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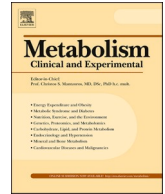
Can liquid biopsies for MASH help increase the penetration of metabolic surgery? A narrative review

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ABSTRACT

This narrative review highlights current evidence on non-invasive tests to predict the presence or absence as well as the severity of metabolic dysfunction-associated steatohepatitis (MASH) and liver fibrosis. Metabolic dysfunction-associated steatotic liver disease (MASLD) is a common condition characterized by fat accumulation in the liver that affects 32 % of the world population. The most severe form of MASLD is MASH in which hepatocyte ballooning and inflammation are present together with steatosis; MASH is often associated with liver fibrosis.

MASH diagnosis is determined by invasive liver biopsy. Hence, there is a critical need for non-invasive MASH tests. Plasma biomarkers for MASH diagnosis generally have low sensitivity (62–66 %), and specificity (78–82 %). Monocyte levels of Perilipin2 (PLIN2) predict MASH with an accuracy of 92–93 %, and sensitivity and specificity of 90–95 % and 88–100 %, respectively. This liquid biopsy test can facilitate the study of MASH prevalence in general populations and also monitor the effects of lifestyle, surgical, and pharmacological interventions. Without any FDA-approved MASH therapeutic, and with metabolic surgery markedly surpassing the efficacy of lifestyle modification, an accurate and reliable liquid biopsy could help more people choose surgery as a treatment for MASH.

1. Introduction

Metabolic dysfunction associated steatotic liver disease (MASLD) is a common disease characterized by fat accumulation in hepatocytes. It affects 32 % of the global population [1,2], with projections indicating that up to 50 % of the population will be affected by 2040 [3].

The prevalence of MASLD is greater in some patient categories, reaching 55 % among people with type 2 diabetes [4] and 75 % among those with obesity [5].

The more severe form of MASLD is metabolic dysfunction-associated steatohepatitis (MASH), which associates steatosis with hepatocyte ballooning and inflammation and, eventually, fibrosis. MASLD has a dynamic behaviour with 2 in 5 patients showing disease progression, 2 in 5 patients showing no changes and 1 in 5 patients presenting with regression [6,7]. Notably, MASLD and especially MASH may progress to cirrhosis and/or hepatocellular carcinoma [8,9]. Moreover, MASH and liver fibrosis are a major cardiovascular risk factor and contribute to all-cause mortality [10].

The diagnosis of MASH is merely histological and several non-invasive scores have been implemented to positively predict steatohepatitis, albeit with suboptimal sensitivities and/or specificities.

The severity of MASH is classified according to the NAFLD Activity

Score (NAS), while liver fibrosis is classified according to the NASH-CRN system [11,12].

Since the diagnosis of MASH is based on an invasive liver biopsy procedure, there is an urgent need to find non-invasive biomarkers for its diagnosis, disease progression, and intervention response monitoring.

As MASLD has a high prevalence in the general population, it would require a comprehensive use of an invasive diagnostic approach, such as liver biopsy. Instead, a test easier to perform, less invasive, scalable and more acceptable to patients but one providing accurate information to guide clinical decisions should be greatly valuable.

This necessity is amplified by evidence that a large number of patients, ranging between 65 % and 73 %, screened for clinical trials did not meet eligibility criteria after liver biopsy [13]. Hence, prebiopsy strategies targeting the right candidates and reducing the number of screen failures are mandatory. In fact, the identification of appropriate and non-invasive biomarkers would increase the enrolment of patients in clinical trials, accelerating the development of therapeutic interventions for MASH, including metabolic surgery.

The ultimate research question is if liquid biopsies for MASH can help increasing the penetration of metabolic surgery.

2. Biomarkers classification

The biomarkers for the assessment of MASH and/or liver fibrosis can be classified into:

a) blood tests;
b) methods that measure the physical properties of the liver, such as stiffness or viscosity;
c) imaging methods that assess liver anatomy.

