+ 1.017 \times \log_{10}$ [GGT (IU/L)] $+ 0.0281 \times$ Age $+ 1.737 \times \log_{10}$ [bilirubin (μmol/L)] – $1.1184 \times$ apolipoprotein (g/L) $+$ $0.301 \times$ Sex (male = 1, female = 0) – 5.54	NA/80%	Rossi et al.[140] Vali et al.[141]	125/3385
ADAPT	Age Pro-C3 Platelets Diabetes	$= e^t +$ Diabetes (yes:1, No:0) Where $t = \log_{10}$[(age × Pro-C3)/ √Platelets]	81% /81%	Daniels et al.[142] Mak et al.[143]	431/1833
FIBC3	Age BMI T2DM Platelet count Pro-C3	$= -5.939 + (0.053 \times$ Age) $+ (0.076 \times$ BMI) $+$ $(1.614 \times$ T2DM) – $(0.009 \times$ Platelets) $+ (0.071 \times$ Pro-C3)	NA/83%	Boyle et al.[144]	NA/449
Enhanced liver fibrosis (ELF)	Hyaluronic acid Pro-C3 TIMP-1	$= -7.412 + 0.681$ ln[HA (μg/L)] $+ 0.775$ ln[Pro-C3 (μg/L)] $+ 0.494$ ln[TIMP-1 (μg/L)]	93% /95%	Guha et al.[145] Karlas et al.[146]	192/41
AST to platelet ratio (APRI)	AST AST upper limit of normal Platelet count	= AST (IU/L) /AST upper limit of normal (IU/L) /Platelets ($\times 10^9$/L)	83% /82%	Wai et al.[147] Siddiqui et al.[148]	270/1904
Fibroscan-AST (FAST) score	LSM CAP AST	$= e^w/(1 + e^w)$ Where $w = -1.65 + 1.07 \times$ ln[LSM (kPa)] $+ 2.66 \times$ $10^{-8} \times CAP^3 - 63.3 \times AST^{-1}$	NA/80%	Newsome et al.[170]	NA/350

Abbreviations: α2M, Alpha(2)-macroglobin; ALT, serum alanine aminotransferase; AST, serum aspartate transaminase; CAP, controlled attenuation parameter; GGT, gamma glutamyltransferase; IFG, impaired fasting glucose; LSM, liver stiffness measurement; MetS, metabolic syndrome; Pro-C3, procollagen III amino terminal peptide; T2DM, type 2 diabetes mellitus; TG, triglycerides; TIMP-1, tissue inhibitor of matrix metalloproteinase.

Transient elastography (TE) measures the elastic shear wave propagation through the liver parenchyma thereby quantifying the liver fibrosis. TE was developed in the 1990s from the field of seismology for the food industry, and it was adapted in the early 2000s for medical use.[163] There are two types of TE: shear wave elastography (SWE) and vibration-controlled transient elastography (VCTE). SWE analyzes the reflection of a produced shear wave, generated by a focused acoustic beam, to determine the degree of liver fibrosis. The VCTE probe administers shockwaves through a mechanical vibrating part to evaluate liver stiffness which is closely associated with liver fibrosis. VCTE also has an ultrasound transducer to measure the attenuation of the mechanical vibration which is associated with the degree of steatosis. In this way, two measurements are performed to obtain the following results: the degree of liver steatosis using controlled attenuation parameter (CAP expressed in dB/m) and the degree of liver fibrosis (also referred to as elasticity [E] expressed in kPa). According to the European Association for the Study of the Liver (EASL), the European Association for the Study of Diabetes (EASD), and the European Association for the Study of Obesity (EASO), TE is an acceptable noninvasive procedure for the identification of cases at low risk of advanced fibrosis/cirrhosis.[111,149] In addition, TE can exclude advanced fibrosis and it has been proposed for initial assessment.[164] VCTE correlates well with hepatic histology in both adults and children,[165,166] and it performs better than the United States for the evaluation of liver steatosis in children.[167] Cut-off values have been determined using matching liver biopsies in 246 patients to classify the degree of fibrosis: 7.2 kPa for significant fibrosis (F2), 8.7 kPa for advanced fibrosis (F3), and 10.3 kPa for cirrhosis (F4).[168] These values are specific for the M probe, but for patients with an increased skin-to-liver capsular distance, for example, patients with obesity, the XL probe has been developed with its own cut-off values: 7.2 kPa for F3 and 7.9 kPa for F4.[169] Recently, a combination of TE and AST measurement has been proposed, the fibroscan-AST (FAST) score,[170] to capture better active fibrotic NASH instead of simple steatosis. TE is a noninvasive technique becoming increasingly available in clinical practice.[168,171,172]

