



Review Article

# Liver Fat Scores for Noninvasive Diagnosis and Monitoring of Nonalcoholic Fatty Liver Disease in Epidemiological and Clinical Studies



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## Abstract

Nonalcoholic fatty liver disease (NAFLD) is strongly associated with the metabolic syndrome and type 2 diabetes and independently contributes to long-term complications. Being often asymptomatic but reversible, it would require population-wide screening, but direct diagnostics are either too invasive (liver biopsy), costly (MRI) or depending on the examiner's expertise (ultrasonography). Hepatosteatosis is usually accommodated by features of the metabolic syndrome (e.g. obesity, disturbances in triglyceride and glucose metabolism), and signs of hepatocellular damage, all of which are reflected by biomarkers, which poorly predict NAFLD as single item, but provide a cheap diagnostic alternative when integrated into composite liver fat indices. Fatty liver index, NAFLD LFS, and hepatic steatosis index are common and accurate indices for NAFLD prediction, but show limited accuracy for liver fat quantification. Other indices are rarely used. Hepatic *fibrosis* scores are commonly used in clinical practice, but their mandatory reflection of fibrotic reorganization, hepatic injury or systemic sequelae reduces sensitivity for the diagnosis of simple steatosis. Diet-induced liver fat changes are poorly reflected by liver fat indices, depending on the intervention and its specific impact of weight loss on NAFLD. This limited validity in longitudinal settings stimu-

lates research for new equations. Adipokines, hepatokines, markers of cellular integrity, genetic variants but also simple and inexpensive routine parameters might be potential components. Currently, liver fat indices lack precision for NAFLD prediction or monitoring in individual patients, but in large cohorts they may substitute nonexistent imaging data and serve as a compound biomarker of metabolic syndrome and its cardiometabolic sequelae.

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## Nonalcoholic fatty liver disease (NAFLD)

NAFLD is defined when hepatic steatosis is present in at least 5% of hepatocytes in liver histology, provided that there are no secondary causes of fatty liver (FL), such as excessive alcohol abuse, steatogenic medications, or specific chronic liver disease (e.g., chronic viral hepatitis C). NAFLD can be histologically classified into nonalcoholic fatty liver (NAFL) without evidence of hepatocellular injury and nonalcoholic steatohepatitis (NASH) with inflammation and ballooning of hepatocytes, which can finally lead to liver fibrosis, cirrhosis, or hepatocellular carcinoma. Environmental factors, such as unhealthy diet or lack of exercise, contribute to the progression of NAFLD, providing linkage to obesity, type 2 diabetes mellitus (DM), dyslipidemia, hypertension, or the entire metabolic syndrome (MetS).<sup>1,2</sup> In general, lipids that cannot be stored in adipocytes are deposited in ectopic tissue, such as liver, muscle, or pancreas.<sup>2</sup> Fatty liver itself results in hepatic insulin resistance (HIR), disinhibition of hepatic glucose production and dysglycemia. Prevalence in the general population has increased to about 25%<sup>3</sup> and there is also a high prevalence in obese children (about 7–8% population-wide and above 30% in obesity-enriched studies).<sup>4</sup> The global presence of NAFLD in patients with DM is 55.5% and attains 68% in Europe.<sup>5</sup> The presence of NAFLD and increasing liver enzyme levels are predictors for DM development<sup>6,7</sup> while the reduction of liver fat reduces the DM risk irrespective of weight loss.<sup>7</sup> Although obesity is the most commonly reported cause of NAFLD, there is a high number of nonobese

**Keywords:** NAFLD; Liver fat indices; Fatty liver index; Prediction; Dietary intervention.

**Abbreviations:** <sup>1</sup>H-MRS, proton magnetic resonance spectroscopy; AI, adaptation index; ALP, alkaline phosphatase; BMI, body mass index; CAP, controlled attenuation parameter; DI, disposition index; DM, type 2 diabetes mellitus; FI, fasting insulin; FL, fatty liver; FLI, fatty liver index, fatty liver index; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; GWAS, genome-wide association studies; HBV, hepatitis B virus; HCV, hepatitis C virus; HIR, hepatic insulin resistance; HSI, hepatic steatosis index; IGI, insulinogenic index; IHL, intrahepatic lipids; IL6, interleukin 6; IL8, interleukin 8; MetS, metabolic syndrome; MRE, magnetic resonance elastography; MRI, magnetic resonance imaging; MRI-PDFF, MRI-estimated proton density fat fraction; MS, metabolic syndrome; NAFL, nonalcoholic fatty liver; NAFLD, nonalcoholic fatty liver disease; NAFLD-LFS, NAFLD liver fat score; NASH, nonalcoholic steatohepatitis; OGIS, oral glucose insulin sensitivity index; PRS, polygenic risk scores; QUICKI, quantitative insulin sensitivity check index; ROC, receiver operating characteristic; SBP, systolic blood pressure; SLD, suspected liver disease; TG, triglycerides; TNF $\alpha$ , tumor necrosis factor alpha; WC, waist circumference; WHR, waist to hip ratio.

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or lean persons with NAFLD<sup>8,9</sup> with comparable long-term risks as obese NAFLD patients.<sup>10</sup> The prevalence of NAFLD in lean persons is estimated by around 10%, the global prevalence of lean NAFLD reaches 4% and within all NAFLD cases, around 20% are neither overweight nor obese.<sup>8,9</sup>

The pathogenesis of NAFLD is influenced by several factors. Genome-wide association studies (GWAS) have found several single nucleotide polymorphisms (SNPs) associated with an increased risk of liver diseases. Loss of function mutations in the genes for the patatin-like phospholipase domain-containing protein 3 (PNPLA3),<sup>11-14</sup> transmembrane 6 superfamily member 2 (TM6SF2),<sup>11-15</sup> and glucokinase regulator (GCKR) increase liver fat.<sup>11</sup> Other SNPs in the membrane bound O-acyltransferase domain-containing 7 (MBOAT7) gene<sup>11,16,17,18</sup> and the 17 $\beta$ -hydroxysteroid dehydrogenase type 13 gene (HSD17B13) have an impact on the risk of NAFLD onset and severity.<sup>19-22</sup>

In the general setting of the metabolic syndrome (MetS), overconsumption or unbalanced distribution of carbohydrates and fats leads to accumulation of lipids in the liver. In interaction with nutrient-specific transcription factors (such as ChREBP and SREBP1), lipogenesis is promoted over lipolysis and  $\beta$ -oxidation.<sup>23,24</sup> Biochemical alteration of those lipids (e.g. peroxidation) augments the burden to liver function beyond simple storage, inducing oxidative and metabolic stress which progresses from simple NAFLD to NASH, fibrosis, cirrhosis and hepatic cancer. The progression from low-grade steatosis to severe hepatic damage is promoted by several independent factors including diet, gut microbiome, genetic background, metabolic comorbidities, and their endocrine sequelae.<sup>25</sup>

The strong correlation between NAFLD and the development of DM underlines the importance of early diagnosis of NAFLD regarding the prevention and dietary intervention in patients with prediabetes or DM. Improvement in hepatic (e.g., HCC) and extrahepatic outcomes can also be achieved by early detection of mild steatosis. In recent studies, the incidence of HCC in patients with NASH cirrhosis was 0.5–2.6% and 0.1–1.3/1,000 patient-years in NAFLD patients without cirrhosis. Early diagnosis of the highly common NAFLD is crucial,<sup>26-28</sup> especially as it is fully treatable at that point. Genetic variations, alterations of lipid and glucose metabolism, clinical features of MetS and the biochemical fingerprint of hepatic damage can be detected in the blood, allowing prediction and monitoring of NAFLD with minimal invasiveness.