The accuracy of biomarkers is evaluated by comparing its sensitivity, specificity, and area under the curve (AUC) with liver histology data. To be reliable, a biomarker should be able to classify correctly at least 80 % of the patients; a test with an AUC lower than 0.80 is considered a poorly accurate test while a test >0.9 is considered excellent [14]. Other important evaluation criteria are the availability and cost of the test as well as its scalability. Moreover, some tests that work well in secondary care setting, where they have been validated against liver biopsy, are less accurate in primary care. For instance, the prevalence of advanced liver fibrosis is much higher in secondary or tertiary care than in the general population [15].

Hence, in the general population biomarkers work better to exclude rather than to diagnose the presence of advanced fibrosis. This observation suggests that at least two tiers of non-invasive fibrosis tests should be done in populations with low prevalence of liver fibrosis [14]. The variability of advanced fibrosis in studies of people with MASLD is very substantial, varying from 3.7 % to 30 % [16]. Modelling studies suggest that having concordant biomarkers improves diagnostic accuracy [17].

The purpose of this narrative review is to highlight current evidence on minimally or non-invasive tests for the diagnosis of presence or absence of MASH and/or liver fibrosis and for the assessment of the severity of these diseases.

Given that the prevalence of severe ($\geq$35 kg/m^2) and morbid ($\geq$40 kg/m^2) obesity in the United States is 23 % and 7.7 % for all adults [18], respectively, and that 2/3 of them have MASLD, it is mandatory to find a test that is either non-invasive or minimally invasive but specific and sensitive for the diagnosis of MASH and/or liver fibrosis. In fact, the NIH guidelines establish that a BMI $\geq$35 kg/m^2 in the presence of an obesity comorbidity represents an indication to metabolic surgery.

2.1. Non-invasive scores, biomarkers, and imaging methods for MASLD diagnosis

A series of scores, such as fatty liver index (FLI), the NAFLD liver fat score (NAFLD-LFS) and the Hepatic steatosis index (HSI), are available for the detection of liver steatosis.

The FLI, NAFLD-LFS and HIS equations are reported in Table 1.

Overall, while FLI, NAFLD-LFS, and HSI showed comparable diagnostic performances in detecting stetatosis >5 % in a retrospective study [19] with AUCs of 0.83, 0.80, and 0.81, respectively, further prospective and validation studies are needed to establish their performance in diagnosing liver steatosis and its severity. It is also worth noting that the diagnostic performance of these indices were evaluated for the detection of liver steatosis using not only liver biopsy but also imaging modalities, such as ultrasound or magnetic resonance (MR) spectroscopy, as the reference standard.

Ultrasound is the most widely used diagnostic for MASLD. According to a large meta-analysis [20] involving 2815 patients with liver biopsy as the reference standard, ultrasound showed pooled sensitivities and specificities for detecting steatosis larger than 20–30 % of 85 % (with a range of 80 %–89 %) and 94 % (with a range of 87 %–97 %), respectively. However, ultrasound has several limitations. Firstly, it can only detect steatosis above a certain threshold, typically 12.5 % to 20 %. Secondly, the result is operator-dependent and obesity can potentially affect its performance.

Magnetic resonance proton density fat fraction (MRI-PDFF) has a high correlation with the histologic fat content of the liver and is considered a valuable tool in clinical practice and research for diagnosing and monitoring liver steatosis [21]. The accuracy and reliability of MRI-PDFF have been validated in various clinical settings and populations [22].

The accuracy of FibroScan controlled attenuation parameter (CAP) in identifying patients with histological-proven liver steatosis is pretty good, although its accuracy decreases with increases in liver fat accumulation. In fact, Eddowes et al. [23] found a CAP AUC of 0.87 (95 % CI 0.82–0.92) for steatosis $\geq$S1 (i.e. 5–33 %), 0.77 (95 % CI 0.71–0.82) for steatosis $\geq$S2 (i.e. 34–66 %), and 0.70 (95 % CI 0.64–0.75) for steatosis = S3 (i.e. >66 %).

2.2. Non-invasive scores, biomarkers, and imaging methods for MASH diagnosis

Table 2 summarizes the biomarkers used to make a diagnosis of MASH.

The ActiTest [24,25] includes several variables – α2-Macroglobulin, apo A1, haptoglobin, total bilirubin, GGT, and ALT – and has a lower than acceptable accuracy as shown in Table 2.