3.3 | NILTs in children

The diagnosis and evaluation of NAFLD in children is based on histological findings and can be supported by laboratory measurements, scores, and imaging techniques.

Biopsy remains the most accurate method to evaluate NAFLD and to grade its inflammatory and fibrosis component.[173] The main pitfall of liver biopsy is that a small sample (1/50.000 of the liver) is analyzed which can result in misclassification due to the inconsistent character of NAFLD. Pediatric biopsies, especially those from prepubertal boys, may differ from those from adults. The latter are characterized by macrovascular steatosis, hepatocyte ballooning, lobular inflammation, and perisinusoidal fibrosis.[174] Histological findings in young patients reveal less ballooning and more portal-based inflammation and fibrosis.[175–178]

As started earlier, laboratory findings based on ALT levels do not permit to make any conclusion on the presence and degree of NASH or NAFLD with fibrosis.[175,179–181] Liver inflammation and NASH may be identified with markers of hepatocyte injury (i.e., cytokeratin 18, leptin, and TNFα), but their accuracy is variable and they are not always available in clinical situations.[182–185] Concerning fibrosis, the number of studies in the young population is very limited. Hyaluronic acid has a fair to good accuracy in detecting minimal-mild and significant fibrosis in children, but more validation studies are needed.[183,186] Plasminogen activator inhibitor-1 (PAI-1) and monocyte chemoattractant protein 1 (MCP-1) may predict advanced fibrosis but this findings have not been validated sufficiently.[187]

Scores have also been developed for the pediatric population in order to evaluate NAFLD. Only the Pediatric NAFLD score has been developed to evaluate steatosis,[188] but it may be of limited value in clinical practice.[189] The pediatric predictive NASH model has an accuracy of 74% to evaluate NASH in children with NAFLD, but it has not been externally validated.[190] Significant liver fibrosis in children cannot be detected accurately using the APRI, NFS, or FIB4 score.[176,191] Only the ELF test may be clinically useful as shown in two pediatric studies evaluating fibrosis compared with liver biopsy.[192,193]

Imaging modalities have been developed and tested to evaluate NAFLD in pediatric patients. Ultrasound is inaccurate to detect fibrosis and unable to detect inflammation in children with NAFLD,[150] but it evaluates steatosis with a sensitivity and a specificity from 84% to 94% and from 32% to 70%, respectively, in 4 pediatric studies.[154,194–196] The CAP also showed good performance compared with liver biopsy with an accuracy of 93% in a pediatric study.[197] Magnetic resonance techniques (i.e., MRI, MRS, 1HMRS, and MRI-PDFF) are the most accurate imaging methods in adults to detect steatosis and comparable results have been found in pediatric studies.[161,198–201] A major drawback is the cost of these techniques which makes them less suitable for daily clinical practice.