## Diagnosis of NAFLD

Liver biopsy is the gold standard for the detection of intrahepatic lipids (IHL) and liver fibrosis. It is also the only direct measure of tissue inflammation. The NAFLD activity score evaluates steatosis, inflammation, and hepatocyte ballooning, while the fibrosis activity score (FAS) provides a semi-quantitative estimation of the grade of fibrosis.<sup>29</sup> Nevertheless, biopsies have limited application in clinical routine due to high costs, sampling errors, and risk to patients. NAFLD can occur as nonhomogeneous patchy steatosis, therefore biopsies can miss highly affected areas. Biopsies have risks of internal bleeding or biliary leakage, especially in patients with liver fibrosis. These patients are at higher risk for progressing liver disease and require a bioptic diagnosis in order to quantify liver damage and to rule out specific (e.g. monogenetic) causes of liver disease. Biopsies should therefore preferably be used on patients with assumed liver fibrosis or steatohepatitis, as the highly prevalent, simple, often asymptomatic NAFLD should not be routinely diagnosed by invasive liver biopsies.<sup>30</sup>

Another option for diagnosing NAFLD that can be used quickly and cost-effectively in the clinical setting is the use of ultrasound-based techniques. This method allows a noninvasive examination of the liver. By observing parameters, such as liver size and shape, parenchyma echogenicity, and imaging of hepatic vessels, steatosis can be staged using various scores and indices. There are also quantitative methods including the controlled attenuation parameter (CAP) based on the Fibro Scan system and the speed of sound estimation that can be used for the evaluation of hepatic fat content. The disadvantages of the conventional ultrasound technique are the low sensitivity of detecting mild steatosis, the dependence of the examination on the observer, and the difficulty using in obese patients, which is often associated with NAFLD.<sup>31</sup> CAP has 87% sensitivity and 91% specificity for mild steatosis, 85% sensitivity and 74% specificity for moderate steatosis, and 76% sensitivity and 58% specificity for severe steatosis.<sup>32</sup> In order to use the Fibro Scan system in obese patients, an XL probe has been developed, detecting the degree of steatosis and fibrosis with higher accuracy than the initial M-probe.<sup>33</sup> As in 20–50% of all HCC cases, not pre-existing cirrhosis is found, monitoring of NAFLD before severe fibrosis is necessary. Also, a rather common scenario of NAFLD monitoring affects patients with pronounced obesity, in which ultrasound examinations are quite complicated. For the sake of HCC prevention, steatosis measurements independent of high-grade fibrosis and obesity are warranted.

Magnetic resonance imaging (MRI) techniques, such as proton magnetic resonance spectroscopy (<sup>1</sup>H-MRS) or MRI-estimated proton density fat fraction (MRI-PDFF) are other noninvasive methods to determine liver fat content.<sup>34</sup> MRI-PDFF has also been shown to provide information about the histological progress accompanying steatosis, which ultrasound-based techniques and computed tomography cannot provide.<sup>35,36</sup> Although MRI-based techniques are highly sensitive and involve no radiation exposure, they have some limitations in clinical practice such as high costs or clinical contraindications (severe obesity, claustrophobia, or metal implants). For those reasons, methods are needed to detect NAFLD rapidly, cost-effectively, noninvasively, and without clinical contraindications. Some biomarkers have already been developed for that purpose, even for the estimation of the amount of hepatic steatosis.

## Liver fat scores

Owing to the increasing prevalence of NAFLD,<sup>3</sup> NASH-related liver transplantation,<sup>37</sup> and the risk of liver, metabolic, and cardiovascular malignancies,<sup>1,2</sup> early diagnosis of NAFLD is important. Reduction of liver fat through lifestyle interventions (e.g. exercise or dietary intervention)<sup>38</sup> can lead to improvement of cardiovascular risk and metabolic status<sup>7</sup> and might also prevent progression to NASH and fibrosis. Liver fibrosis scores including FIB-4, the NAFLD fibrosis score (NFS), BMI-ALT/AST-Ratio-DM (BARD) score, and the enhanced liver fibrosis score (ELF) are capable of differentiating liver fibrosis from nonfibrotic NAFLD. However, they are limited in their prediction of simple steatosis and early stage NAFLD,<sup>39-41</sup> as they require markers reflecting fibrotic reorganization (Hyaluronic acid, tissue inhibitor of matrix metalloproteinase (TIMP)-1, procollagen III amino-terminal propeptide (PIIINP); ELF score), hepatic injury (AST/ALT ratio; FIB-4 and APRI) or extrahepatic sequelae (thrombocyte count; FIB-4, APRI), which are not elevated in nonfibrotic NAFLD. Scores specifically designed to detect simple steatosis are reviewed below. The most relevant simple tools using parameters of routine blood samples to

detect hepatic steatosis are the fatty liver index (FLI),<sup>42</sup> the NAFLD liver fat score (NAFLD-LFS)<sup>43</sup> and the hepatic steatosis index (HSI).<sup>44</sup> The parameters relevant for NAFLD are summarized in Table 1.<sup>42-44</sup>

### FLI

Bedogni *et al.*<sup>42</sup> developed the FLI, which is based on data from the Dionysos Nutrition and Liver Study. The study population included 497 (males, *n*=305) Italian subjects, 18 to 75 years of age, with suspected liver disease (SLD, *n*=224) compared with a population without SLD (*n*=287). Alanine aminotransferase (ALT) levels >30 U/L or gamma-glutamyl transferase (GGT) >35 U/L were necessary to define SLD, and subjects with viral hepatitis (HBV, HCV) were excluded from the study. NAFLD was diagnosed by ultrasonography. After identifying independent predictors of FL, the FLI was calculated by the level of TG, GGT, body mass index (BMI), and waist circumference (WC). Hepatic steatosis was ruled out with an FLI of <30 (sensitivity 87%) and ruled in with an FLI≥60 (specificity 86%). The main limitation of this study was the selection of ultrasonography as the diagnostic gold standard method for the detection of NAFLD, which has a poor significance and cannot precisely detect hepatic steatosis.<sup>42</sup>

### NAFLD-LFS

Kotronen *et al.*<sup>43</sup> was the first study to introduce the NAFLD liver fat score (NAFLD-LFS). It included 470 (males, *n*=221) Finnish individuals, 18-75 years of age with (*n*=111) or without DM (*n*=359) who were divided into an estimation group and a validation group. Except for obesity or DM, no known acute or chronic disease and alcohol consumption less than 20 g per day were criteria to be included in the study. Subjects using antihypertensives influencing glucose metabolism (β-blockers and thiazides), thiazolidinediones or those being currently pregnant were excluded. Liver fat was measured by <sup>1</sup>H-MRS. The presence of the MetS and DM, fasting serum insulin, aspartate aminotransferase (AST) and the AST/alanine aminotransferase (ALT) ratio were the strongest predictors of FL and were used for the calculation. Firstly, the liver fat risk score was developed predicting NAFLD starting from values greater than -0.640 (sensitivity 86%, specificity 71%). Secondly, an algorithm was generated that calculated the estimated percentage liver fat content, cross-validated by <sup>1</sup>H-MRS measurement of the liver fat content. The calculated liver fat percentage was strongly correlated with the liver fat measured in the MRS (*r*=0.70) both in the validation group (*R*<sup>2</sup>=0.45) and in all subjects (*R*<sup>2</sup>=0.49). Additional genotyping was performed for SNPs in the PNPLA3 gene (rs738409, adiponutrin gene; see above). Although this was a strong predictor of NAFLD, integrating the SNP as cofactor of the prediction equation provided no significant improvement over other indices.<sup>43</sup>

### HSI

Lee *et al.*<sup>44</sup> based their calculation of the HSI on data from Korean subjects who underwent routine health checkups. Of 21,130 participants, 3,591 were excluded because of chronic liver diseases (hepatitis B, C, excessive alcohol consumption, certain medications). After adjusting for sex (70% male) and age (mean=52.2 years), 10,724 subjects (5,362 with NAFLD) were randomly assigned to derivation and validation cohorts. The HSI algorithm considers the presence of DM, female sex, BMI, and the AST/ALT ratio as predictors of NAFLD. NAFLD is

Table 1. Calculation of fatty liver index (FLI), liver fat score (LFS) and hepatic steatosis index (HSI)

