Hindawi

Review Article

Advance of Serum Biomarkers and Combined Diagnostic Panels in Nonalcoholic Fatty Liver Disease

Yuping Zeng ,[1] He He ,[1] and Zhenmei An [2]

[1]*Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu, China*
[2]*Department of Endocrine and Metabolism, West China Hospital, Sichuan University, Chengdu, China*

Correspondence should be addressed to Zhenmei An; azmhxnfm@163.com

Nonalcoholic fatty liver disease (NAFLD) affects approximately 25-30% population worldwide, which progresses from simple steatosis to nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma, and has complications such as cardiovascular events. Liver biopsy is still the gold standard for the diagnosis of NAFLD, with some limitations, such as invasive, sampling deviation, and empirical judgment. Therefore, it is urgent to develop noninvasive diagnostic biomarkers. Currently, a large number of NAFLD-related serum biomarkers have been identified, including apoptosis, inflammation, fibrosis, adipokines, hepatokines, and omics biomarkers, which could effectively diagnose NASH and exclude patients with progressive fibrosis. We summarized serum biomarkers and combined diagnostic panels of NAFLD, to provide some guidance for the noninvasive diagnosis and further clinical studies.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) has recently been proposed to rename metabolic-associated fatty liver disease (MAFLD) [1, 2]. The diagnosis criteria have changed from "exclusive," that is, excluding liver steatosis caused by excessive alcohol intake, virus, drugs, etc., to "definitive," that is, the existence of evidence of liver steatosis (imaging, serum biomarkers, or histopathology) and the combination of overweight/obesity, type 2 diabetes (T2DM), or metabolic dysfunction [1, 2]. This renaming emphasizes the diagnostic value of metabolic disorders, such as obesity and diabetes, in NAFLD. However, related renaming is controversial, so this paper continued the traditional nomenclature to describe the disease.

With the improvement of lifestyle, the prevalence of obesity increased yearly [3], including related comorbidities, such as T2DM, hyperlipidemia, NAFLD, and metabolic syndrome (MS). Overweight patients account for about 1.5 million worldwide, and up to 90% of obese patients have a combination of NAFLD [4]. Research has reported that T2DM is one of the strongest risk factors for NAFLD [5].

NAFLD could progress from simple steatosis (SS) to nonalcoholic steatohepatitis (NASH) and even to fibrosis, cirrhosis, and end-stage hepatocellular carcinoma (HCC) [6, 7]. NAFLD increases twofold higher risks of metabolic syndrome/diabetes and is independently associated with cardiovascular events and extrahepatic complications [5, 8]. With the pandemic of obesity, the number of NASH-related death will increase by 178% by 2030 [9], becoming the most common cause of liver-related death in the future. Therefore, there is an imperative need for early screening, early diagnosis, and early treatment of NAFLD, to reduce the disease burden and socioeconomic pressure.

At present, the gold standard for the diagnosis of NAFLD is liver biopsy, which is an invasive operation that has the risks of bleeding, pain, and death, sampling deviation, poor representation, pathologist's empirical judgment, and poor patient compliance [10]. Clinically, the diagnosis of NAFLD mainly depends on ultrasound. However, ultrasound has a low sensitivity in obese patients and is not suitable for large-scale population screening [10]. Additionally, NAFLD could be reversed early on with lifestyle intervention or physical activity. Nevertheless, the current difficulties in

the early diagnosis of NAFLD limit the implementation of treatment plans and miss the best time for treatment. Therefore, it is crucial to investigate early noninvasive diagnostic biomarkers and monitor the disease progression of NAFLD. This review summarized the advance of serum biomarkers and combined diagnostic panels in the diagnosis and staging of NAFLD, to provide some guidance for the noninvasive diagnosis and further clinical studies.

2. Apoptosis Biomarkers

The most studied NAFLD-related serum biomarker is the hepatocyte apoptosis product, cytokeratin-18 (CK-18) [11, 12], accounting for about 5% of liver proteins [13]. The sensitivity (SEN) of CK-18 M30 and CK-18 M65 for the diagnosis of NASH was 70% and 63.6%, the specificity (SPE) was 83.7% and 89.4%, and the area under the curve (AUC) was 0.71 and 0.81, respectively, indicating an excellent diagnostic value of CK-18 in NAFLD [14]. However, for patients with progressive fibrosis, the SEN of CK-18 was only 54%, with an SPE of 85% and an AUC of 0.68 [15], suggesting insufficient SEN of CK-18 for monitoring the fibrosis progression; therefore, CK-18 should be utilized in combination with other biomarkers (Table 1).

Chuah et al. [16] found that the combination of CK-18, aspartate aminotransferase (AST), and homeostasis model assessment (HOMA) (MACK-3) significantly improved the accuracy of the diagnosis of NASH. The SEN was 84.2%, the SPE was 81.4%, and the AUC was 0.81. Gao et al. [17] combined MACK-3, MS, and platelets (PLT) to establish a nomogram, which improved the diagnostic efficacy in patients with progressive fibrosis, with an NPV of 94.7%. The combination of CK-18, ALT, and MS had an AUC of 0.88 for the diagnosis of NASH in obese subjects [18]. Thus, these studies suggested that CK-18 could be an effective diagnostic biomarker for NASH, especially in combination with other biomarkers. These combined diagnostic panels could monitor the disease progression of NAFLD.

3. Inflammatory Biomarkers

Excessive accumulation of triglycerides (TG) in hepatocytes could further develop into ballooning, inflammation, and fibrosis. TG content [29] did not correlate with the severity of NAFLD, while the precursors or intermediates such as palmitate, diacylglycerol, and ceramide could cause mitochondrial dysfunction as well endoplasmic reticulum stress, resulting in hepatocyte damage and release of proinflammatory cytokines. Therefore, inflammatory factors are possible diagnostic biomarkers for patients with NASH. Currently, alanine aminotransferase (ALT) and AST are used clinically as biomarkers of inflammatory damage in hepatocytes. For NAFLD patients undergoing bariatric surgery, ALT and AST could be used to monitor disease progression and assess the clinical benefits of treatment strategies [30]. However, NAFLD patients could also exhibit normal ALT levels and even decrease in patients with progressive fibrosis [31], suggesting that ALT alone cannot be relied upon to determine the severity of NAFLD. Several studies have proposed that

combined diagnostic panels using routine indicators could effectively diagnose NAFLD [32, 33] (Table 2). All of these models have high diagnostic accuracy and are routinely available clinically, but the specific cut-offs need to be further optimized.

Golgi protein 73 (GP73) is mainly expressed in bile duct epithelial cells but hardly expressed in hepatocytes [34]. Serum GP73 levels [34] were elevated in NASH patients and increased with the severity of inflammation, with an AUC of 0.89 for the diagnosis of NASH. Kar et al. [35] found that interleukin-6 (IL-6) and vascular cell adhesion molecule 1 (VCAM-1) could effectively differentiate the severity of NASH, with AUCs of 0.83 and 0.87. IL-8 levels were increased in obese patients with NASH and could be used as a potential diagnostic biomarker [36]. The chemokine C-X-C motif chemokine 10 (CXCL10) was elevated in NASH patients, and the AUC for the diagnosis of SS was 0.81, for NASH was 0.77 [37]. In summary, IL-6, VCAM-1, IL-8, and CXCL10 are potentially inflammatory diagnostic biomarkers for NASH, but these biomarkers are not disease-specific and may be disturbed by systemic inflammation, so a comprehensive clinical judgment is needed. Besides, serum GP73 might become a more specific inflammatory biomarker for NASH, but the diagnostic value needs to be externally validated in multicenter studies.

4. Fibrosis Biomarkers

NASH-related fibrosis could progress into hepatic decompensation and end-stage HCC, significantly increasing the risks of liver-related mortality and extrahepatic complications [45]. Therefore, it is necessary to screen for the risk of advanced fibrosis in NAFLD patients before bariatric surgery [46]. PLT are fundamental molecules in the development of fibrosis, and antiplatelet therapy reduced the incidence and mortality of NASH [47], indicating that PLT could be a diagnostic and therapeutic target for NASH. Numerous studies have constructed combined diagnostic models including PLT with high SPE in the diagnostic of progressive fibrosis (Table 3) [10, 32, 45]. Siddiqui et al. [48] evaluated the diagnostic efficacy of the NAFLD fibrosis score (NFS), fibrosis-4 (FIB-4), and AST/platelet ratio index (APRI) for NASH-related fibrosis with C-statistics of 0.80, 0.78, and 0.76 and NPV of 93%, 91%, and 91%, respectively. Udelsman et al. [49] revealed that NFS had better performance in excluding advanced fibrosis with an NPV of 99% in obese individuals. Studies have also reported the best diagnostic performance of the Hepamet fibrosis score (HFS) in advanced fibrosis among NAFLD patients [50]. However, Higuera-de-la-Tijera et al. concluded that HFS had a low positive predictive value of 36.7% in the Mexican population [51]. Importantly, NAFLD/NASH is a pandemic prothrombotic condition strongly associated with CVD risks [50, 52, 53]. Therefore, these noninvasive diagnostic panels of NAFLD severity could also assess the hepatological and cardiometabolic risks in NAFLD patients [50]. Taken together, combined diagnostic panels could be invoked as exclusion indicators for advanced fibrosis, but large-scale validation is still needed [54].

TABLE 1: Combined diagnostic panels of apoptosis biomarkers.