Finally, two pediatric studies showed excellent accuracy to evaluate the presence of any, significant and advanced fibrosis using TE in children with histologically proven NAFLD.[166,202] The XL probe has not been validated in a pediatric population. MRE outperforms TE in adults, but a pediatric two-center study showed a fair and good accuracy for detecting any and significant fibrosis, respectively, in children with biopsy-proven NAFLD.[203] Finally, acoustic radiation force impulse imaging (ARFI) showed a sensitivity and specificity of 79%–82% and of 45%–77%, respectively, to establish fibrosis in two pediatric studies with children with liver disease other than NAFLD.[204,205]

3.4 | Future developments for serum-based markers of NAFDL-NASH

Omics-based biomarkers including proteomics, metabolomics, and lipidomics are emerging for patients with NAFLD. Different proteins have been identified but none is specific for NASH.[206,207] Altered levels of bile acids and glutathione have been reported during NAFLD onset.[208] Lipidomic-based markers have also been published.[209–211]

TABLE 2 Cut-off values for steatosis

Study	Cutoff (dB/m)	Sensitivity	Specificity	AUROC
Steatosis stage ≥ S1				
Wan et al. (2021)[234]	326	nc	nc	nc
Agarwal et al. (2020)[a][235]	330.5	0.672	0.667	0.73
Agarwal et al. (2020)[b][235]	276	0.909	0.717	0.87
Barsamian et al. (2020)[237]	294	0.77	0.68	0.77
Shalimar et al. (2020)[236]	285	0.928	0.875	0.96
Somda et al. (2019)[239]	255	0.88	0.76	0.83
Eddowes et al. (2019)[241]	302	0.80	0.83	0.87
Naveau et al. (2017)[238]	298	0.78	0.83	0.81
de Barros et al. (2016)[233]	214	nc	nc	nc
Steatosis stage ≥ S2				
Eilenberg et al. (2021)[242]	350	nc	nc	0.703
Wan et al. (2021)[234]	393	1	0.43	nc
Agarwal et al. (2020)[a][235]	350	0.647	0.638	0.67
Agarwal et al. (2020)[b][235]	321	0.667	0.815	0.71
Barsamian et al. (2020)[237]	326	0.74	0.75	0.78
Shalimar et al. (2020)[236]	340	0.652	0.644	0.667
Somda et al. (2019)[239]	288	0.81	0.73	0.86
Eddowes et al. (2019)[241]	331	0.70	0.76	0.77
Garg et al. (2018)[240]	336	0.739	0.755	0.74
Naveau et al. (2017)[238]	303	0.90	0.69	0.83
de Barros et al. (2016)[233]	251	nc	nc	nc
Steatosis stage ≥ S3				
Eilenberg et al. (2021)[242]	353.5	nc	nc	0.738
Wan et al. (2021)[234]	360	nc	nc	nc
Agarwal et al. (2020)[a][235]	358.5	0.889	0.709	0.734
Naveau et al. (2017)[238]	326	0.83	0.71	0.84
Shalimar et al. (2020)[236]	355	0.778	0.673	0.724
Somda et al. (2019)[239]	297	0.66	0.59	0.79
Eddowes et al. (2019)[241]	337	0.72	0.63	0.70
Garg et al. (2018)[240]	357	1	0.778	0.82
de Barros et al. (2016)[233]	296	nc	nc	nc

Abbreviations: AUROC, area under the receiver operating characteristic; nc, not communicated.
[a]Pre-operative measurement.
[b]Postoperative measurement.

These studies are often limited by their size and their ability to distinguish different grades of steatosis. One example is the oxNASH panel which includes different parameters like linoleic acid and 13-HODE (hydroxyoctadecadienoic acid) and has a sensitivity and specificity of 81% and 97%, respectively, to detect NASH.[212]

Cytokeratin-18 (CK-18) is a promising biomarkers for hepatic apoptosis and NASH. It has a sensitivity and specificity of 66% and 82%, respectively.[164] The combination with fibroblast growth factor 12 (FGF12) improves both sensitivity and specificity above 90%.[213] In subjects with obesity, CK-18 combined with adiponectin and resistin showed an accuracy between 73% and 91% to detect NASH.[214] The derived form of CK-18, M30, combined with the serum apoptosis-mediating surface antigen sFas, had an accuracy of 79%–93%.[215]