Publication	Index	Calculation	Cutoff value	Gold standard in primary publication	AUROC, sensitivity, specificity
Bedogni <i>et al.</i> <sup>42</sup>	Fatty liver index (FLI)	$FLI = (e^{0.953 \cdot \log_e(TG)} + 0.139 \cdot BMI + 0.718 \cdot \log_e(GGT) + 0.053 \cdot WC - 15.745) / (1 + e^{0.953 \cdot \log_e(TG)} + 0.139 \cdot BMI + 0.718 \cdot \log_e(GGT) + 0.053 \cdot WC - 15.745)} * 100$	FLI < 30 rules out, FLI ≥ 60 rules in	Ultrasonography	Se.: 87% (lower cutoff)/Sp.: 86% (upper cutoff)
Kotronen <i>et al.</i> <sup>43</sup>	NAFLD liver fat score (LFS)	$NAFLD-LFS = -2.89 + 1.18 \cdot MetS (yes=1/no=0) + 0.45 \cdot DM (yes=2/no=0) + 0.15 \cdot FI (mU/L) + 0.04 \cdot fs-AST (U/L) - 0.94 \cdot AST/ALT$	LFS > -0.640 rules in, LFS < -0.640 rules out	1H-MRS	AUROC=0.88; Se.: 86%/Sp.: 71%
	Liver fat (%)	$Liver\ fat\ (\%) = 10^{(-0.805 + 0.282 \cdot MetS (yes=1/no=0) + 0.078 \cdot DM (yes=2/no=0) + 0.525 \cdot \log(FI [mU/L]) + 0.521 \cdot \log(AST [U/L]) - 0.454 \cdot \log(AST/ALT))}$		1H-MRS	-
Lee <i>et al.</i> <sup>44</sup>	Hepatic steatosis index (HSI)	$HSI = 8 \cdot ALT/AST + BMI (+2, if DM; +2, if female)$	HSI < 30.0 rules out, HSI > 36.0 rules in	Ultrasonography	Se.: 92.5%/Sp.: 92.4%

ALT, alanine amino transferase; AST, aspartate amino transferase; BMI, body mass index; DM, type 2 diabetes mellitus; FI, fasting insulin; GGT, gamma-glutamyl transferase; MetS, metabolic syndrome; TG, triglycerides; WC, waist circumference.

ruled out at an HSI value of <30.0 (sensitivity 92.5%) and ruled in at a value of >36.0 (specificity 92.4%). As with the FLI, the validation study for HSI is limited by the diagnostic ambiguity of ultrasound analysis, which was used as gold standard. Moreover, the development in an Asian population questions the significance of HSI in Caucasians<sup>44</sup> as there is evidence for ethnic differences in the accuracy of liver fat scores<sup>45</sup> and in the appearance of NAFLD.<sup>46</sup>

### Other indices

A variety of other indices were established in the past decades, but were rarely used in other studies. This is due to their complex algorithm structure, specific and rather expensive score elements and/or their nonsuperiority over the previously mentioned indices (Table 2).<sup>47–65</sup> Based on the FLI, Kantartzis *et al.*<sup>47</sup> developed an extended FLI which could predict NAFLD more accurately by adding stimulated TG and glucose levels from an oral glucose tolerance test and including rs738409 (PNPLA3) in the equation.<sup>47</sup> The extended FLI was capable of predicting changes in liver fat with higher accuracy than the original FLI (n=213; standardized beta coefficient: 0.23–0.29). External validation of the extended FLI was not found in the literature. Because of ethnic differences in the accuracy of liver fat scores, other indices have been designed for various ethnic groups.

Wang *et al.*<sup>48</sup> developed the Zhejiang University (ZJU) index for Chinese subjects, which was validated against ultrasound and biopsy diagnoses. The ZJU index also might be a tool to classify NAFLD into different stages, including NASH. It was externally validated in some Asian populations, predicting NAFLD with moderate-to-high AUROCs (0.69–0.96),<sup>66–71</sup> and equal or better predictive power than scores developed in Western populations.<sup>72,73</sup>

Bhatt *et al.*<sup>49</sup> developed two Indian fatty liver indices (IFLIs) calculated by either clinical variables (IFLI-C) or by both clinical and biochemical variables (IFLI-CB), and validated by ultrasonography<sup>49</sup> IFLI-C and IFLI-CB performed better than the FLI and liver fat (%), but similar to NAFLD-LFS. To our knowledge, the IFLI indices have not been replicated or used in later studies.

Brandi *et al.*<sup>50</sup> proposed an index derived from a classification tree, which was validated by ultrasonography-based NAFLD diagnosis. Following the method, NAFLD can be ruled in or ruled out. The particular algorithm has never been replicated or even used in clinical trials.

Jamali *et al.*<sup>51</sup> developed the NAFLD/NASH discriminating score based on histological data from liver biopsies. Given the rather specific and expensive parameters, the usability in clinical routine is very limited.

The indices by Yip *et al.*<sup>52</sup> were tested against <sup>1</sup>H-MRS measurements as gold standard. The simplest NAFLD ridge score was proposed as the best choice, but has not been frequently used in other studies.

The lipid accumulation product (LAP) was introduced as new parameter to reflect obesity-related (e.g., cardiovascular) risks by integrating both WC and triglycerides (TG), including consideration of sex differences,<sup>53</sup> and testing the index against ultrasound sonography. Lacking clear cut-offs<sup>54</sup> and being outperformed by NAFLD-LFS and HSI in high-risk settings and by FLI in population-based settings,<sup>74</sup> the LAP has limited usability for NAFLD prediction.

The original SteatoTest includes costly parameters that limit routine use.<sup>55</sup> The simpler version (SteatoTest-2) neglects BMI and bilirubin as typical sources of real-world confounding and performs comparably well.<sup>56</sup>

The Framingham Steatosis Index (FSI) by Long *et al.*,<sup>57</sup>

the Korean K-NAFLD score,<sup>58</sup> and the NAFL screening score (NSS)<sup>59</sup> use parameters similar to the FLI, NAFLD-LFS, and HSI.

Uric acid is a component found in the NAFL risk score,<sup>60</sup> and the score by Pan *et al.*,<sup>61</sup> which integrate data on anthropometric characteristics, metabolism, and diet. History of gout and ferritin levels are novel parameters used in a score based on the Study of Health in Pomerania (SHIP). It also integrates more common items (age, AST, ALT, WC, BMI, TG) and performed well in comparison to HSI and FLI.<sup>62</sup>

Ethnicity can be an important confounder of score reliability. Several studies reported a poorer performance of the HSI in non-Asian cohorts. In order to address this issue for multiethnic populations the score by Ruhl *et al.*,<sup>63</sup> the Dallas Steatosis Index (DSI),<sup>64</sup> the NAFLD-MESA index and the NAFLD clinical index were developed and outperformed the original FLI.<sup>65</sup>

### External validation of common liver fat scores for prediction of NAFLD and liver fat content

A number of recent studies have evaluated available liver fat scores for clinical practice. A summary of predictive values for the indices from original and external validation studies is shown in Table 3.<sup>36,42–44,75–81</sup> Koot *et al.*<sup>75</sup> found that these scores are poor predictors of NAFLD in obese children.<sup>30</sup> The cohort (119 severely obese children (14.3±2.1 years of age, BMI z-score 3.35±0.35; 47% NAFLD cases) was investigated with MR spectroscopy as gold standard; FLI, NAFLD-LFS, and HSI were assessed. As these scores were developed for adult populations, their poor performance in pediatric patients is not entirely unexpected. However, the pediatric prediction score did not outperform the others either.

Another study<sup>76</sup> aimed at validating the FLI in 168 healthy adults and another 168 adults with components of the MetS by measuring IHL by <sup>1</sup>H-MRS. This study showed that FLI can detect presence of NAFLD in the individual but doubted its accuracy in predicting the degree of hepatic steatosis/actual liver fat content. Thus, in accord with its original designation, the FLI can primarily be used to identify patients with possible steatosis in order to perform further diagnostics or to roughly classify groups of patients.

Kabisch *et al.*<sup>77,78</sup> showed that the FLI and NAFLD-LFS had a highly significant correlation with IHL at the baseline visit of their intervention studies, replicating their capability to predict NAFLD and to mirror actual liver fat content with good precision (AUROCs around 0.73; r-values around 0.5).<sup>77,78</sup> Both cohorts included participants with prediabetes or overt DM and a considerable proportion of NAFLD patients, allowing for plausible correlations.

A similar approach was adopted by Kahl *et al.*,<sup>79</sup> who reported only moderate accuracy of the scores that did not allow quantification of liver fat content. However, in that study, predominantly nonobese persons with low liver fat content were included, limiting the replication of the index performances.