Models	Variables	AUC	Diagnostic efficacy	Ref
MACK-3	CK-18 M30, AST, HOMA	NASH: 0.81; fibrosis: 0.80	NASH: cut-off: ≤0.167 and ≥0.551, SEN: 84.2%, SPE: 81.4%; fibrosis: cut-off: ≤0.134, SEN: 100%, SPE: 43.8%	[16]
Nomogram	MACK-3, MS, PLT	Fibrosis: 0.79	Fibrosis: cut-off: ≤137 and ≥180, NPV: 94.7%, SPE: 93.2%	[17]
G-NASH	CK-18 M30, GP73	NASH: 0.85	NASH: accuracy: 62%, non-NASH: accuracy: 100%	[19]
Nice model	CK-18, ALT, MS	NASH: 0.88	NASH: cut-off: 0.14, SEN: 84%, SPE: 86%	[18]
—	CK-18 M30, ALT, PLT, TG	NASH: 0.92	NASH: cut-off: 0.361, SEN: 89%, SPE: 86%	[20]
FIC-22	CK-18 M30, FIB-4	NASH: 0.82; fibrosis: 0.78	NASH: cut-off: 1, SEN: 89.1%, SPE: 62.5%; fibrosis: cut-off: 1, SEN: 87.4%, SPE: 56.1%	[21]
FICK-3	CK-18 M30, FIB-4, HOMA	NAFLD 0.84; fibrosis: 0.95	—	[22]
NASH diagnostic™	CK-18 M30, adiponectin, resistin	NASH: 0.91	NASH: cut-off: 0.2272, SEN: 95.45%, SPE: 70.21%	[14]
—	CK-18 M65, IL-6, adiponectin	NASH: 0.90	NASH: SEN: 84.5%, SPE: 85.7%	[23]
—	CK-18 M30, FGF-21	NASH: 0.94	NASH: SEN: 92%, SPE: 85%	[24]
CHeK	CK-18 M30, GGT, age, HbA1c, adiponectin	NASH: 0.73	—	[25]
—	CK-18 M30, sFas	NASH: 0.93	NASH: cut-off: -0.5509, SEN: 88%, SPE: 89%	[26]
	sFasL, chemokine 2, race	NASH: 0.71; fibrosis: 0.75	NASH: cut-off: OR = 1.56, SEN: 63.64%, SPE: 86.67%; fibrosis: cut-off: OR = 6.29, SEN: 59.38%, SPE: 80.00%	[27]
—	CK-18 M30, cathepsin D	NASH: 0.998	—	[28]

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; AUC: area under the curve; SEN: sensitivity; SPE: specificity; CK-18: cytokeratin-18; AST: aspartate aminotransferase; HOMA: homeostasis model assessment; MS: metabolic syndrome; PLT: platelet; GP73: golgi protein 73; ALT: alanine aminotransferase; TG: triglyceride; FIB-4: fibrosis-4; IL-6: interleukin-6; FGF-21: fibroblast growth factor-21; GGT: γ-glutamyl transpeptidase; HbA1c: glycosylated hemoglobin.

TABLE 2: Combined diagnostic models of routine indicators.

Models	Variables	AUC	Diagnostic efficacy	Ref
FLI	BMI, waist, TG, GGT	NAFLD: 0.84	NASH: cut-off: <30 and ≥60, SEN: 87%, SPE: 86%	[38]
HIS	ALT/AST, BMI, gender, diabetes	NAFLD: 0.81	NAFLD: cut-off: <30 and >36, SEN: 92.5%, SPE: 92.4%	[39]
NSS	Age, BMI, TG, FPG, ALT/AST, UA	Men: 0.83; women: 0.86	Men: cut-off: 33, SEN: 79.86%, SPE: 66.13%; women: cut-off: 29, SEN: 89.39%, SPE: 68.98%	[40]
ION	WHR, TG, ALT, HOMA	NAFLD: 0.77	NAFLD: cut-off: <11, SEN: 81%, SPE: 56%; cut-off: ≥22, SEN: 60%, SPE: 82%	[41]
NAFLD liver fat score	MS, diabetes, insulin, AST, AST/ALT	NAFLD: 0.87	NAFLD: cut-off: -0.640, SEN: 86%, SPE: 71%	[42]
HAIR score	HBP, ALT, insulin, GLU	NASH: 0.68	NASH: cut-off: 3, SEN: 57%, SPE: 77%	[43]
SteatoTest-2	A2M, ApoA1, HP, GGT, TC, GLU, ALT, AST, age, gender	SS: 0.68	SS: SEN: 95.5%, PPV: 97.0%	[44]
NASHTest-2	A2M, ApoA1, HP, TBIL, GGT, TC, TG	NASH: 0.59	NASH: SEN: 83.3%, SPE: 37.5%	[44]

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; SS: simple steatosis; AUC: area under the curve; SEN: sensitivity; SPE: specificity; BMI: body mass index; TG: triglycerides; GGT: γ-glutamyl transpeptidase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; FPG: fasting glucose; UA: uric acid; WHR: waist-hip ratio; HOMA: homeostasis model assessment; MS: metabolic syndrome; HBP: high blood pressure; GLU: glucose; A2M: alpha-2 macroglobulin; APOA1: apolipoprotein A1; HP: haptoglobin; TC: total cholesterol; TBIL: total bilirubin.

Alpha-2 macroglobulin (A2M), hyaluronic acid (HA), and tissue inhibitor of metalloproteinase-1 (TIMP1) are specific biomarkers of fibrosis. The AUCs for the diagnosis of NASH-related fibrosis were 0.77, 0.81, and 0.78, respectively [55]. Combining these three indicators, the AUC was 0.87 [55]. Furtherly combining HA, procollagen III N-terminal peptide (PIIINP), and TIMP-1 (ELF model), the AUC could reach 0.95, with an SEN of 87% and SPE of 93%, indicating the high clinical application value in the diagnosis of fibrosis, but the model was not sensitive for early fibrosis [56]. Type IV collagen is differentially expressed in patients with NASH and fibrosis, suggesting that it could be a possible biomarker to distinguish NASH from early fibrosis [57]. Type III procollagen (PRO-C3) also showed high expression in fibrosis

TABLE 3: Combined diagnostic panels of fibrosis biomarkers.

Models	Variables	AUC	Diagnostic efficacy	Ref
FibroTest	A2M, APOA1, HP, TBIL, GGT	Advanced fibrosis: 0.79	—	[44]
NFS	Age, BMI, diabetes, AST/ALT, PLT, ALB	Advanced fibrosis: 0.88	Advanced fibrosis: cut-off: <-1.455, SEN: 82%, SPE: 77%; cut-off: >0.676, SEN: 51%, SPE: 98%	[61]
FIB-4	Age, AST, PLT, ALT	Advanced fibrosis: 0.78	Advanced fibrosis: cut-off: <1.3, SEN: 82%, SPE: 57%; cut-off: ≥2.67, SEN: 36%, SPE: 93%	[62]
HFS	Sex, age, diabetes, GLU, INS, HOMA, AST, ALB, PLT	Advanced fibrosis: 0.85	Advanced fibrosis: cut-off: <0.12, SEN: 70.7%, SPE: 80.9%; cut-off: ≥0.47, SEN: 38%, SPE: 98%	[63]
APRI	AST/PLT	Moderate fibrosis: 0.73; advanced fibrosis: 0.76	Moderate fibrosis: cut-off: 0.84, SEN: 65%, SPE: 71%; advanced fibrosis: cut-off: 0.84, SEN: 75%, SPE: 65%	[48]
ARR	AST/ALT	Moderate fibrosis: 0.65; advanced fibrosis: 0.68	Moderate fibrosis: cut-off: 0.81, SEN: 54%, SPE: 68%; advanced fibrosis: cut-off: 0.85, SEN: 54%, SPE: 73%	[48]
BARD score	BMI ≥ 28 kg/m^2, AST/ALT≥0.8, diabetes	Advanced fibrosis: 0.81	Advanced fibrosis: PPV: 43%, NPV: 96%	[64]
FibroMeter NAFLD	Age, weight, GLU, ALT, AST, PLT, ferritin	Significant fibrosis: 0.94	Significant fibrosis: SEN: 78.5%, SPE: 95.9%	[65]
BAAT score	Age, BMI, ALT, TG	Mild fibrosis: 0.68; advanced fibrosis: 0.62	Mild fibrosis: cut-off: 2.00, SEN: 90.4%, SPE: 35%; advanced fibrosis: cut-off: 2.00, SEN: 94.9%, SPE: 23.8%	[66]
AP index	Age, PLT	Advanced fibrosis: 0.88	—	[67]
CDS	PLT, AST/ALT, INR	Advanced fibrosis: 0.95	Advanced fibrosis: cut-off: 5.00, SEN: 89%, SPE: 90%	[67]
HALT-C model	PLT, AST/ALT, INR	Advanced fibrosis: 0.91	Advanced fibrosis: cut-off: 0.7-0.8, SEN: 89%, SPE: 83%	[67]
Hepascore	Age, gender, TBIL, GGT, A2M, HA	Moderate fibrosis: 0.73; advanced fibrosis: 0.81	Moderate fibrosis: cut-off: 0.44, SEN: 50.5%, SPE: 88.3%; advanced fibrosis: cut-off: 0.37, SEN: 75.5%, SPE: 84.1%	[68]
—	A2M, HA, TIMP1	Advanced fibrosis: 0.87	Advanced fibrosis: cut-off: 17, SEN: 84.8%, SPE: 72.3%	[55]
ELF	HA, PIIINP, TIMP-1	Advanced fibrosis: 0.95	Advanced fibrosis: cut-off: 9.8, SEN: 86.7%, SPE: 92.5%	[56]
FibroMeter^{V2G}	AST, urea, PLT, PT, HA, A2M	Advanced fibrosis: 0.80	Advanced fibrosis: cut-off: ≥0.434, SEN: 68.3%, SPE: 75.6%	[69]
CA index	Type IV collagen, AST	NASH: 0.86; fibrosis: 0.92	—	[70]
FM-fibro index	VCAM1, HA	Moderate fibrosis: 0.85; advanced fibrosis: 0.92	—	[70]
FM-fibro index	VCAM1, type IV collagen	Moderate fibrosis: 0.86; advanced fibrosis: 0.92	—	[70]
FM-fibro index	Type IV collagen, HA	Moderate fibrosis: 0.86; advanced fibrosis: 0.91	—	[70]
ADAPT	Age, diabetes, PRO-C3, PLT	Advanced fibrosis: 0.86	Advanced fibrosis: cut-off: >6.3287, PPV: 48.4%, NPV: 96.6%	[59]
FIB-C3	Age, BMI, diabetes, PLT, PRO-C3	Advanced fibrosis: 0.89	Advanced fibrosis: cut-off: >-0.4, SEN: 83%, SPE: 80%	[71]
ABC3D	Age, BMI, diabetes, PLT, PRO-C3	Advanced fibrosis: 0.88	Advanced fibrosis: cut-off: >3, SEN: 77%, SPE: 82%	[71]