The NashTest (Table 2) calculation is based on 13 biochemical and clinical variables (age, sex, height, weight, serum levels of triglycerides (TGs), cholesterol, a-macroglobulin, apolipoprotein A1, haptoglobin, GGT, ALT, AST, and total bilirubin). Yet, only 30 % of patients can be correctly classified by the test [25].

NashTest2 [25] is an evolution of NashTest and includes α2-Macroglobulin, apo A1, haptoglobin, total bilirubin, GGT, AST, cholesterol, and triglycerides to calculate a score for presence/absence of MASH prediction (Table 2). However, this test also shows low accuracy, sensitivity, and specificity [26].

The Magnetic Resonance Elastography (MRE) [27,28] is a cost analysis, and the availability of the device is limited. While it shows a good AUC and specificity, its sensitivity is low 65 % (95 % CI: 46–80) (Table 2).

Fibrosis-4 [FIB-4] score, AST-to-platelet ratio index [APRI], and ELF score, originally designed to detect liver fibrosis, have been also used for MASH diagnosis. The FIB-4 index results from the following equation:

$$FIB - 4 = \frac{age \cdot AST}{Platelet\ count \cdot \sqrt{ALT}}$$

A FIB-4 meta-analysis of six studies for the risk of fibrosis including 3557 subjects showed an AUC of 0.81 (95 % CI: 0.77–0.84), sensitivity of 0.57 (95 % CI: 0.39–0.74) classified as low certainty due to high inconsistency, and specificity of 0.89 (95 % CI: 0.77–0.95) with

Table 1

Non-invasive algorithms for MASLD diagnosis.

Fatty liver index (FLI)	$FLI = logistic(0.953 \cdot ln(TG) + 0.139 \cdot BMI + 0.718 \cdot ln(\gamma GT) + 0.053 \cdot WC - 15.745) \cdot 100$	TG = triglycerides, BMI = body mass index, γGT = gamma-glutamyl transferase, WC = waist circumference
NAFLD liver fat score (NAFLD-LFS)	$NAFLD - LF = -2.89 + 1.18 \cdot MS(yes = 1, no = 0) + 0.45 \cdot T2DM\ (yes = 2, no = 0) + 0.15 \cdot I_0 + 0.04 \cdot AST - 0.94 \cdot \frac{AST}{ALT}$	MS = metabolic syndrome, T2DM = type 2 diabetes mellitus, AST = aspartate transaminase, ALT = alanine transaminase
Hepatic steatosis index (HSI)	$HSI = 8 \cdot \frac{ALT}{AST} + BMI + 2\ if\ T2DM\ and + 2\ if\ female$	AST = aspartate transaminase, ALT = alanine transaminase, T2DM = type 2 diabetes mellitus

Table 2

Blood biomarkers for diagnosis of MASH.

Blood biomarkers	Mechanism	Method	AUC	Sensitivity	Specificity	References and type of study	Validation studies AUC	Sensitivity	Specificity	References and type of study	Limitations
ActiTest	α2-Macroglobulin, apo A1, haptoglobin, total bilirubin, GGT, ALT	Routine analyses	0.68	43.4 %	85.5 %	24 Cross sectional (3 cohorts)	0.70	74 %	62 %	25 Cross-sectional (1 cohort)	Lower than acceptable
NashTest	13 biochemical and clinical variables (age; sex; height; weight; and serum levels of triglycerides (TGs), cholesterol, a-macroglobulin, apolipoprotein A1, haptoglobin, GGT, ALT, AST, and total bilirubin)	Routine analyses	Overall, only 30 % of patients were correctly classified by the test			25 Cross-sectional (1 cohort)	0.33	92.9 %	33.7 %	24 Cross sectional (3 cohorts)	Low accuracy
NashTest-2	α2-Macroglobulin, apo A1, haptoglobin, total bilirubin, GGT, AST, cholesterol, and triglycerides	Routine analyses	0.69	71 %	60 %	25 Cross-sectional (1 cohort)	0.70	92 %	20 %	26 cross sectional (1 cohort)	Low accuracy, sensitivity and specificity
MRE	Magnetic Resonance	MRI with dedicated software	0.83	65 %	83 %	25	0.89	87.4 %	74.3 %	27 meta-analysis	Expensive, limited availability
PLIN2 (HeparDx) NASH yes/not	PLIN2, diabetes, triglycerides, ALT, waist circumference	Flow cytometry	0.98	95 %	90 %	33 Cross sectional (2 cohorts)	0.98	88 %	100 %	33 Cross sectional (2 cohorts)	Inexpensive, but flow cytometry is not widely available
PLIN2 (HeparDx) NAS ≥ 4	PLIN2, diabetes, triglycerides, ALT, waist circumference	Flow cytometry	0.98	100 %	93 %	33 Cross sectional (2 cohorts)	The analysis was done in the two cohorts merged			33 Cross sectional (2 cohorts)	Inexpensive, but flow cytometry is not widely available