Unlike other studies, Motamed *et al.*<sup>80</sup> reported a very high predictive power of FLI, with NAFLD diagnosed by ultrasound rather than <sup>1</sup>H-MRS. The study did not find significant differences between the FLI and WC as a single factor for the diagnosis of NAFLD. In the development of the FLI,<sup>42</sup> WC was already identified as the strongest predictor of NAFLD, along with BMI. The excellent replication of the FLI performance in this study can be attributed to the large cohort of more than 5,000 middle-aged patients with high prevalence of NAFLD.

A retrospective analysis of 324 liver biopsies from middle-

Table 2. Calculation of less common liver fat indices

Publication	Index	Calculation	Cutoff values	Gold standard in primary publication	AUROC, sensitivity, specificity
Kantartzis et al. <sup>47</sup>	Extended FLI	Extended FLI: $(x/1+x) \times 100$ ; $x = e^{0.4508 \times \log_e(TG) + 0.0621 \times BMI + 0.4022 \times \log_e(GGT) + 0.0454 \times (WC) + 4.8874 \times (\text{fold-change } TG_{OGTT}) + 2.4134 \times \log_e(2\text{-h glucose}) - 1.1143 \times (rs738409 C>G \text{ variant of PNPLA3}; C=1 \text{ and } XG=0) - 19.1367$	FLI < 30 rules out, FLI ≥ 60 rules in	1H-MRS	AUROC=0.86; Se.: 78% (lower cutoff)/Sp.: 92% (upper cutoff)
Wang et al. <sup>48</sup>	ZJU index	ZJU: BMI (kg/m <sup>2</sup> )+FPG (mmol/L)+TG (mmol/L)+3×ALT (IU/L)/AST (IU/L) ratio (+2, if female)	ZJU < 32.0 rules out, ZJU > 38.0 rules in	Ultrasound and liver biopsy	AUROC=0.822; Se.: 92.2% (lower cutoff)/Sp.: 93.4% (upper cutoff)
Bhatt et al. <sup>49</sup>	Indian Fatty Liver Index (IFLI-C)	IFLI-C: 1 (double chin)+15.5 (SBP)+13.8 (buffalo hump); SBP: 1 if > 120/80; 0 if otherwise	IFLI-C ≥ 1 rules in	Ultrasound	AUROC=0.650, Se.: 65%/Sp.: 62%
	Indian Fatty Liver Index (IFLI-CB)	IFLI-CB: TG (1 if ≥ 150 mg/dL; 0 if otherwise)+12×(FLI; 1 if > 2.7 μU/mL; 0 if otherwise)+16×(SBP; 1 if > 120/80 mmHg; 0 if otherwise)+18×(buffalo hump)	IFLI-CB ≥ 28 rules in	Ultrasound	AUROC=0.719, Se.: 64%/Sp.: 67%
Birjandi et al. <sup>50</sup>	Iranian classification tree method	classification tree using BMI, WHR, TG, FPG, SPB, and ALT		Ultrasound	AUROC=0.78, Se.: 74%/Sp.: 83%
Jamali et al. <sup>51</sup>	NAFLD discriminant score	NAFLD discriminant score: $[(-0.298 \times \text{adiponectin}) + (0.022 \times \text{TNF-}\alpha) + (1.021 \times \text{Log visfatin}) + (0.709 \times \text{Log IL-6}) + 1.154]$	Score > -0,29 rules in	Ultrasound	AUROC=0.94, Se.: 91%/Sp.: 83%
Yip et al. <sup>52</sup>	NAFLD Ridge Score	NAFLD ridge score: $-0.614 + 0.007 \times \text{ALT} - 0.214 \times \text{HDL} + 0.053 \times \text{TG} + 0.144 \times \text{HbA1c} + 0.032 \times \text{WBC} + 0.132 \times \text{hypertension}$ (yes=1; no=0)	Score < 0.24 rules out, Score > 0.44 rules in	1H-MRS	AUROC=0.87; Se.: 92% (lower cutoff)/Sp.: 90% (upper cutoff)
	NAFLD logit score	NAFLD logit score: $e^{7.338 + 0.046 \times \text{ALT} - 1.277 \times \text{HDL} + 0.486 \times \text{TG} + 0.911 \times \text{HbA1c} + 0.207 \times \text{WBC} + 0.589 \times \text{hypertension}}$ (yes=1; no=0)/(1+ $e^{7.338 + 0.046 \times \text{ALT} - 1.277 \times \text{HDL} + 0.486 \times \text{TG} + 0.911 \times \text{HbA1c} + 0.207 \times \text{WBC} + 0.589 \times \text{hypertension}}$ (yes=1; no=0))	Score < 0.19 rules out, Score > 0.45 rules in	1H-MRS	AUROC=0.87; Se.: 90% (lower cutoff)/Sp.: 90% (upper cutoff)
	NAFLD AdaBoost score	Combination of decision tree and weighted scores for ALT, HDL, HbA1c, WBC, and TG in one sum	Score < -0.76 rules out, Score > 0.05 rules in	1H-MRS	AUROC=0.88; Se.: 91% (lower cutoff)/Sp.: 90% (upper cutoff)
	NAFLD decision tree score	classification tree using ALT, TG, WBC, ALT and HbA1c	Score < 0.27 rules out, Score > 0.57 rules in	1H-MRS	AUROC=0.89; Se.: 95% (lower cutoff)/Sp.: 92% (upper cutoff)
Kahn et al. <sup>53</sup>	Lipid accumulation product (LAP)	LAP: $(WC-65) \times TG$ for men; $(WC-58) \times TG$ for women; interpretation based on logarithmic derivation		ultrasound	AUROC=0.78-0.80, Se. and Sp. not given
Bedogni et al. <sup>54</sup>	SteatoTest	total bilirubin, GGT, α2m, haptoglobin, ALT, apolipoprotein AI, BMI, total cholesterol, TG, FPG, age and sex for adjustment		Liver biopsy	AUROC=0.79; several reports on Se. and Sp.

(continued)

Table 2. (continued)

Publication	Index	Calculation	Cutoff values	Gold standard in primary publication	AUROC, sensitivity, specificity
Poynard et al. <sup>56</sup>	SteatoTest-2	GGT, $\alpha$ 2m, haptoglobin, ALT, apolipoprotein AI, total cholesterol, TG, FPG; age and sex for adjustment	0,40	Liver biopsy	AUROC=0.77–0.87; Se.: 79%; Sp.: 50%
Long et al. <sup>57</sup>	Framingham Steatosis Index (FSI)	FSI: $-7.981+0.011 \times \text{age} - 0.146 \times \text{sex}$ (female=1, male=0)+ $0.173 \times \text{BMI} + 0.007 \times \text{TG} + 0.593 \times \text{hypertension}$ (yes=1, no=0)+ $0.789 \times \text{diabetes}$ (yes=1, no=0)+ $1.1 \times \text{ALT}/\text{AST}$ ratio $\geq 1.33$ (yes=1, no=0).		CT	AUROC=0.845; several reports on Se. and Sp.
Jeong et al. <sup>58</sup>	K-NAFLD score	K-NAFLD-S.: $0.913 \times \text{sex}$ (female=2, male=1)+ $0.089 \times \text{WC} + 0.032 \times (\text{SBP} + \text{FPG}) + 0.007 \times \text{TG} + 0.105 \times \text{ALT} - 20.929$	Score < -3.285 rules out, Score > 0.884 rules in	NAFLD-LFS	AUROC=0.929; Se. and Sp. not given
Zhou et al. <sup>59</sup>	NAFL screening score (NSS)	age, BMI, TG, ALT, AST, FPG, uric acid	Score < 29 (female) or < 33 (male) rules out	Ultrasound	AUROC=0.825–0.861
Zhou et al. <sup>60</sup>	NAFL risk score	BMI, TG, GGT, ALT, AST, LDL, HDL, uric acid	Cutoff of 7 (male) and 13 (female) for high 4-year risk	Ultrasound	AUROC=0.739–0.823
Pan et al. <sup>61</sup>	Not named	BMI, WC, TG, HDL, ALT, diabetes, hyperuricemia, intake of tubers, and fried food	Specific nomogram; score < 180 rules out, Score > 340 rules in	Ultrasound	AUROC=0.834; Se: 99%; Sp. 94%
Meffert et al. <sup>62</sup>	Study of health in pomerania score	Age, AST, ALT, WC, BMI, TG, ferritin, history of gout		Ultrasound	AUROC=0.860
Ruhl et al. <sup>63</sup>	USA fatty liver index	Age, WC, FI, FPG, GGT, ethnicity	US FLI < 10 rules out, US FLI > 30 rules in	Ultrasound	AUROC=0.80, Se.: 86% (lower cutoff)/Sp.: 88% (upper cutoff)
McHenry et al. <sup>64</sup>	Dallas steatosis index (DSI)	ALT, BMI, age, sex, TG, FPG, diabetes, hypertension, ethnicity		1H-MRS	AUROC=0.824
Rodriguez et al. <sup>65</sup>	NAFLD-MESA index	age, sex, ethnicity, diabetes, smoking history, BMI, GGT, TG	> 21 rules in	CT	AUROC=0.83; Se. 75%; Sp. 72%
	NAFLD clinical index	age, sex, ethnicity, diabetes, smoking history, BMI	> 19 rules in	CT	AUROC=0.78; Se. 80%; Sp. 60%