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; AUC: area under the curve; SEN: sensitivity; SPE: specificity; A2M: alpha-2 macroglobulin; APOA1: apolipoprotein A1; HP: haptoglobin; TBIL: total bilirubin; GGT: γ-glutamyl transpeptidase; BMI: body mass index; ALT: alanine aminotransferase; AST: aspartate aminotransferase; PLT: platelet; ALB: albumin; GLU: glucose; TG: triglyceride; INR: international normalized ratio; HA: hyaluronic acid; TIMP1: tissue inhibitor of metalloproteinases-1; PIIINP: procollagen III N-terminal peptide; PT: prothrombin time; VCAM1: vascular cell adhesion molecule 1; PRO-C3: procollagen type III.

patients and increased with the degree of fibrosis [58]. Daniels et al. [59] constructed ADAPT score by incorporating age, diabetes, PRO-C3, and PLT, and the AUC for diagnosing progressive fibrosis was 0.86 and was better than the conventional APRI, FIB-4, and NFS scores. Kanno et al. [60] found that aldo-keto reductase family 1 member B10

(AKR1B10) was highly positively correlated with the fibrosis stage and thus could be a possible diagnostic and prognostic biomarker. In conclusion, A2M, HA, TIMP1, PIIINP, type IV collagen, PRO-C3, and AKR1B10 are biomarkers that directly respond to fibrosis in NASH, but these biomarkers remain to be investigated in patients with other chronic liver

diseases, and further validation is needed for their extension in the clinical setting.

5. Adipokines and Hepatokines

Adipokines and hepatokines [72] are involved in the dialogue between the liver and adipocytes, including adiponectin, visfatin, resistin, adipocyte fatty acid-binding protein (AFABP), angiopoietin-like proteins-1,2,3,4,6,8 (ANGPTL), fetuin-A,B, FGF-1,2,19,21, heparin, and retinol-binding protein (RBP-4) [45]. The expression levels of resistin, visfatin, and RBP-4 did not differ between NASH and SS patients after correction of BMI or waist; therefore, the diagnostic value of these biomarkers alone for NAFLD needs to be further explored [73]. A combined diagnostic panel combining adiponectin, visfatin, TNF-α, and IL-6 could effectively differentiate NASH and SS with a SEN of 90% and SPE of 66%[74], suggesting the possible usage as an early screening model for NASH. Fibroblast growth factor-21 (FGF-21) was reported to be increased in obese adolescents and independently correlated with NAFLD [75]. Overall, adipokines and hepatokines were potential diagnostic biomarkers of NAFLD, but the diagnostic accuracy is not yet high enough, and these biomarkers are not yet widely used in clinical practice.

6. Omics Biomarkers

6.1. Genomics. PNPLA3 rs738409 and TM6SF2 rs58542926 are the most reported NAFLD-related single nucleotide polymorphisms (SNPs) [76]. PNPLA3 and TM6SF2 significantly increased the risk of inflammation and fibrosis progression in NAFLD, even after the correction of insulin resistance [77]. Therefore, they could be used as valid diagnostic biomarkers for NASH. Research also reported that polygenetic risk scores combining PNPLA3, GCKR, and TM6SF2 were significantly associated with an increased risk of NAFLD in obese patients [78]. Hyysalo et al. [79] combined PNPLA3, AST, and insulin to establish the "NASH score" model, and the AUC for the diagnosis of NASH was 0.77. Koo et al. [80] developed the "NASH PT score" with an AUC of 0.86 for the diagnosis of NASH. Therefore, combining genetic information and routine indicators could effectively predict NASH. Other reported NASH-related SNPs included DYSF, MBOAT7, LYPLAL1, PPP1R3B, HSD17B13, PYGO1, and GATAD2A [76, 81], and "NAFLD liver fat score," "NASH ClinLipMet score," and "HCC risk score" [82].

6.2. Epigenomics. DNA methylation affects the gene expression levels and is associated with the heterogeneity of NAFLD [83]. Research showed that the methylation levels of peroxisome proliferator-activated receptor γ (PPARγ) in plasma-free DNA could differentiate the severity of NAFLD and was a potential noninvasive biomarker for NAFLD [84]. It has further been reported that the methylation status of 22 CpG correlated with the degree of steatosis [85]. Hyun et al. [86] detailed and summarized the association of methylation status of genes such as SLC7A11, ACSL4, and CPT1C in

peripheral blood with NASH. All in all, these studies suggested that combined diagnostic panels might assist clinicians in diagnosing NASH and predicting disease progression, but further external validation is required.

6.3. Transcriptomics. MicroRNA (miRNA) mainly regulates downstream gene expression at the posttranscriptional level. miRNA-122 and miRNA-34a [87] were the most studied in NASH. The AUC of miRNA-122 for the diagnosis of NAFLD was 0.82 and the AUC of miRNA-34a for the diagnosis of NASH was 0.78. Therefore, miRNA-122 and miRNA-34a are reliable diagnostic biomarkers for NASH, but related studies are still in the preliminary stage. The diagnostic value of other miRNAs including miRNA-33, miRNA-192, miRNA-21, miRNA-375, miRNA-221, and miRNA-222 needs to be validated in larger cohorts [88].

Long noncoding RNA (lncRNA) regulates gene expression mainly through chromatin modification, activation/ repression of transcriptional enhancers, and targeting miR-NAs (ceRNAs) [89]. A study reported that LeXis has an AUC of 0.74, an SEN of 54.3%, and an SPE of 100% for the diagnosis of NASH [89]. Microarray analysis [90] identified lncPRYP4-3 as a potential diagnostic biomarker for NAFLD and revealed its interaction with PRS4Y2. Di Mauro et al. [91] found that the AUC of combined TGFB2/TGFB2-OT1 and FIB-4 was 0.89 for the diagnosis of NASH-related fibrosis. Another study showed that XLOC_014172 and LOC149086 were elevated expressed in patients with HCC and therefore might be used as biomarkers of HCC progression in NASH [92]. Other lncRNAs including FLRL6, FLRL2, lncSTR, lncARS, lnc18q22.2, MEG3, and PVT1 might be potential diagnostic biomarkers for NAFLD [93–95].

CircroRNA- (circRNA-) related studies have focused on its competition with miRNAs to bind target genes and thus regulate downstream gene expression. Overexpression of circRNA_002581 [96] significantly attenuated the inhibitory effect of miR-122 on CPEB1, which is involved in the pathogenesis of NASH through the CPEB1-PTEN-AMPK-mTOR signaling pathway. circRNA_0046367 suppressed the inhibitory effect of miR-34a on PPARα, thereby promoting the expression of downstream lipid metabolism-related genes [97]. circRNA_0046366 expression levels were reduced in NASH patients and highly correlated with oxidative stress, lipotoxicity, and NAFLD disease severity [98], which could be a potential diagnostic biomarker and therapeutic target for NASH. Other circRNAs including circScd1 and circRNA_0071410 were also engaged in the process of lipoatrophy and fibrosis in NAFLD [95].

6.4. Proteomics. Yu C et al. [99] identified six biomarkers to establish a combined diagnostic model with 89% SEN and 83% SPE for the diagnosis of NAFLD, and further prospective cohorts found that patients with high levels of hemoglobin were more likely to develop NAFLD, making hemoglobin a potential diagnostic and predictive biomarker for NAFLD. Another study [100] found that ALDOB, APOM, LGALS3BP, PIGR, VTN, and AFM were significantly differentially expressed in NAFLD and control

TABLE 4: Combined diagnostic models of omics biomarkers.