Several other markers for hepatic fibrosis have also emerged: hyaluronic acid (HA), serum procollagen III amino-terminal peptide (P3NP) and its neo-epitope Pro-C3, and the tissue inhibitor of metalloprotease-1 (TIMP1). HA, represented in the accumulation of extra cellular matrix, has an accuracy of 87% for F2 and of 92% for F4.[216] P3NP is a reliable biomarker for fibrosis,[217] and elevated Pro-C levels correlate with NASH and fibrosis.[218] TIMP1 may be the best biomarker for patients with obesity with an accuracy of 97% to detect NASH-related fibrosis.[219]

TABLE 3 Cut-off values for elasticity

Study	Cutoff (kPa)	Sensitivity	Specificity	AUROC
Fibrosis stage ≥ F2				
Eilenberg et al. (2021)[242]	6.3	0.788	0.537	0.687
Agarwal et al. (2020)[a][235]	7.95	0.738	0.689	0.747
Agarwal et al. (2020)[b][235]	7.5	0.667	0.75	0.834
Eddowes et al. (2019)[241]	8.2	0.71	0.70	nc
Garg et al. (2018)[240]	7.25	0.7	0.587	0.65
Naveau et al. (2014)[243]	7.6	0.73	0.78	0.81
Wan et al. (2021)[234]	9.0	nc	nc	nc
Barsamian et al. (2020)[237]	8.1	nc	nc	0.83
Weiss et al. (2016)[244]	7.1	nc	nc	nc
de Barros et al. (2016)[233]	7.9	nc	nc	nc
Fibrosis stage ≥ F3				
Eilenberg et al. (2021)[242]	12.6	0.538	0.915	0.786
Agarwal et al. (2020)[1][235]	8.95	0.895	0.759	0.854
Agarwal et al. (2020)[2][235]	12	0.6	0.616	0.835
Eddowes et al. (2019)[241]	9.7	0.71	0.75	0.80
Garg et al. (2018)[240]	12.5	0.636	0.877	0.83
Naveau et al. (2014)[243]	7.6	1	0.74	0.85
Barsamian et al. (2020)[237]	8.7	nc	nc	0.90
Weiss et al. (2016)[244]	9.5	nc	nc	nc
de Barros et al. (2016)[233]	9.6	nc	nc	nc
Fibrosis stage ≥ F4				
Agarwal et al. (2020)[1][235]	14.25	0.889	0.873	0.916
Eddowes et al. (2019)[241]	13.6	0.85	0.79	0.89
Weiss et al. (2016)[244]	12.5	nc	nc	nc

Abbreviation: AUROC: area under the receiver operating characteristic.
[a]Pre-operative measurement.
[b]Postoperative measurement; nc: not communicated.

Novel developments using noncoding messenger RNAs (mRNAs), long-noncoding RNAs (lncRNAs), circular RnAs (circRNAs), and microRNAs (miRNAs) in patients with NAFLD- NASH are promising.[220] Subjects with severe obesity and NASH show upregulated miR-122 and miR-192 compared with those with simple steatosis.[221]

Altogether, so far the best performing marker for the diagnosis of liver fibrosis at an early stage in subjects with obesity seems to be FIB-4. While the search for useful serum markers is still ongoing, promising imaging techniques are being developed like TE which is the most accurate tool for the lowest costs.