BMI, body mass index; FI, fasting insulin; FPG, fasting plasma glucose; GGT, gamma-glutamyl transferase; HbA1c, hemoglobin A1c; IL6/8, interleukin 6/8; SBP, systolic blood pressure; TG, triglycerides; TNFo, tumor necrosis factor  $\alpha$ ; WBC, white blood cell count;  $\alpha$ 2m,  $\alpha$ 2-macroglobulin; WC, waist circumference.

**Table 3. Area under the receiver operating characteristic curve (AUROC), 95% confidence interval (CI), sensitivity (%) and specificity (%) for fatty liver index (FLI), NAFLD liver fat score LFS and hepatic steatosis index (HSI) for prediction of NAFLD in original and external validation studies**

<b>Index</b>	<b>Study</b>	<b>AUROC (CI)</b>	<b>Sensitivity, %</b>	<b>Specificity, %</b>	
Fatty liver index (FLI) <sup>42</sup>	Original study		87	86	
	Validation study		70	60	
		Koot <i>et al.</i> <sup>75</sup>	0.71 (0.61–0.80)		
		Cuthbertson <i>et al.</i> <sup>76</sup>	0.79 (0.74–0.84)	95	91
		Kabisch <i>et al.</i> <sup>77</sup>	0.786		
		Kabisch <i>et al.</i> <sup>78</sup>	0.731		
NAFLD liver fat score (LFS) <sup>43</sup>	Original study		76	83	
	Validation study		77	71	
		Kahl <i>et al.</i> <sup>79</sup>	0.72 (0.59–0.85)		
		Motamed <i>et al.</i> <sup>80</sup>	0.86 (0.85–0.88)		
		Fedchuk <i>et al.</i> <sup>81</sup>	0.83 (0.72–0.91)	76	87
		Koot <i>et al.</i> <sup>75</sup>	0.88 (0.84–0.92)	86	71
Hepatic steatosis index (HSI) <sup>44</sup>	Original study		65	87	
	Validation study		92.5	92.4	
		Kabisch <i>et al.</i> <sup>77</sup>	0.77		
		Kabisch <i>et al.</i> <sup>78</sup>	0.75		
		Kahl <i>et al.</i> <sup>79</sup>	0.70 (0.53–0.87)	35	91
		Fedchuk <i>et al.</i> <sup>81</sup>	0.80 (0.69–0.88)	65	87
	Koot <i>et al.</i> <sup>75</sup>	0.81 (0.80–0.82)	92.5	92.4	
	Kabisch <i>et al.</i> <sup>78</sup>	0.68 (0.59–0.78)	67	62	
	Kahl <i>et al.</i> <sup>79</sup>	0.77			
	Kahl <i>et al.</i> <sup>79</sup>	0.79 (0.68–0.90)	100	75	
	Fedchuk <i>et al.</i> <sup>81</sup>	0.81 (0.71–0.88)	61	93	

**Table 4. Correlation of liver fat scores and type 2 diabetes-related outcomes in external validation studies**

Study	Outcome	FLI	NAFLD-LFS	HSI
Kahl <i>et al.</i> <sup>79</sup>	ISComp_In	r=-0.62***	r=-0.71***	r=-0.53***
	OGIS	r=-0.62***	r=-0.51***	r=-0.50***
	QUICKI	r=-0.55***	r=-0.68***	r=-0.42***
	DI_In	r=0.47***	r=0.57***	r=0.48***
	B-cell func_In	r=0.57***	r=0.57***	r=0.47***
	AI	r=0.34***	r=0.35***	r=0.33**
	IGI_CP_In	r=-0.02	r=0.05	r=0.02
	IGI_Ins_In	r=0.16	r=0.26*	r=0.19
Hep_Extr_In	r=-0.39***	r=-0.55***	r=-0.42***	
Gastaldelli <i>et al.</i> <sup>91</sup>	Glucose concentration	R=0.34****		
	Ln (Insulin concentration)	R=0.62****		
	Ln (Clamp FFA concentration)	R=0.41****		
	Ln (Peripheral IS)	R=-0.43****		
Bozkurt <i>et al.</i> <sup>92</sup>	PGDM – IS vs. NGT	p=0.104		
	PGDM – IR vs. NGT	p<0.001		
	PGDM-IS vs. PGDM-IR	p=0.006		
Balkau <i>et al.</i> <sup>93</sup>	Incident diabetes (men)	≤ 57.67: OR=1; >57.67: OR=4.46	≤ -1.15: OR=1; > -1.15: OR=4.88	
	Incident diabetes (women)	≤ 21.64: OR=1; >21.64: OR=11.58	≤ -1.82: OR=1; > -1.82: OR=12.48	
Unalp-Arida <i>et al.</i> <sup>83</sup>	Diabetes	L: HR=0.5; I: HR=2.1; H: HR=7.4	L: HR=0.6; I: HR=5.2; H: HR=16.8	L: HR=0.6; I: HR=2.0; H: HR=6.9

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001; \*\*\*\*p<0.0005. r, correlation coefficient; R, correlation coefficients of univariate analysis. AI, adaptation index; B-cell func\_In, fasting β-cell function; DI, disposition index; Hep\_Extr\_In, hepatic insulin extraction; IGI\_CP, insulinogenic C-peptide index; IGI\_Ins, insulinogenic indices; IR, insulin resistance; IS, insulin sensitivity; ISComp, Matsuda's index; NGT, control group (p-values from Fisher protected least significant difference test); OGIS, oral glucose insulin sensitivity index; OR, odds ratio; PGDM, previous gestational diabetes; QUICKI, quantitative insulin sensitivity check index.

aged, overweight-to-obese patients (64% males) that categorized NAFLD as mild (≥5–33%), moderate (>33–66%), and severe (>66%) steatosis, showed that all indices were capable of accurately ruling NAFLD in or out and correlated strongly with insulin resistance. However, they were limited in the quantification of steatosis, which makes the markers questionable for describing changes in liver fat. On the other hand, liver biopsies can fail to reflect the average steatosis in the entire organ.<sup>81</sup>

#### Liver fat scores in prediction models for non-NAFLD outcomes

Elevated liver fat content and NAFLD are strongly associated with hepatic insulin resistance, hypertriglyceridemia, and chronic cardiometabolic complications such as T2DM and CVD.<sup>7,82–84</sup> In the TULIP study, NAFLD predicted failure in even compliant dietary intervention in patients with prediabetes.<sup>85</sup> Other diabetes prevention studies indicated that prediabetes patients with NAFLD had better responses to treatment that improving insulin resistance.<sup>77,86–89</sup> NAFLD predicts CVD and mortality independently of glycemic metabolism.<sup>90</sup> Therefore, it is of interest if liver fat scores have the same predictive potential for metabolic sequelae. In a study by Kahl *et al.*,<sup>79</sup> FLI and NAFLD-LFS were also inversely correlated with parameters of insulin sensitivity such as the

quantitative insulin sensitivity check index (QUICKI), describing fasting insulin sensitivity as well as the oral glucose insulin sensitivity index (OGIS), and Matsuda's index (ISComp) for dynamic insulin sensitivity. Fasting β-cell function and parameters describing post-load insulin secretion such as the disposition index (DI), adaptation index (AI), and insulinogenic indices (IGI\_Ins) positively correlated with fatty liver indices (Table 4).<sup>79</sup> However, this positive correlation may be a spurious relationship as the study included participants without T2DM. In such a healthy cohort, low insulin secretion is mainly triggered secondarily by fatty liver and hepatic insulin resistance. The study population did not include patients with advanced insulin-deficient diabetes. The correlation of fatty liver scores, especially FLI, with DM and insulin resistance (Table 4) has already been shown in a number of studies, and emphasizes the strong association between DM and NAFLD.<sup>90–93</sup>