Models	Variables	AUC	Diagnostic efficacy	Ref
NASH score	PNPLA3, insulin, AST	NASH: 0.77	NASH: cut-off: >-1.054, SEN: 75%, SPE: 74%	[79]
NASH PT score	PNPLA3, TM6SF2, diabetes, AST, HOMA-IR, hsCRP	NASH: 0.86	NASH: cut-off: >-0.785, SEN: 91.0%, SPE: 58.1%	[80]
—	PNPLA3, GCKR, GATAD2A	NASH: 0.65	—	[76]
Extended FLI	PNPLA3, BMI, waist, TG, GGT, 2-hour GLU, 2-hour TG/TG	NAFLD: 0.86	NAFLD: cut-off: ≥60, SEN: 48.6%, SPE: 91.93%	[116]
CI+SNP	Weight, waist, BMI, AST/ALT, TG, FPG, APOC3	NAFLD: 0.90	NAFLD: cut-off: >0.2253, SEN: 86.21%, SPE: 82.23%	[117]
NIS4	miRNA-34a, A2M, YKL-40, HbA1c	NASH: 0.80	NASH: cut-off: <0.36, SEN: 80.8%, SPE: 65.2%; cut-off: ≥0.63, SEN: 45.2%, SPE: 90.4%	[118]
—	miRNA-122, miRNA-192, miRNA-21, CK-18	NASH: 0.83	NASH: cut-off: >0.2253, SEN: 86.21%, SPE: 82.23%	[119]
—	miRNA-122, miRNA-1290, miRNA-192, miRNA-27b	NAFLD: 0.86	NAFLD: SEN: 85.55%, SPE: 73.3%	[120]
—	TGFB2/TGFB2-OT1, FIB-4	Advanced fibrosis: 0.89	Advanced fibrosis: SEN: 80%, SPE: 87.5%	[91]
—	TGFB2/TGFB2-OT1, LSM	Advanced fibrosis: 0.89	Advanced fibrosis: SEN: 80%, SPE: 90.6%	[91]
—	m/z: 2760, 2957, 2967, 5814, 6306, 15, 124 Da	—	SEN: 89%, SPE: 83%	[99]
—	N184_A3G3F1S3+AFP/ N241_A3G3F1S3+AFP	HCC: 0.84	HCC: cut-off: 2.25, SEN: 70%, SPE: 83%; cut-off: 2.75, SEN: 70%, SPE: 87%	[121]
GlycoNASHTest	Log(NGA2F/NA2)	NASH: 0.74; advanced fibrosis: 0.87	Advanced fibrosis: SEN: 89.5%, SPE: 71.4%	[122]
—	11 TGs	SS: 0.90	SS: SEN: 98%, SPE: 78%	[104]
—	29 TGs	NASH: 0.96	NASH: SEN: 91%, SPE: 95%	[123]
—	TAG 46:1, TAG 48:1, TAG 50:1, SM d32:0, SM d38:0	Nonobesity NAFLD: 0.92; NASH: 0.81	—	[124]
—	DAG 34:1, DAG 40:7, DAG 40:8, TAG 46:1, TAG 48:1, TAG 50:2, SM d36:0	Obesity NAFLD: 0.97; NASH: 0.81	—	[124]
—	5-HETE, 7,17-DHDPA, adrenal acid, arachidonic acid, eicosapentaenoic acid, 16-HDOHE, 9-HODE	Mild fibrosis: 0.74	—	[125]
—	BMI, age, gender, ALT, TAG	NASH: 0.83	—	[126]
—	EV CD14+, HA, PIIINP, TIMP-1	Advanced fibrosis: 0.95	Advanced fibrosis: cut-off: -0.8687, SEN: 88.9%, SPE: 94.1%	[113]
—	EV CD16+, HA, PIIINP, TIMP-1	Advanced fibrosis: 0.97	Advanced fibrosis: cut-off: -0.3435, SEN: 88.9%, SPE: 88.2%	[113]
—	EV UB2L3, EV Fas	NASH: 0.77	NASH: SEN: 75%, SPE: 83%	[127]

NAFLD: nonalcoholic fatty liver disease; NASH: nonalcoholic steatohepatitis; AUC: area under the curve; SEN: sensitivity; SPE: specificity; AST: aspartate aminotransferase; HOMR: homeostasis model assessment; hsCRP: high-sensitivity C-reactive protein; TG: triglycerides; GGT: γ-glutamyl transpeptidase; GLU: glucose; BMI: body mass index; ALT alanine aminotransferase. FPG: fasting glucose; A2M: alpha-2 macroglobulin; HbA1c: glycated hemoglobin; TAG: triacylglycerol; DAG: diacylglycerol; DM: sphingomyelin; CK-18: cytokeratin-18; AFP: alpha-fetoprotein; HA: hyaluronic acid; TIMP1: tissue inhibitor of metalloproteinases-1; PIIINP: procollagen III N-terminal peptide; EV: extracellular vesicle.

patients by plasma protein profiling, but the clinical application needs to be further investigated. Younossi et al. [101] revealed that A2M and coagulation factor V were highly correlated with NASH-related fibrosis, suggesting that they were potential biomarkers of fibrosis progression.

6.5. Metabonomics and Lipidomics. Serum metabolomics revealed that pyroglutamic acid was effective in differentiating patients with NASH and SS with a SEN of 72%, SPE of 85%, and AUC of 0.82 [102]. A study found monounsaturated TG as a specific biomarker for NASH [103]. The AUC for the combination of 11 TGs to distinguish between healthy controls and SS was 0.90, and the combination of 22 TGs to distinguish between SS and NASH was 0.95, suggesting that lipids have a high application value in the diagnosis of NAFLD [104]. Further validating the serum lipid profiles [105], the C16:1n7/C16:0 had an AUC of 0.71 for the diagnosis of NASH and 0.69 for fibrosis patients. A study also
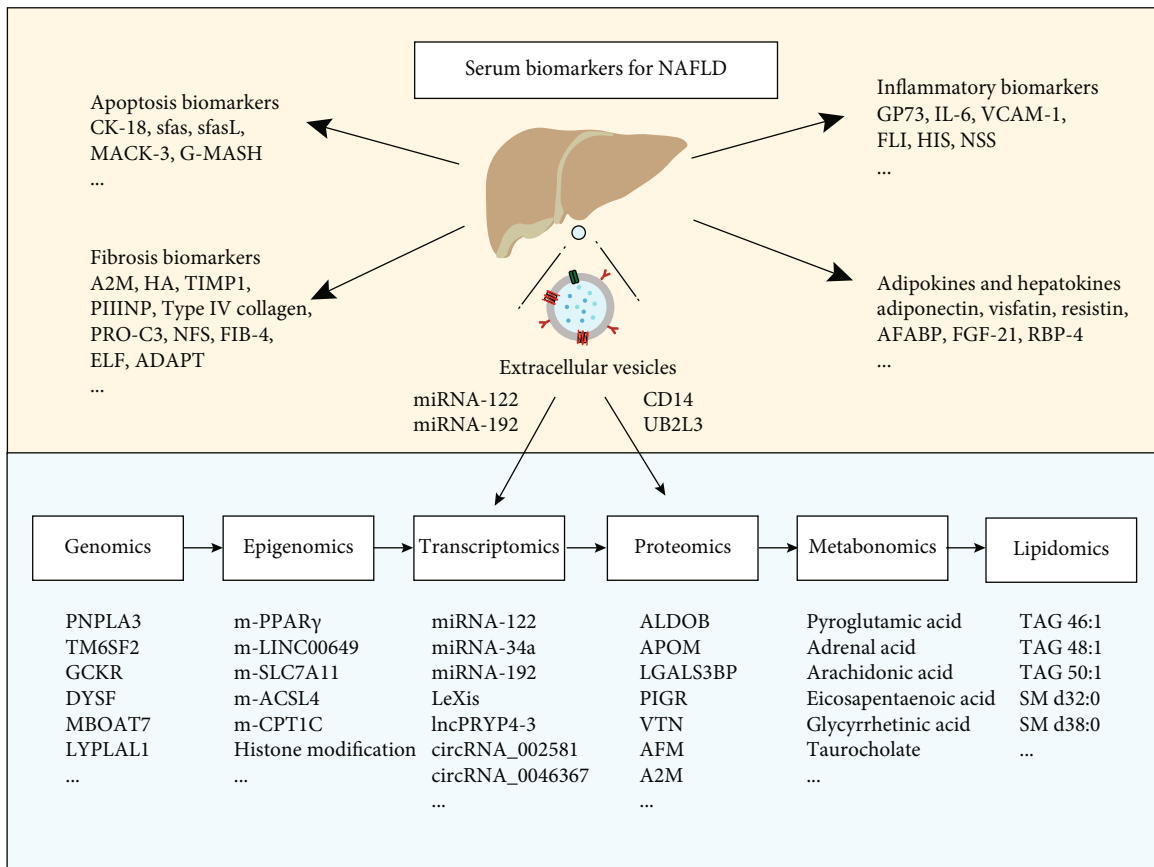
Figure 1: Serum diagnostic biomarkers for NAFLD.

found that serum phosphatidylcholine and sphingomyelin levels were markedly increased in SS and NASH patients [106]. Beyoglu and Idle [107] summarized in detail the metabolomic and lipidomic biomarkers associated with NAFLD, including fatty acids, 5-HETE, 8-HETE, 15-HETE, glycyrrhetinic acid, and taurocholate.