moderate certainty [29]. When applied to MASH diagnosis the predictivity is very poor and far worse than for liver fibrosis.

The Aspartate aminotransferase [AST]-to-platelet ratio index [APRI] is calculated as:

$$APRI = \frac{\left(\frac{AST}{Normal\ AST\ Upper\ Limit}\right)}{Platelet\ Count} * 100$$

APRI has a low AUC of 0.68 (CI 0.63–0.72), sensitivity 0.53 and specificity 0.75 [30].

The AST/ALT ratio shows an AUC of 0.53 (CI 0.48–0.59) and a good sensitivity of 0.88, but a very low specificity 0.25 [30].

PRO—C3, a serum biomarker correlated with the formation of type III collagen, has a low AUC of 0.74 (CI 0.69–0.78), a low sensitivity of 0.56, and an acceptable specificity of 0.82 [30].

NIS4 is an algorithm comprising four independent biomarkers: miR-34a-5p, alpha-2 macroglobulin (A2M), YKL-40 (or chitinase 3-like protein 1 [CHI3L1]), and glycated haemoglobin (HbA1c) [31]. The discovery cohort in [31] included 239 prospectively recruited patients with biopsy-confirmed MASH, i.e. a NAS value ≥3, and a fibrosis stage 0–3. This algorithm was validated in 2 independent cohorts, the RESOLVE-IT with 475 patients and Angers including 227 patients [31]. The AUC of

NIS4 was 0.80 (95 % CI 0.73–0.85) in the discovery cohort and 0.83, (95 % CI 0.79–0.86) and 0.76 (95 % CI 0.69–0.82) in the two validation cohorts. However, the test's sensitivity was poor with a value of 0.63 (CI 57.8–68.0) for classifying subjects as without MASH and 0.51 (CI 45.3–56.1) for classifying subjects as having MASH. Since sensitivity is the ability of a test to correctly identify patients with a disease, NIS4, registered by GENFIT, is effective only in about half the patients giving a fifty-fifty chance.

NIS2+ [32] includes two biomarkers, miR-34a-5p and YKL-40. It was intended to simplify NIS4 but its performance remains poor having an AUC of 0.79 (CI 0.723, 0.849) in the training cohort and 0.81 (CI 0.795–0.832) in the validation cohort, a sensitivity of 0.65 (CI 0.54–0.74) and a specificity of 0.61 (CI 0.58–0.64), respectively [32].

Perilipin-2 (PLIN2) levels in circulating monocytes was tested as a predictor of histological MASH [33] and this test, HeparDx, is now being developed by Metadeq, Inc. Monocyte PLIN2 levels had an AUCs of 0.98 (CI 0.95–1) and 0.97 (CI 0.95–1) in the discovery and in the validation cohorts, with a sensitivity and specificity of 95 % and 90 % in the discovery cohort and of 88 % and 100 % in the validation cohort, respectively [33]. The Olden algorithm identified monocyte PLIN2 as the most important variable in classifying subjects according to NAS levels. The classification had an accuracy of 85 % in the discovery and 85 % in the validation cohort. Critically, PLIN2 was able to discriminate among various grades of MASH severity. The AUC of NAS < 3 was 0.97 (CI 0.96–1), NAS =3 was 0.84 (CI 0.76–0.92) and NAS ≥ 4 was 0.98 (CI 0.96–1) [33]. The PLIN2 biomarker can be used in community and population studies permitting to investigate the real prevalence of MASH. Moreover, since it requires only a blood sample, it is a potentially valuable tool for population-based and prevention studies in children as well.