Other studies have found an association of FLI with hepatic and cardiovascular diseases and cancer.<sup>82,91,94</sup> A recent study in an US population reported that none of the scores correlated with increased cardiovascular mortality.<sup>83</sup> NAFLD-LFS and FLI were associated only with increased liver disease mortality and NAFLD-LFS was also associated with increased diabetes mortality. Correlations of liver fat scores with hepatic, cardiometabolic, cardiovascular, and cancer outcomes are shown in Table 5.<sup>82,83,91,94</sup>



**Table 5. Correlation between liver fat scores and risk parameters for cardiovascular and hepatic diseases and cancer from external validation studies**

Study	Outcome	FLI	NAFLD-LFS	HSI
Gastaldelli <i>et al.</i> <sup>91</sup>	Systolic blood pressure	R=0.39****		
	Diastolic blood pressure	R=0.35****		
	CCA IMT	R=0.30****		
	Framingham score	R=0.34****		
	Ln (LDL cholesterol)	R=0.33****		
	Ln (HDL cholesterol)	R=-0.50****		
Calori <i>et al.</i> <sup>94</sup>	15-year hepatic-related mortality	HR=1.036****		
	15-year CVD mortality	HR=1.007**		
	15-year cancer mortality	HR=1.006*		
	15-year all-cause mortality	HR=1.006**		
Lerchbaum <i>et al.</i> <sup>82</sup>	All-cause mortality	Q1: HR=1.0; Q2: HR=1.14; Q3: HR=1.11; Q4: HR=1.26		
	Cardiovascular mortality	Q1: HR=1.0; Q2: HR=1.28; Q3: HR=1.35; Q4: HR=1.32		
	Cancer mortality	Q1: HR=1.0; Q2: HR=1.10; Q3: HR=0.75; Q4: HR=1.01		
	Non-cardiovascular mortality	Q1: HR=1.0; Q2: HR=0.98; Q3: HR=0.88; Q4: HR=1.27		
Unalp-Arida <i>et al.</i> <sup>83</sup>	All-cause mortality	L: HR=19.0; I: HR=32.1; H: HR=42.2	L: HR=23.8; I: HR=41.3; H: HR=48.9	L: HR=28.5; I: HR=29.2; H: HR=35.1
	Cardiovascular disease	L: HR=6.0; I: HR=13.1; H: HR=19.0	L: HR=9.2; I: HR=18.2; H: HR=27.2	L: HR=11.3; I: HR=12.5; H: HR=16.0
	Neoplasms	L: HR=5.2; I: HR=11.9; H: HR=13.5	L: HR=7.5; I: HR=13.4; H: HR=12.4	L: HR=8.7; I: HR=9.5; H: HR=10.3
	Liver disease	L: HR=0.4; I: HR=0.6; H: HR=2.5	L: HR=0.5; I: HR=1.6; H: HR=5.5	L: HR=0.8; I: HR=0.8; H: HR=1.7

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0005$ . Fat probability (low, L; intermediate, I; High, H); Framingham score, prediction score for coronary heart disease (CHD);  $r$ , correlation coefficient; Quartiles for FLI levels: Q1 (< 31.0); Q2 (31.0–53.7); Q3 (53.8–75.6); Q4 (>75.6); R, regression coefficients of univariate analysis. CCA, common carotid artery; CVD, cardiovascular disease; HR, Cox proportional hazard ratio (without adjustment); IMT, carotid intima media thickness.

### Usability of liver fat scores as monitoring tools for interventional changes in liver fat

The predictive power of the indices is also of interest for longitudinal studies in which IHL is reduced, e.g., by lifestyle interventions (Table 6).<sup>47,77,78,95–97</sup> Keating *et al.*<sup>95</sup> performed a lifestyle intervention trial with either an 8-week exercise or a 12-week nutraceutical intervention. They evaluated whether some surrogate markers, including FLI, HSI, NAFLD-LFS and WC, indicated changes in liver fat measured by <sup>1</sup>H-MRS. At baseline, the scores correlated weakly or moderately with measured liver fat content. During intervention, only changes of FLI, HSI, and in particular the single parameter WC correlated with changes in IHL.<sup>95</sup>

Another trial found that FLI values were significantly lower after 9 months of a low-fat, high-fiber lifestyle intervention. In contrast, HSI did not detect changes in liver fat.<sup>47</sup> Other predictors of change in liver fat were 2-h TG and TG fold-change measured at 2 h during OGTT (TG<sub>OGTT</sub>) as the strongest predictor. Kabisch *et al.*<sup>77</sup> evaluated the power of FLI and NAFLD-LFS comparing two randomized lifestyle interventions

(low-carb and low-fat diets) in patients with prediabetes. IHL were measured by <sup>1</sup>H-MRS. The scores strongly correlated with IHL at baseline, however both scores only correlated moderately with liver fat with a low-fat but not with a low-carb diet. Liver fat content decreased significantly in both diet groups without a significant difference between the groups. However, in contrast to the low-fat group, changes of IHL in the low-carb group only correlated with changes of two parameters (fasting insulin, ALT) used to calculate the indices. In the low-fat group, changes in body weight correlated with changes of liver fat. As reduction of liver fat under low-carb conditions is mainly independent of body weight reduction and correlates with different parameters than in the low-fat group, the mechanisms of IHL reduction might vary with different diets or other treatments. Similar results were reported in DM patients on a high-protein diet, in which the reduction in liver fat was also found to be independent of weight reduction. Only FLI correlated weakly with changes in liver fat, but at baseline all indices were significantly correlated with IHL. Apart from WC, none of the index parameters (body weight, WC, fasting insulin, TG,

Table 6. Correlation of changes in liver fat scores with changes in liver fat content after lifestyle interventions

Study	FLI	NAFLD-LFS	HSI
Keating <i>et al.</i> <sup>95</sup>	r=0.466**	r=0.117	r=0.245*
Kantartzis <i>et al.</i> <sup>47</sup>	BL: 70.39±3.01; FU: 63.35±3.44. p=0.002		BL: 41.62±0.65; FU: 41.45±0.83. p=0.059
Kabisch <i>et al.</i> <sup>77</sup>	Low fat: r=0.499***; Low carb: r=0.075	Low fat: r=0.438**; Low carb: r=0.257	
Kabisch <i>et al.</i> <sup>78</sup>	r=0.342	r=-0.058	r=-0.049
Vilar-Gomez <i>et al.</i> <sup>96</sup>		ΔCCI=-1.95±0.22***; ΔUC=0.47±0.41	
Arslanow <i>et al.</i> <sup>97</sup>	Δ%=-21.3 (-74.0-0.0)***		

\*p<0.05; \*\*p<0.01; \*\*\*p<0.001. ΔCCI/UC, change in liver fat after 1 year of continuous care intervention or standard diabetes care; Δ%, percentage change in liver fat after 2 weeks hypocaloric high-fiber and high-protein diet; r=Spearman correlation coefficient. BL, baseline; FU, follow-up; Low fat/carb, changes in liver fat after 3 weeks of low fat or low carb dietary intervention.