Taken together, omics have identified many new NAFLD-related serum biomarkers. It is critical to the early screen obese patients for the risks of NASH and fibrosis progression by these biomarkers (Table 4), while the specific mechanisms and diagnostic value need to be further explored [108]. Due to methodological limitations and reproducibility of results, the identification of omics biomarkers has not yet been widely used in clinical practice.

7. Extracellular Vesicles

Extracellular vesicles (EV) are membrane vesicles released by cells, including exosomes, microvesicles, and apoptotic vesicles, which carry a variety of genetic information such as mRNA, noncoding RNA, lipids, and proteins, and are involved in intercellular signaling transduction [109]. EV remains relatively stable in plasma and is, therefore, a reliable diagnostic biomarker for NASH [110]. Lipid-induced damage to hepatocytes significantly increased the release of exosomes [109], which carried TNF-related ligands that interacted with macrophages and induced inflammatory responses. Besides, exosomes derived from hepatocytes could interact with hepatic stellate cells, thus participating in the pathogenesis of progressive fibrosis [109]. Exosomal miRNA-122 is significantly elevated in the NASH model and involved in the macrophage-induced inflammatory response and is, therefore, a reliable diagnostic biomarker for NASH [111, 112]. EV CD14$^+$ and EV CD16$^+$ significantly increased the accuracy of the ELF model to diagnose severe fibrosis with AUCs of 0.95 and 0.97, respectively [113]. Ban et al. [114] summarized NAFLD-related exosome biomarkers, including CD4, CD8, CD14, CD15, TER119, CD41, CD62P, miRNA-122, and miRNA-192. Shabangu et al. [115] also concluded that cytochrome P450 2E1, toll-like receptor-9, homocysteine, and PPARγ in EV could be used as potential diagnostic biomarkers for NAFLD.

8. Conclusions

In conclusion, a large number of NAFLD-related serum biomarkers and combination diagnostic panels have been reported (Figure 1). CK-18 is the most studied NASH-related biomarker with high diagnostic efficacy, especially in combination with other biomarkers. The combined diagnostic models of fibrosis such as NFS, FIB-4, and ELF could effectively exclude patients with fibrosis progression, so they could be used as routine screening indicators in clinical practice. Omic biomarkers such as PNPLA3, TM6SF2,

miRNA-122, miRNA-34a, and EVs could effectively diagnose patients with NASH, but the practical application still needs further validation. There are no valid and reliable serum biomarkers to differentiate SS and NASH; therefore, it is urgent to explore noninvasive, highly sensitive, highly specific, and clinically accessible biomarkers to identify the severity of NAFLD. Especially in obese patients, it is important to early screen for inflammatory and fibrotic progression of NAFLD and monitor the outcome of bariatric surgery.

Data Availability

No data were used to support this study.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

References

[1] M. Eslam, P. N. Newsome, S. K. Sarin et al., "A new definition for metabolic dysfunction-associated fatty liver disease: an international expert consensus statement," *Journal Of Hepatology*, vol. 73, no. 1, pp. 202–209, 2020.

[2] M. Eslam, A. J. Sanyal, J. George et al., "MAFLD: a consensus-driven proposed nomenclature for metabolic associated fatty liver disease," *Gastroenterology*, vol. 158, no. 7, pp. 1999–2014, 2020.

[3] M. Blüher, "Obesity: global epidemiology and pathogenesis," *Nature Reviews Endocrinology*, vol. 15, no. 5, pp. 288–298, 2019.

[4] S. A. Polyzos, J. Kountouras, and C. S. Mantzoros, "Obesity and nonalcoholic fatty liver disease: from pathophysiology to therapeutics," *Metabolism*, vol. 92, pp. 82–97, 2019.

[5] G. Targher, K. E. Corey, C. D. Byrne, and M. Roden, "The complex link between NAFLD and type 2 diabetes mellitus - mechanisms and treatments," *Nature Reviews Gastroenterology & Hepatology*, vol. 18, no. 9, pp. 599–612, 2021.

[6] J. Zhou, F. Zhou, W. Wang et al., "Epidemiological features of NAFLD from 1999 to 2018 in China," *Hepatology*, vol. 71, no. 5, pp. 1851–1864, 2020.

[7] C. D. Byrne and G. Targher, "NAFLD as a driver of chronic kidney disease," *Journal of Hepatology*, vol. 72, no. 4, pp. 785–801, 2020.

[8] G. Targher, C. D. Byrne, and H. Tilg, "NAFLD and increased risk of cardiovascular disease: clinical associations, pathophysiological mechanisms and pharmacological implications," *Gut*, vol. 69, no. 9, pp. 1691–1705, 2020.

[9] Z. Younossi, F. Tacke, M. Arrese et al., "Global perspectives on nonalcoholic fatty liver disease and nonalcoholic steatohepatitis," *Hepatology*, vol. 69, no. 6, pp. 2672–2682, 2019.

[10] J. H. Zhou, J. J. Cai, Z. G. She, and H. L. Li, "Noninvasive evaluation of nonalcoholic fatty liver disease: current evidence and practice," *World Journal of Gastroenterology*, vol. 25, no. 11, pp. 1307–1326, 2019.

[11] A. E. Feldstein, A. Wieckowska, A. R. Lopez, Y.-C. Liu, N. N. Zein, and A. J. McCullough, "Cytokeratin-18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study," *Hepatology*, vol. 50, no. 4, pp. 1072–1078, 2009.

[12] A. Wieckowska, N. N. Zein, L. M. Yerian, A. R. Lopez, A. J. McCullough, and A. E. Feldstein, "In vivo assessment of liver cell apoptosis as a novel biomarker of disease severity in non-alcoholic fatty liver disease," *Hepatology*, vol. 44, no. 1, pp. 27–33, 2006.

[13] M. Kawanaka, K. Nishino, J. Nakamura et al., "Correlation between serum cytokeratin-18 and the progression or regression of non-alcoholic fatty liver disease," *Annals of Hepatology*, vol. 14, no. 6, pp. 837–844, 2015.

[14] Z. M. Younossi, M. Jarrar, C. Nugent et al., "A novel diagnostic biomarker panel for obesity-related nonalcoholic steatohepatitis (NASH)," *Obesity Surgery*, vol. 18, no. 11, pp. 1430–1437, 2008.

[15] K. Cusi, Z. Chang, S. Harrison et al., "Limited value of plasma cytokeratin-18 as a biomarker for NASH and fibrosis in patients with non-alcoholic fatty liver disease," *Journal of Hepatology.*, vol. 60, no. 1, pp. 167–174, 2014.

[16] K. H. Chuah, W. N. I. Wan Yusoff, P. Sthaneshwar, N. R. Nik Mustapha, S. Mahadeva, and W. K. Chan, "MACK-3 (combination of hoMa, Ast and CK18): a promising novel biomarker for fibrotic non-alcoholic steatohepatitis," *Liver International.*, vol. 39, no. 7, pp. 1315–1324, 2019.

[17] F. Gao, J. F. Huang, K. I. Zheng et al., "Development and validation of a novel non-invasive test for diagnosing fibrotic non-alcoholic steatohepatitis in patients with biopsy-proven non-alcoholic fatty liver disease," *Journal of Gastroenterology and Hepatology*, vol. 35, no. 10, pp. 1804–1812, 2020.

[18] R. Anty, A. Iannelli, S. Patouraux et al., "A new composite model including metabolic syndrome, alanine aminotransferase and cytokeratin-18 for the diagnosis of non-alcoholic steatohepatitis in morbidly obese patients," *Alimentary Pharmacology & Therapeutics*, vol. 32, no. 11-12, pp. 1315–1322, 2010.

[19] K. I. Zheng, W. Y. Liu, X. Y. Pan et al., "Combined and sequential non-invasive approach to diagnosing non-alcoholic steatohepatitis in patients with non-alcoholic fatty liver disease and persistently normal alanine aminotransferase levels," *BMJ Open Diabetes Research and Care*, vol. 8, no. 1, 2020.

[20] W. Cao, C. Zhao, C. Shen, and Y. Wang, "Cytokeratin 18, alanine aminotransferase, platelets and triglycerides predict the presence of nonalcoholic steatohepatitis," *PloS One*, vol. 8, no. 12, article e82092, 2013.

[21] T. Tada, T. Kumada, H. Toyoda, T. Saibara, M. Ono, and M. Kage, "New scoring system combining the FIB-4 index and cytokeratin-18 fragments for predicting steatohepatitis and liver fibrosis in patients with nonalcoholic fatty liver disease," *Biomarkers*, vol. 23, no. 4, pp. 328–334, 2018.

[22] M. A. Mohammed, N. M. Omar, S. A. Mohammed, A. M. Amin, and D. F. Gad, "FICK-3 score combining fibrosis-4, insulin resistance and cytokeratin-18 in predicting non-alcoholic steatohepatitis in NAFLD Egyptian patients," *Pakistan Journal of Biological Sciences: PJBS*, vol. 22, no. 10, pp. 457–466, 2019.

[23] M. Grigorescu, D. Crisan, C. Radu, M. D. Grigorescu, Z. Sparchez, and A. Serban, "A novel pathophysiological-based panel of biomarkers for the diagnosis of nonalcoholic

steatohepatitis," *Journal of Physiology and Pharmacology*, vol. 63, no. 4, pp. 347–353, 2012.

[24] L. He, L. Deng, Q. Zhang et al., "Diagnostic value of CK-18, FGF-21, and related biomarker panel in nonalcoholic fatty liver disease: a systematic review and meta-analysis," *Biomed Research International*, vol. 2017, Article ID 9729107, 12 pages, 2017.