4 | TE IN THE BARIATRIC WORKUP

The most common problem reported for the measurements with TE is the occurrence of false positive results. This can be partly caused by liver steatosis[222] or, in 20% of the measurements, by generalized obesity with high subcutaneous fat accumulation.[223] A measurement with VCTE can be considered as correctly done, and thus reliable for interpretation, if the interquartile range/median ratio (IQR/M) ≤ 0.30 or IQR/M > 0.30 with a liver stiffness evaluation <7.1 kPa.[224] Different factors also play a role in the determination of the correct value. For example, extrahepatic cholestasis, a limited operator experience, and the presence of ascites have been cited as confounding parameters for the measurement.[168,223] Also, in the presence of congestive heart failure, right-sided heart failure, and acute inflammation or edema, the results may be less accurate.[225–228] In any event, TE measurement has a high intra-observer and interobserver level of agreement,[229] making it very useful in clinical practice.

An XL probe for the Fibroscan™ has been developed to address the misclassification problem caused by obesity. This new probe aims to lower the failure rate and to improve the accuracy of the measurement. This development brought new problems about the possible cut-off values that physicians need to use. Several studies have shown that the XL probe can be used for a correct evaluation of the liver fibrosis of patients with obesity, with cut-off values being generally

1.5 to 2.0 kPa lower than with the M probe.[223,230,231] There is some literature aiming to define the position of TE for the diagnosis of fibrosis or steatosis stage, but, to the best of our knowledge, little is known about the XL probe for patients enrolled for bariatric surgery.[232]

European guidelines do not provide any cut-off values for different stages of steatosis using TE. For fibrosis, the following cut-off values have been recommended: 7.2 kPa for F3 and 7.9 kPa for F4 when using a XL probe. However, these recommendations are based on the study of individuals without obesity.[111,149]

We identified 12 recent studies which evaluated NASH with an XL probe in patients referred for bariatric surgery[233–244] (Tables 2 and 3). Ten provided cut-off values for steatosis stages and nine for fibrosis stages. Based on these studies, the best defined stages for steatosis were S1, S2, and S3 with cut-off values from 214 to 330.5, 251 to 393, and 296 to 360 dB/m, respectively. The fibrosis stages identified as F2, F3, and F4 were best defined with cut-off values from 6.3 to 9.0, 7.6 to 12.6, and 12.5 to 14.25 kPa, respectively. Ten out of the 12 studies confirmed their findings with liver biopsies.

Several reports used references from previous studies;[168,233,244–246] others included a relatively low number of histological validations[234] or they defined different cutoff values for the same stage of liver steatosis or fibrosis.[235] Different factors may also affect the TE measurement and its accuracy: The presence of diabetes mellitus, high triglyceride levels, a great probe-to-capsule distance, and a higher BMI have been mentioned.

The cut-off values proposed in these studies differ from those mentioned by the European guidelines. While the cut-off values identified are supported by several studies, the numbers of subjects included are still relatively low, especially when considering the fact that validation with liver biopsies is necessary. Therefore, larger studies including liver biopsy are still necessary.

5 | CONCLUSION

Obesity as a chronic disease is a worldwide growing problem which is associated with comorbidities. The occurrence of NAFLD/NASH is clearly related to obesity and almost ubiquitous in severe obesity. NAFLD/NASH can lead to severe complications such as cirrhosis or HCC. An early diagnosis of NAFLD/NASH is warranted to prevent progression and severe complications. Several diagnostic tools are available to detect and quantify fibrosis. Biomarkers are the first choice to distinguish the major part of patients with a mild form of NAFLD from those at risk for liver fibrosis. For this purpose, algorithms are simple, applicable, and also inexpensive tests that can be used to recognize serious cases of fibrosis. In addition, technological development currently allows to help establish the diagnosis, especially for patients with an indeterminate score. Different techniques are available to monitor steatosis and fibrosis in patients with severe obesity before and after bariatric surgery. Nevertheless, some questions remain regarding the availability, the ease of use, the cost, and the correct use of cut-off values for each method.

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CONFLICT OF INTEREST

The authors declared no conflicts of interest.

ORCID

Willy Theel https://orcid.org/0000-0003-3639-0963
Elisabeth F. C. van Rossum https://orcid.org/0000-0003-0120-4913

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