AST, ALT, AST/ALT ratio, GGT) were correlated with changes in liver fat. Correlation of WC with IHL can be explained by the simultaneous reduction of visceral and hepatic fat.<sup>78</sup> As anthropometric parameters of obesity such as BMI or body weight are important index components, the lack of correlation of weight reduction with liver fat reduction is probably the crucial reason for the poor performance of the indices. As the intervention was performed in subjects with T2DM and reduced insulin secretion, insulin levels do not necessarily reflect improvement in fatty liver. Although TG and GGT were among the most representative parameters for changes in liver fat, there was also no significant correlation with liver fat reduction. The high-protein diet improved lipid and aminotransferase levels, which is why their changes may have appeared beyond steatosis and therefore lacked correlation with liver fat reduction.<sup>78</sup> High aminotransferase levels may also have been influenced by pre-existing hepatic inflammation, NASH, or fibrosis.

In a study comparing a comprehensive continuous care intervention (CCI) with a nutritional ketosis diet and standard diabetes care treatment (UC), NAFLD-LFS was significantly improved in patients undergoing CCI in comparison to the UC patients.<sup>96</sup> Similar to the results of Kabisch *et al.*,<sup>77</sup> NAFLD-LFS changed progressively with higher weight reduction in the diet (≥10%). The study was limited by the lack of IHL data from imaging or biopsy.<sup>96</sup> During a short-term hypocaloric high-fiber and high-protein diet, liver fat measured by CAP and FLI significantly decreased in the study cohort, with the reduction being stronger in patients with weight loss ≥5%.<sup>97</sup> The correlation between CAP and FLI was not determined. However, CAP at baseline correlated with some parameters of the FLI (BMI, WC), but not TG or GGT.

The limited accuracy of liver fat indices in general can also be explained by treatment effects. Even in cross-sectional designs, antidiabetic treatment, lipid-lowering drugs, antihypertensive medication, inflammatory disorders, specific liver conditions without linkage to NAFLD (Morbus Meulengracht), and of course unreported excess alcohol intake may confound parameters of NAFLD scores.<sup>98</sup>

### Potential biomarkers of novel liver fat indices

The accuracy of indices in cross-sectional studies is sometimes considerably lower than the reported accuracy in the original studies. Even at moderate or high precision, their usefulness for indicating liver fat content has been questioned. Therefore, they should be used only for predicting the *possible* presence of NAFLD, assessment of the risk of liver malignancy or cardiovascular disease and selection of patients for detailed examination.

In longitudinal studies, the indices correlated with the measured liver fat before the intervention, while changes in IHL not clearly indicated by the indices during and after the interventions. Even moderate correlations appeared only in the case of strong changes in body weight, as all common indices are mainly based on BMI and/or WC. As liver fat reduction can be achieved independent of body weight reduction especially in low-carb or high-protein diets or due to medication, the indices are presumably useless as monitoring tools when used with these treatments.

The most important parameters included in the equations were BMI, TG, WC, GGT, FPG, fasting insulin, AST, ALT, AST/ALT and SBP. Although fasting insulin and parameters of insulin sensitivity (QUICKI, OGIS, ISComp), β-cell function and post-load insulin resistance (DI, AI, IGI) were associated with liver fat scores and IHL in recent studies,<sup>77,79,81,91-93,99</sup> fasting insulin is only included in the NAFLD-LFS.<sup>43</sup> High vari-

ability among insulin test kits complicates the utility of insulin as a parameter for liver fat scores. Moreover, the pulsatile insulin release requires multiple, i.e. costly blood draws in short intervals.<sup>100</sup> FPG is used as a parameter in some more recently introduced scores<sup>50,55,56,58,59,63,64</sup> for being rarely reported as a possible predictor for NAFLD in the literature. A few studies found a significant positive association between the triglyceride and glucose index (TyG), calculated as  $\text{Ln}[\text{TG}(\text{mg/dL}) \times \text{FPG}(\text{mg/dL})]$ , and NAFLD.<sup>101-103</sup> Moreover, according to the literature, TyG-related parameters, such as TyG-BMI ( $=\text{TyG} \times \text{BMI}$ ), TyG-WC ( $=\text{TyG} \times \text{WC}$ ), have been more reliable predictors than the classical TyG and former parameters.<sup>103,104</sup> Abnormal glucose tolerance, defined as either impaired glucose tolerance (2 h glucose between 7.8 and 11.0 mmol/L) or DM (2h glucose  $\geq 11.1$  mmol/L or FPG  $\geq 7.0$  mmol/L) was reported as a significant predictor for steatohepatitis and fibrosis in NAFLD patients.<sup>105</sup> Another study showed significantly increased fasting glucose and HbA1c levels in subjects with steatosis compared to subjects without steatosis.<sup>106</sup>

In addition to the above parameters, other predictors associated with NAFLD have been discussed. The role of adipokines (especially adiponectin) in the pathogenesis of NAFLD has been investigated in many studies. In rat models<sup>107,108</sup> as well as in human subjects with NAFLD,<sup>51,109,110</sup> the concentration of adiponectin was significantly decreased and adiponectin gene polymorphisms associated with NAFLD were found.<sup>111,112</sup> One publication investigated the association between serum retinol-binding protein 4 (RBP4) and NAFLD.<sup>113</sup> In this study, they found lower RBP4 levels in patients with NASH than in patients with simple steatosis. Nevertheless, the differences found were not significant and there was no correlation of RBP4 with BMI, HOMA, FPG, or fasting insulin. A similar lack of correlation was reported in subjects with DM,<sup>114</sup> probably owing to medication effects.<sup>98</sup>

In a meta-analysis, fetuin-A and fetuin-B concentrations were significantly higher in subjects with NAFLD and fetuin-A played a role in the process of simple steatosis to NASH.<sup>115</sup> Another approach found a significant association between elevated fetuin-A concentrations and increased FLI, ALT and AST<sup>116</sup> and a prospective cross-sectional study found an independent correlation of increased fetuin-A concentrations with NAFLD.<sup>117</sup> One study discussed progranulin as a potential predictor for NAFLD, as progranulin was significantly higher in patients with NAFLD and positively correlated with total cholesterol and LDL cholesterol.<sup>41</sup> Although these markers could be used to predict NAFLD, their measurement is too expensive for routine clinical use. Less expensive parameters are needed.

FIB-4, NFS, BARD, or ELF calculate fibrosis risk in patients with NAFLD using specific markers of extracellular matrix production.<sup>118</sup> Typically, they cannot be used as early predictors for simple nonprogressed steatosis. However, serum cytokeratin-18, as one of those matrix markers, is also strongly related to simple NAFLD and ALT levels in adults<sup>119,120</sup> and children,<sup>121</sup> providing sensitivity and specificity of over 97% for detection of moderate-to-high steatosis.<sup>119</sup> A parameter correlated with histological classifications of hepatic steatosis, is plasma cathepsin D (CatD), which was found to be significantly decreased after gastric bypass surgery in subjects with NASH.<sup>122</sup> Another study reported a positive association of insulin resistance (HOMA-IR and plasma insulin levels) with CatD in subjects with NAFLD.<sup>123</sup> In contrast, CatD had only weak effectiveness in indicating changes in NAFLD and NASH in an Asian population, again underscoring ethnic differences in the parameters of NAFLD.<sup>124</sup> Again, quantification of cathepsin D is expensive and possibly not cost-effective for

NAFLD screening.