[25] A. Canbay, J. Kälsch, U. Neumann et al., "Non-invasive assessment of NAFLD as systemic disease-a machine learning perspective. PLoS One. 2019; 14(3): e0214436. Pub Med PMID: 30913263. Pubmed Central PMCID: PMC6435145 to declare," *PloS One*, vol. 14, no. 3, article e0214436, 2019.

[26] T. I. A. R. Tamimi, H. M. Elgouhari, N. Alkhouri et al., "An apoptosis panel for nonalcoholic steatohepatitis diagnosis," *Journal of Hepatology*, vol. 54, no. 6, pp. 1224–1229, 2011.

[27] S. Page, A. Birerdinc, M. Estep et al., "Knowledge-based identification of soluble biomarkers: hepatic fibrosis in NAFLD as an example," *PloS one*, vol. 8, no. 2, article e56009, 2013.

[28] S. M. Walenbergh, T. Houben, T. Hendrikx et al., "Plasma cathepsin D levels: a novel tool to predict pediatric hepatic inflammation," *Official journal of the American College of Gastroenterology ACG*, vol. 110, no. 3, pp. 462–470, 2015.

[29] N. Tanaka, T. Kimura, N. Fujimori, T. Nagaya, M. Komatsu, and E. Tanaka, "Current status, problems, and perspectives of non-alcoholic fatty liver disease research," *World Journal of Gastroenterology*, vol. 25, no. 2, pp. 163–177, 2019.

[30] G. Bower, T. Toma, L. Harling et al., "Bariatric surgery and non-alcoholic fatty liver disease: a systematic review of liver biochemistry and histology," *Obesity Surgery*, vol. 25, no. 12, pp. 2280–2289, 2015.

[31] V. A. Piazzolla and A. Mangia, "Noninvasive diagnosis of NAFLD and NASH," *Cells*, vol. 9, no. 4, article 1005, 2020.

[32] E. Tsai and T. P. Lee, "Diagnosis and evaluation of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis, including noninvasive biomarkers and transient elastography," *Clinics in Liver Disease*, vol. 22, no. 1, pp. 73–92, 2018.

[33] L. Castera, M. Friedrich-Rust, and R. Loomba, "Noninvasive assessment of liver disease in patients with nonalcoholic fatty liver disease," *Gastroenterology*, vol. 156, no. 5, pp. 1264–1281, 2019.

[34] L. Wang, M. Yao, S. Liu et al., "Serum Golgi protein 73 as a potential biomarker for hepatic necroinflammation in population with nonalcoholic steatohepatitis," *Disease Markers*, vol. 2020, Article ID 6036904, 7 pages, 2020.

[35] S. Kar, S. Paglialunga, S. H. Jaycox, R. Islam, and A. H. Paredes, "Assay validation and clinical performance of chronic inflammatory and chemokine biomarkers of NASH fibrosis," *PLoS One*, vol. 14, no. 7, article e0217263, 2019.

[36] T. Auguet, L. Bertran, J. Binetti et al., "Relationship between IL-8 circulating levels and TLR2 hepatic expression in women with morbid obesity and nonalcoholic steatohepatitis," *International Journal of Molecular Sciences*, vol. 21, no. 11, 2020.

[37] Z. Xu, X. Zhang, J. Lau, and J. Yu, "C-X-C motif chemokine 10 in non-alcoholic steatohepatitis: role as a pro-inflammatory factor and clinical implication," *Expert Reviews in Molecular Medicine*, vol. 18, article e16, 2016.

[38] G. Bedogni, S. Bellentani, L. Miglioli et al., "The fatty liver index: a simple and accurate predictor of hepatic steatosis in the general population," *BMC Gastroenterology*, vol. 6, pp. 1–7, 2006.

[39] J. H. Lee, D. Kim, H. J. Kim et al., "Hepatic steatosis index: a simple screening tool reflecting nonalcoholic fatty liver disease," *Digestive and Liver Disease*, vol. 42, no. 7, pp. 503–508, 2010.

[40] Y. J. Zhou, Y. F. Zhou, J. N. Zheng et al., "NAFL screening score: a basic score identifying ultrasound-diagnosed non-alcoholic fatty liver," *Clinica Chimica Acta*, vol. 475, pp. 44–50, 2017.

[41] M. Otgonsuren, M. J. Estep, N. Hossain et al., "Single non-invasive model to diagnose non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH)," *Journal of Gastroenterology and Hepatology*, vol. 29, no. 12, pp. 2006–2013, 2014.

[42] A. Kotronen, M. Peltonen, A. Hakkarainen et al., "Prediction of non-alcoholic fatty liver disease and liver fat using metabolic and genetic factors," *Gastroenterology*, vol. 137, no. 3, pp. 865–872, 2009.

[43] F. Bril, M. J. McPhaul, M. P. Caulfield et al., "Performance of plasma biomarkers and diagnostic panels for nonalcoholic steatohepatitis and advanced fibrosis in patients with type 2 diabetes," *Diabetes Care*, vol. 43, no. 2, pp. 290–297, 2020.

[44] L. L. Lardi, R. M. Lul, G. Z. Port et al., "Fibromax and inflamatory markers cannot replace liver biopsy in the evaluation of non-alcoholic fatty liver disease," *Minerva Gastroenterologica e Dietologica*, vol. 68, no. 1, pp. 85–90, 2022.

[45] V. W. Wong, L. A. Adams, V. de Ledinghen, G. L. Wong, and S. Sookoian, "Noninvasive biomarkers in NAFLD and NASH - current progress and future promise," *Nature Reviews Gastroenterology & Hepatology*, vol. 15, no. 8, pp. 461–478, 2018.

[46] A. Antipass, A. Austin, S. Awad, D. Hughes, and I. Idris, "Evaluation of liver function tests and risk score assessment to screen patients for significant liver disease prior to bariatric and metabolic surgery," *Obesity Surgery*, vol. 30, no. 7, pp. 2840–2843, 2020.

[47] M. Malehmir, D. Pfister, S. Gallage et al., "Platelet GPIbα is a mediator and potential interventional target for NASH and subsequent liver cancer," *Nature Medicine*, vol. 25, no. 4, pp. 641–655, 2019.

[48] M. S. Siddiqui, G. Yamada, R. Vuppalanchi et al., "Diagnostic accuracy of noninvasive fibrosis models to detect change in fibrosis stage," *Clinical Gastroenterology And Hepatology*, vol. 17, no. 9, pp. 1877–1885, 2019.

[49] B. V. Udelsman, K. Corey, M. M. Hutter, D. C. Chang, and E. R. Witkowski, "Use of noninvasive scores for advanced liver fibrosis can guide the need for hepatic biopsy during bariatric procedures," *Surgery for Obesity and Related Diseases*, vol. 17, no. 2, pp. 292–298, 2021.

[50] S. Ballestri, A. Mantovani, E. Baldelli et al., "Liver fibrosis biomarkers accurately exclude advanced fibrosis and are associated with higher cardiovascular risk scores in patients with NAFLD or viral chronic liver disease," *Diagnostics*, vol. 11, no. 1, 2021.

[51] F. Higuera-de-la-Tijera, J. Córdova-Gallardo, E. Buganza-Torio et al., "Hepamet fibrosis score in nonalcoholic fatty liver disease patients in Mexico: lower than expected positive predictive value," *Digestive Diseases and Sciences*, vol. 66, no. 12, pp. 4501–4507, 2021.

[52] J. Cai, X. J. Zhang, Y. X. Ji, P. Zhang, Z. G. She, and H. Li, "Nonalcoholic fatty liver disease pandemic fuels the upsurge in cardiovascular diseases," *Circulation Research*, vol. 126, no. 5, pp. 679–704, 2020.

[53] M. Spinosa and J. G. Stine, "Nonalcoholic fatty liver disease-evidence for a thrombophilic state?," *Current Pharmaceutical Design*, vol. 26, no. 10, pp. 1036–1044, 2020.

[54] J. Hernandez Roman and M. S. Siddiqui, "The role of noninvasive biomarkers in diagnosis and risk stratification in nonalcoholic fatty liver disease," *Endocrinology, Diabetes & Metabolism*, vol. 3, no. 4, article e00127, 2020.

[55] R. Loomba, A. Jain, A. M. Diehl et al., "Validation of serum test for advanced liver fibrosis in patients with nonalcoholic steatohepatitis," *Clinical Gastroenterology and Hepatology*, vol. 17, no. 9, pp. 1867–1876, 2019.

[56] L. Miele, T. De Michele, G. Marrone et al., "Enhanced liver fibrosis test as a reliable tool for assessing fibrosis in nonalcoholic fatty liver disease in a clinical setting," *The International Journal of Biological Markers*, vol. 32, no. 4, pp. e397–e402, 2017.

[57] M. Mizuno, T. Shima, H. Oya et al., "Classification of patients with non-alcoholic fatty liver disease using rapid immunoassay of serum type IV collagen compared with liver histology and other fibrosis markers," *Hepatology Research*, vol. 47, no. 2, pp. 216–225, 2017.

[58] Y. I. Luo, A. Oseini, R. Gagnon et al., "An evaluation of the collagen fragments related to fibrogenesis and fibrolysis in nonalcoholic steatohepatitis," *Scientific Reports*, vol. 8, no. 1, article 12414, 2018.

[59] S. J. Daniels, D. J. Leeming, M. Eslam et al., "ADAPT: an algorithm incorporating PRO-C3 accurately identifies patients with NAFLD and advanced fibrosis," *Hepatology*, vol. 69, no. 3, pp. 1075–1086, 2019.

[60] M. Kanno, K. Kawaguchi, M. Honda et al., "Serum aldo-keto reductase family 1 member B10 predicts advanced liver fibrosis and fatal complications of nonalcoholic steatohepatitis," *Journal Of Gastroenterology*, vol. 54, no. 6, pp. 549–557, 2019.

[61] P. Angulo, J. M. Hui, G. Marchesini et al., "The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD," *Hepatology*, vol. 45, no. 4, pp. 846–854, 2007.

[62] Q. M. Anstee, E. J. Lawitz, N. Alkhouri et al., "Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR trials," *Hepatology*, vol. 70, no. 5, pp. 1521–1530, 2019.

[63] J. Ampuero, R. Pais, R. Aller et al., "Development and validation of Hepamet fibrosis scoring system-a simple, noninvasive test to identify patients with nonalcoholic fatty liver disease with advanced fibrosis," *Clinical Gastroenterology and Hepatology*, vol. 18, no. 1, pp. 216–225, 2020.

[64] S. A. Harrison, D. Oliver, H. L. Arnold, S. Gogia, and B. A. Neuschwander-Tetri, "Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease," *Gut*, vol. 57, no. 10, pp. 1441–1447, 2008.

[65] P. Calès, F. Lainé, J. Boursier et al., "Comparison of blood tests for liver fibrosis specific or not to NAFLD," *Journal of Hepatology*, vol. 50, no. 1, pp. 165–173, 2009.

[66] M. S. Siddiqui, K. R. Patidar, S. Boyett, V. A. Luketic, P. Puri, and A. J. Sanyal, "Performance of non-invasive models of fibrosis in predicting mild to moderate fibrosis in patients with non-alcoholic fatty liver disease," *Liver International*, vol. 36, no. 4, pp. 572–579, 2016.

[67] H. Fujii, M. Enomoto, W. Fukushima et al., "Noninvasive laboratory tests proposed for predicting cirrhosis in patients with chronic hepatitis C are also useful in patients with non-alcoholic steatohepatitis," *Journal of Gastroenterology*, vol. 44, no. 6, pp. 608–614, 2009.

[68] L. A. Adams, J. George, E. Bugianesi et al., "Complex non-invasive fibrosis models are more accurate than simple models in non-alcoholic fatty liver disease," *Journal Of Gastroenterology and Hepatology*, vol. 26, no. 10, pp. 1536–1543, 2011.

[69] M. Guillaume, V. Moal, C. Delabaudiere et al., "Direct comparison of the specialised blood fibrosis tests Fibro Meter (V2G) and enhanced liver fibrosis score in patients with non-alcoholic fatty liver disease from tertiary care centres," *Alimentary Pharmacology & Therapeutics*, vol. 50, no. 11-12, pp. 1214–1222, 2019.

[70] T. Okanoue, H. Ebise, T. Kai et al., "A simple scoring system using type IV collagen 7S and aspartate aminotransferase for diagnosing nonalcoholic steatohepatitis and related fibrosis," *Journal of Gastroenterology*, vol. 53, no. 1, pp. 129–139, 2018.

[71] M. Boyle, D. Tiniakos, J. M. Schattenberg et al., "Performance of the PRO-C3 collagen neo-epitope biomarker in non-alcoholic fatty liver disease," *JHEP Reports*, vol. 1, no. 3, pp. 188–198, 2019.

[72] O. Kucukoglu, J. P. Sowa, G. D. Mazzolini, W. K. Syn, and A. Canbay, "Hepatokines and adipokines in NASH-related hepatocellular carcinoma," *Journal of Hepatology*, vol. 74, no. 2, pp. 442–457, 2020.

[73] S. A. Polyzos, J. Kountouras, V. Polymerou, K. G. Papadimitriou, C. Zavos, and P. Katsinelos, "Vaspin, resistin, retinol-binding protein-4, interleukin-1alpha and interleukin-6 in patients with nonalcoholic fatty liver disease," *Annals of Hepatology*, vol. 15, no. 5, pp. 705–714, 2016.

[74] R. Jamali, A. Arj, M. Razavizade, and M. H. Aarabi, "Prediction of nonalcoholic fatty liver disease via a novel panel of serum adipokines," *Medicine*, vol. 95, no. 5, article e2630, 2016.

[75] C. Giannini, A. E. Feldstein, N. Santoro et al., "Circulating levels of FGF-21 in obese youth: associations with liver fat content and markers of liver damage," *The Journal of Clinical Endocrinology and Metabolism*, vol. 98, no. 7, pp. 2993–3000, 2013.

[76] T. Kawaguchi, T. Shima, M. Mizuno et al., "Risk estimation model for nonalcoholic fatty liver disease in the Japanese using multiple genetic markers," *PLoS One*, vol. 13, no. 1, article e0185490, 2018.

[77] B. K. Koo, S. K. Joo, D. Kim et al., "Additive effects of PNPLA3 and TM6SF2 on the histological severity of nonalcoholic fatty liver disease," *Journal of Gastroenterology and Hepatology*, vol. 33, no. 6, pp. 1277–1285, 2018.

[78] A. Di Costanzo, L. Pacifico, C. Chiesa et al., "Genetic and metabolic predictors of hepatic fat content in a cohort of Italian children with obesity," *Pediatric Research*, vol. 85, no. 5, pp. 671–677, 2019.

[79] J. Hyysalo, V. T. Männistö, Y. Zhou et al., "A population-based study on the prevalence of NASH using scores validated against liver histology," *Journal of Hepatology*, vol. 60, no. 4, pp. 839–846, 2014.

[80] B. K. Koo, S. K. Joo, D. Kim et al., "Development and validation of a scoring system, based on genetic and clinical factors, to determine risk of steatohepatitis in Asian patients with nonalcoholic fatty liver disease," *Clinical Gastroenterology and Hepatology*, vol. 18, no. 11, pp. 2592–2599, 2020.

[81] Q. M. Anstee, R. Darlay, S. Cockell et al., "Genome-wide association study of non-alcoholic fatty liver and steatohepatitis in a histologically characterised cohort," *Journal of Hepatology*, vol. 73, no. 3, pp. 505–515, 2020.

[82] U. Vespasiani-Gentilucci, P. Gallo, C. Dell'Unto, M. Volpentesta, R. Antonelli-Incalzi, and A. Picardi, "Promoting genetics in non-alcoholic fatty liver disease: combined risk score through polymorphisms and clinical variables," *World Journal of Gastroenterology*, vol. 24, no. 43, pp. 4835–4845, 2018.

[83] A. Bayoumi, H. Gronbaek, J. George, and M. Eslam, "The epigenetic drug discovery landscape for metabolic-associated fatty liver disease," *Trends in Genetics*, vol. 36, no. 6, pp. 429–441, 2020.

[84] T. Hardy, M. Zeybel, C. P. Day et al., "Plasma DNA methylation: a potential biomarker for stratification of liver fibrosis in non-alcoholic fatty liver disease," *Gut*, vol. 66, no. 7, pp. 1321–1328, 2017.

[85] J. Ma, J. Nano, J. Ding et al., "A peripheral blood DNA methylation signature of hepatic fat reveals a potential causal pathway for nonalcoholic fatty liver disease," *Diabetes*, vol. 68, no. 5, pp. 1073–1083, 2019.

[86] J. Hyun and Y. Jung, "DNA Methylation in nonalcoholic fatty liver disease," *International Journal of Molecular Sciences*, vol. 21, no. 21, article 8138, 2020.

[87] C. H. Liu, J. Ampuero, A. Gil-Gómez et al., "mi RNAs in patients with non-alcoholic fatty liver disease: a systematic review and meta-analysis," *Journal of Hepatology*, vol. 69, no. 6, pp. 1335–1348, 2018.

[88] M. Gjorgjieva, C. Sobolewski, D. Dolicka, M. C. de Sousa, and M. Foti, "mi RNAs and NAFLD: from pathophysiology to therapy," *Gut*, vol. 68, no. 11, pp. 2065–2079, 2019.

[89] J. G. Park, G. Kim, S. Y. Jang et al., "Plasma long noncoding RNA is a potential diagnostic marker for non-alcoholic steatohepatitis," *Life*, vol. 10, no. 10, 2020.

[90] H. Yang, Q. Li, L. Zhang et al., "Lnc PRYP4-3 serves as a novel diagnostic biomarker for dissecting subtypes of metabolic associated fatty liver disease by targeting RPS4Y2," *Clinical and Experimental Medicine*, vol. 20, no. 4, pp. 587–600, 2020.

[91] S. Di Mauro, A. Scamporrino, S. Petta et al., "Serum coding and non-coding RNAs as biomarkers of NAFLD and fibrosis severity," *Liver International*, vol. 39, no. 9, pp. 1742–1754, 2019.

[92] A. Hanson, D. Wilhelmsen, and J. K. DiStefano, "The role of long non-coding RNAs (lnc RNAs) in the development and progression of fibrosis associated with nonalcoholic fatty liver disease (NAFLD)," *Noncoding RNA*, vol. 4, no. 3, 2018.