Recent studies have reported a relationship between iron metabolism and body composition and NAFLD.<sup>125-135</sup> A prospective study reported an association between a high serum iron-to-ferritin ratio with healthy body composition and reduced risk of fatty liver progression in young adult women <45 years of age, but not in middle-aged women  $\geq 45$  years of age.<sup>126</sup> A study of obese male pediatric patients found that serum ferritin was more strongly linked to liver fat content and inflammation than body iron status was.<sup>127</sup> Furthermore, increased serum ferritin levels predicted the risk of NASH and fibrosis development in adult patients with NAFLD.<sup>128</sup> A similar association of serum ferritin with parameters of liver health (liver fat content, ALT, hepatic iron) and with glucose and lipid metabolism also found this parameter might be a predictor of NAFLD.<sup>130</sup> The best area under the curve (AUC) for the prediction of hepatic steatosis was found by combining blood ferritin, FPG, and ALT. The SHIP NAFLD score is the only index using ferritin.<sup>62</sup>

Another possible predictor of NAFLD is alkaline phosphatase (ALP), which has been reported as an independent predictor of DM.<sup>5</sup> An experimental study investigated the role of tissue nonspecific alkaline phosphatase (TNAP) in mice with TNAP<sup>+/-</sup> haplodeficiency (absence of an allele of TNAP) and found that the mice developed hepatic steatosis similar to that induced by a diet deficient in methionine and choline (MCD).<sup>136</sup> Acetylcholine (ACh) might also participate in hepatic steatosis and fibrosis progression as it induced fibrogenesis in hepatic stellate cells in vitro as well as in human whole-liver samples of NASH fibrosis via muscarinic ACh receptors.<sup>137</sup> Furthermore, serum cholinesterase activity was significantly higher in patients with NAFLD<sup>138,139</sup> and DM<sup>140</sup> in previous studies. In contrast, a study of DM patients with or without NAFLD found decreased cholinesterase activity in those with hepatic steatosis.<sup>141</sup> Cholinesterase activity may have been elevated at earlier stages of the disease and only had decreased activity in cases with advanced liver cell damage, which may explain the controversial results. This fact indicates that cholinesterase activity may also serve as an indicator of the severity of steatosis. To investigate the predictive power of ALP and cholinesterase for NAFLD, further studies are needed.

Uric acid has been considered as a possible predictor of NAFLD,<sup>142</sup> and was reported to be positively correlated with NAFLD risk<sup>143,144</sup> and to induce hepatic fat accumulation and insulin resistance.<sup>145</sup> NSS and NAFL risk scores are the first indices implementing that parameter as a continuous parameter.<sup>59,60</sup> The German SHIP NAFLD score integrates history of gout,<sup>62</sup> and another score developed in China uses a binary expression for hyperuricemia.<sup>61</sup> As none of those scores are widely used, uric acid is still a parameter of interest for novel indices and may help to develop scores that outperform others with respect to longitudinal reflection of liver fat changes.

As mentioned above, SNPs influence the risk of chronic liver and heart diseases, the factors PNPLA3, TM6SF2, MBOAT7, GCKR, and HSD17B13 have key involvement.<sup>11-15,17-22</sup> In addition to those genetic variants, GWAS found several further genetic factors that were significantly associated with NAFLD.<sup>146</sup> PYGO1 is a protein contributing to the Wnt signaling pathway. Absence in a homologue PYGO2 in mice resulted in increased adiposity and impaired glucose tolerance.<sup>147</sup> The rs429358-C variant defines the main three alleles of apolipoprotein E (ApoE) in combination with rs7412. Although all three alleles appeared to be protective against NAFLD, one simultaneously increased cardiovascular and metabolic risk via enhanced hepatic VLDL secretion. Furthermore, increased ApoE serum levels correlated with higher FLI.<sup>148</sup>

Mitochondrial amidoxime reducing component 1 (MARC1) and sorting and assembly machinery component (SAMM50) encode for proteins located in the outer mitochondrial membrane.<sup>149,150</sup> Missense variants in the MARC 1 protein protected against all-cause cirrhosis and were associated with lower levels of hepatic fat, NAFLD risk and aminotransferase levels.<sup>149,151</sup> SAMM50 SNPs were associated with NAFLD and might be involved in the progression of NAFLD.<sup>150</sup> The neurocan core protein (NCAN), a proteoglycan involved in remodeling central nervous system, is also expressed in the liver. NCAN function is linked to hepatic steatosis, lobular inflammation, and fibrosis. protein phosphatase 1 regulatory subunit 3B (PPP1R3B) has a variant associated with CT-diagnosed but not histologically diagnosed hepatic steatosis (rs4240624) and one predicting severe NAFLD on ultrasound (rs61756425). Tribbles pseudokinase 1 (TRIB1) was associated with increased ALT and NAFLD diagnosed histologically or by ultrasound.<sup>148</sup>

Other genetic factors were not identified by GWAS, but in other studies or meta-analyses increased risk of NAFLD was linked to a loss of function in the phosphatidylethanolamine N-methyltransferase (PEMT), nine ERLIN1-CHUK-CWF19L1 variants, a mitochondrial transport protein (MTTP) polymorphism, and a superoxide dismutase 2 (SOD2) variant. A polymorphism in the uncoupling protein 2 (UCP) was associated with reduced risk of NASH and higher hepatic protein levels.<sup>152</sup>

To improve the prognostic accuracy of genetic risk factors, they are often combined to polygenic risk scores (PRS). Di Costanzo *et al.*<sup>153</sup> developed a 4-SNP-PRS (TM6SF2, GCKR, PNPLA3, MBOAT7) with a high predictive value of NAFLD and found that PPP1R3B and MBOAT7 could have an impact on the severity of NAFLD. The same SNPs were combined with clinical fibrosis scores (NFS, Fib-4, aspartate aminotransferase-to-platelet ratio, BARD, and the Forns score) and improved the prediction of severe liver disease in subjects with metabolic risk factors.<sup>154</sup> An 11-SNP risk score (PNPLA3, HSD17B13, TM6SF2, GATAD2A, GCKR, SUGP1, SAMM50, ERLIN1-CHUK-CWF19L1, MBOAT7, TRIB1) was developed using data from multiple ethnic groups. The resulting GRS was significantly associated with NAFLD in several ethnic groups (Latinos, Japanese Americans, Native Hawaiians, Whites, African Americans) and had higher accuracy in patients with NAFLD cirrhosis. The impact of PPP1R3B and MBOAT7 on the severity of NAFLD was also found in that study.<sup>155</sup> Gao *et al.*<sup>156</sup> combined PNPLA3 and HSD17B13 with sex, MetS, HOMA-IR, and serum AST levels to predict NASH. The developed nomogram could be used in both groups with or without pre-diabetes or MetS. The identified SNPs and the PRS show that NAFLD is a polygenic condition whose risk is best assessed by combining different genetic variants, especially PNPLA3, TM6SF2, MBOAT7, GCKR, and HSD17B13, with metabolic and clinical factors. SNPs should be further considered as accurate markers of NAFLD and could be used in new liver fat scores.

## Conclusions

Existing liver fat scores can be used as biomarkers to capture the probable presence or absence of NAFLD at baseline. Given the limited reliability and precision, a confirmed diagnosis for individuals in clinical practice or clinical research as the basis for therapeutic decisions or study inclusion is not possible. Liver fat indices are not capable of replacing imaging as a more accurate method, and they cannot clearly quantify the grade of NAFLD. However, they may serve as useful tools for larger cohorts, such as in epidemiological studies where

imaging data is not available owing to high cost or the historic nature of certain cohorts. In those settings, FLI, NAFLD-LFS, and HSI are most common scores and are a practical, cheap and *post-hoc* available approach for risk stratification of metabolic, cardiovascular diseases, or liver malignancy. Their performance for the prediction of NAFLD is consistently replicated. Noninvasive indices for NASH may be even more warranted, as noninflammatory NAFLD has a rather low risk for long-term complications, while identifying the smaller portion of NASH patients among all NAFLD cases would improve their disease management.

The power of the indices as monitoring tools for interventions has rarely been validated. However, the existing longitudinal studies show that even the well-established indices have poor power in representing changes in liver fat in the context of lifestyle interventions, especially when interventional benefits are mainly independent of weight loss. That awaits development of new indices predicting liver fat changes irrespective of the performed diet. Ideally, these should be able to predict liver fat in both cross-sectional and longitudinal settings.

Moreover, it would be useful to have an index that would allow quantification of liver fat and thus help to categorize NAFLD into mild, moderate, and severe types. In addition to the parameters that have been used in the indices, other parameters may be considered as candidates for the diagnosis of NAFLD. While parameters such as adipokines, RBP-4, fetuin-A/B, progranulin are too expensive, and liver fibrosis variables are not considered for early diagnosis of NAFLD, parameters such as FPG, ferritin, ALP, cholinesterase, or uric acid could be other favorable predictors for the diagnosis of NAFLD and should be further investigated. Genetic variants and polygenic risk scores are potential tools to stratify the NAFLD risk and should also be considered for new liver fat scores.

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## Conflict of interest

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## Author contributions

Wrote the paper and are guarantors of this work (MR, SK), conceptualized the publication (SK), provided supervision, reviewed the manuscript and, acquired financial support by public funding (AFHP, JS).

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