[93] S. A. Sulaiman, N. I. A. Muhsin, and R. Jamal, "Regulatory non-coding RNAs network in non-alcoholic fatty liver disease," *Frontiers in Physiology*, vol. 10, article 279, 2019.

[94] B. Atanasovska, S. S. Rensen, M. R. van der Sijde et al., "A liver-specific long noncoding RNA with a role in cell viability is elevated in human nonalcoholic steatohepatitis," *Hepatology*, vol. 66, no. 3, pp. 794–808, 2017.

[95] A. E. Botello-Manilla, N. C. Chavez-Tapia, M. Uribe, and N. Nuno-Lambarri, "Genetics and epigenetics purpose in nonalcoholic fatty liver disease," *Expert Review of Gastroenterology & Hepatology*, vol. 14, no. 8, pp. 733–748, 2020.

[96] X. Jin, J. Gao, R. Zheng et al., "Antagonizing circ RNA_002581-mi R-122-CPEB1 axis alleviates NASH through restoring PTEN-AMPK-mTOR pathway regulated autophagy," *Cell Death & Disease*, vol. 11, no. 2, pp. 1–13, 2020.

[97] X.-Y. Guo, J.-N. Chen, F. Sun, Y.-Q. Wang, Q. Pan, and J.-G. Fan, "circ RNA_0046367 prevents hepatoxicity of lipid peroxidation: an inhibitory role against hepatic steatosis," *Oxidative Medicine And Cellular Longevity*, vol. 2017, Article ID 3960197, 16 pages, 2017.

[98] Y. Chien, P. H. Tsai, Y. H. Lai et al., "Circular RNA as novel biomarkers in liver diseases," *Journal of the Chinese Medical Association*, vol. 83, no. 1, pp. 15–17, 2020.

[99] C. Yu, C. Xu, L. Xu, J. Yu, M. Miao, and Y. Li, "Serum proteomic analysis revealed diagnostic value of hemoglobin for nonalcoholic fatty liver disease," *Journal of Hepatology*, vol. 56, no. 1, pp. 241–247, 2012.

[100] L. Niu, P. E. Geyer, N. J. Wewer Albrechtsen et al., "Plasma proteome profiling discovers novel proteins associated with non-alcoholic fatty liver disease," *Molecular Systems Biology*, vol. 15, no. 3, article e8793, 2019.

[101] Z. M. Younossi, A. Karrar, M. Pierobon et al., "An exploratory study examining how nano-liquid chromatography-mass spectrometry and phosphoproteomics can differentiate patients with advanced fibrosis and higher percentage collagen in non-alcoholic fatty liver disease," *BMC Medicine*, vol. 16, no. 1, article 170, 2018.

[102] S. Qi, D. Xu, Q. Li et al., "Metabonomics screening of serum identifies pyroglutamate as a diagnostic biomarker for nonalcoholic steatohepatitis," *Clinica Chimica Acta*, vol. 473, pp. 89–95, 2017.

[103] P. Puri, M. M. Wiest, O. Cheung et al., "The plasma lipidomic signature of nonalcoholic steatohepatitis," *Hepatology*, vol. 50, no. 6, pp. 1827–1838, 2009.

[104] R. Mayo, J. Crespo, I. Martinez-Arranz et al., "Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: results from discovery and validation cohorts," *Hepatology Communications*, vol. 2, no. 7, pp. 807–820, 2018.

[105] K. Yamada, E. Mizukoshi, T. Seike et al., "Serum C16:1n7/C16:0 ratio as a diagnostic marker for non-alcoholic steatohepatitis," *Journal of Gastroenterology and Hepatology*, vol. 34, no. 10, pp. 1829–1835, 2019.

[106] S. Tiwari-Heckler, H. Gan-Schreier, W. Stremmel, W. Chamulitrat, and A. Pathil, "Circulating phospholipid patterns in NAFLD patients associated with a combination of metabolic risk factors," *Nutrients*, vol. 10, no. 5, 2018.

[107] D. Beyoğlu and J. R. Idle, "Metabolomic and lipidomic biomarkers for premalignant liver disease diagnosis and therapy," *Metabolites*, vol. 10, no. 2, article 50, 2020.

[108] N. Perakakis, K. Stefanakis, and C. S. Mantzoros, "The role of omics in the pathophysiology, diagnosis and treatment of non-alcoholic fatty liver disease," *Metabolism*, no. article 154320, 2020.

[109] P. B. Devhare and R. B. Ray, "Extracellular vesicles: novel mediator for cell to cell communications in liver pathogenesis," *Molecular Aspects of Medicine*, vol. 60, pp. 115–122, 2018.

[110] A. Eguchi and A. E. Feldstein, "Extracellular vesicles in non-alcoholic and alcoholic fatty liver diseases," *Liver Research*, vol. 2, no. 1, pp. 30–34, 2018.

[111] Z. Zhao, L. Zhong, P. Li et al., "Cholesterol impairs hepatocyte lysosomal function causing M1 polarization of macrophages via exosomal mi R-122-5p," *Experimental Cell Research*, vol. 387, no. 1, article 111738, 2020.

[112] P. Hirsova, S. H. Ibrahim, A. Krishnan et al., "Lipid-induced signaling causes release of inflammatory extracellular vesicles from hepatocytes," *Gastroenterology*, vol. 150, no. 4, pp. 956–967, 2016.

[113] J. A. Welsh, E. Scorletti, G. F. Clough, N. A. Englyst, and C. D. Byrne, "Leukocyte extracellular vesicle concentration is inversely associated with liver fibrosis severity in NAFLD," *Journal of Leukocyte Biology*, vol. 104, no. 3, pp. 631–639, 2018.

[114] L. A. Ban, N. A. Shackel, and S. V. McLennan, "Extracellular vesicles: a new frontier in biomarker discovery for non-alcoholic fatty liver disease," *International Journal of Molecular Sciences*, vol. 17, no. 3, article 376, 2016.

[115] C. S. Shabangu, J. F. Huang, H. H. Hsiao, M. L. Yu, W. L. Chuang, and S. C. Wang, "Liquid biopsy for the diagnosis of viral hepatitis, fatty liver steatosis, and alcoholic liver diseases," *International Journal of Molecular Sciences*, vol. 21, no. 10, article 3732, 2020.

[116] K. Kantartzis, I. Rettig, H. Staiger et al., "An extended fatty liver index to predict non-alcoholic fatty liver disease," *Diabetes & Metabolism*, vol. 43, no. 3, pp. 229–239, 2017.

[117] H. Yang, G. Chen, C. Song et al., "A novel index including SNPs for the screening of nonalcoholic fatty liver disease among elder Chinese: a population-based study," *Medicine*, vol. 97, no. 13, article e0272, 2018.

[118] S. A. Harrison, V. Ratziu, J. Boursier et al., "A blood-based biomarker panel (NIS4) for non-invasive diagnosis of non-alcoholic steatohepatitis and liver fibrosis: a prospective derivation and global validation study," *The Lancet Gastroenterology & Hepatology.*, vol. 5, no. 11, pp. 970–985, 2020.

[119] P. P. Becker, M. Rau, J. Schmitt et al., "Performance of serum micro RNAs -122, -192 and -21 as biomarkers in patients with non-alcoholic steatohepatitis," *PloS one*, vol. 10, no. 11, article e0142661, 2015.

[120] Y. Tan, G. Ge, T. Pan, D. Wen, and J. Gan, "A pilot study of serum micro RNAs panel as potential biomarkers for diagnosis of nonalcoholic fatty liver disease," *PloS One*, vol. 9, no. 8, article e105192, 2014.

[121] J. Zhu, J. Huang, J. Zhang et al., "Glycopeptide biomarkers in serum haptoglobin for hepatocellular carcinoma detection in patients with nonalcoholic steatohepatitis," *Journal of Proteome Research*, vol. 19, no. 8, pp. 3452–3466, 2020.

[122] C. Chen, H. Schmilovitz-Weiss, X. E. Liu et al., "Serum protein N-glycans profiling for the discovery of potential biomarkers for nonalcoholic steatohepatitis," *Journal of Proteome Research*, vol. 8, no. 2, pp. 463–470, 2009.

[123] N. Perakakis, S. A. Polyzos, A. Yazdani et al., "Non-invasive diagnosis of non-alcoholic steatohepatitis and fibrosis with the use of omics and supervised learning: a proof of concept study," *Metabolism*, vol. 101, article 154005, 2019.

[124] Y. Jung, M. K. Lee, P. Puri et al., "Circulating lipidomic alterations in obese and non-obese subjects with non-alcoholic fatty liver disease," *Alimentary Pharmacology & Therapeutics*, vol. 52, no. 10, pp. 1603–1614, 2020.

[125] C. Caussy, J. C. Chuang, A. Billin et al., "Plasma eicosanoids as noninvasive biomarkers of liver fibrosis in patients with nonalcoholic steatohepatitis," *Therapeutic Advances in Gastroenterology*, vol. 13, article 1756284820923904, 2020.

[126] R. X. Yang, C. X. Hu, W. L. Sun et al., "Serum monounsaturated triacylglycerol predicts steatohepatitis in patients with non-alcoholic fatty liver disease and chronic hepatitis B," *Scientific Reports*, vol. 7, no. 1, article 10517, 2017.

[127] D. Povero, H. Yamashita, W. Ren et al., "Characterization and proteome of circulating extracellular vesicles as potential biomarkers for NASH," *Hepatology Communications*, vol. 4, no. 9, pp. 1263–1278, 2020.