An extensive and punctual review of role multiomic analyses in the pathophysiology, diagnosis and treatment of MASLD inclusive of MASH has been recently published [34]. Interestingly, the addition of gene variants, such as PNPLA3 rs738409 that is associated with liver steatosis, does not improve the diagnostic accuracy of commonly used diagnostic scores (see those in Tables 2 and 3) and are not accurate markers of liver histologic features [34]. Micro-RNAs (miRNA), such as miR-122, are unable to diagnose the presence of MASH [34]. Proteomics and lipidomics add complexity in analysis and rise costs, but with a poor gain in sensitivity and specificity for MASH diagnosis [34]. Therefore, although omics have a relevant role in pathophysiology studies, their relevance in MASH diagnosis is still unclear.

2.3. Non-invasive scores, biomarkers, and imaging methods for liver fibrosis diagnosis

Liver fibrosis in MASH ranges between F1 and F3; F3 is histologically characterized by portal-portal bridging fibrosis. Non-invasive biomarkers have shown high effectiveness in ruling out advanced fibrosis or cirrhosis, typically exhibiting negative predictive values (NPVs) >90 % in many cases. These biomarkers, which include various blood tests and imaging techniques, provide a valuable tool to screen individuals and exclude the likelihood of advanced liver fibrosis.

However, their positive predictive values (PPVs) are often more modest. This means that when these biomarkers indicate the presence of fibrosis or cirrhosis, there is a higher chance of false positives compared to ruling out the condition. Consequently, additional diagnostic evaluations may be required to confirm the presence and stages of fibrosis accurately.

Furthermore, non-invasive biomarkers typically lack the ability to accurately discriminate individual fibrosis stages. While they can provide an overall assessment of fibrosis severity (e.g., low, moderate, high), they may not offer the same level of precision as invasive procedures such as liver biopsy.

Advancements in non-invasive testing techniques continue to evolve and researchers are actively working to improve the accuracy and specificity of these biomarkers. Ongoing research aims to enhance the positive predictive values and refine the ability to differentiate among different stages of fibrosis, which could lead to more precise and reliable non-invasive diagnostic tools in the future.

A normal AST:ALT ratio should be <1, however while ALT circulating levels decrease as soon as liver fibrosis progresses AST levels remain constant; thus, an AST to ALT ratio >1 suggests the presence of cirrhosis [35]. But although AST to ALT ratio <0.8 can rule out the presence of advanced fibrosis, this ratio is unable to predict the presence and severity of liver fibrosis [36,37]. A significant limitation of scores using transaminases is that ALT levels fall with age, with a consequent increase of the AST to ALT score that is unrelated to the presence of liver fibrosis [38].

The BARD score combines the presence of type 2 diabetes with BMI and AST to ALT ratio and was created to assess the likelihood of advanced fibrosis in patients with MASLD [37]. A BARD score < 2 has a high negative predictive value of over 96 %.

However, it is important to note that most patients with MASLD often exceed the BARD score threshold of 2 due to the high prevalence of obesity and type 2 diabetes in this population. The higher BARD score in many individuals with MASLD limits the score's utility in clinical practice as it has a lower PPV. The limitations of the BARD score's PPV highlight the challenge of accurately identifying advanced fibrosis in patients with MASLD using non-invasive scoring systems alone. Additional diagnostic evaluations, such as imaging techniques or liver biopsy, may be necessary to confirm the presence and stage of fibrosis in patients with higher BARD scores [37].

It is important for healthcare professionals to consider the overall clinical context and use multiple diagnostic tools and assessments to make informed decisions regarding the presence and severity of liver fibrosis in patients with MASLD.

NAFLD Fibrosis Score (NFS)

$$NFS = -1.675 + 0.037 \cdot age(year) + 0.094 \cdot BMI\left(\frac{kg}{m^2}\right) + 1.13 \cdot IFG \, or \, diabetes \, (yes$$

$$= 1, no = 0) + 0.99 \cdot \frac{AST}{ALT} - 0.013 \cdot platelet \, count\left(\frac{x10^9}{l}\right) - 0.66 \cdot albumin\left(\frac{g}{dl}\right)$$

According to the reported data [39], the NFS achieves an AUC of 0.81 with confidence intervals of 0.71 and 0.91. This indicates that the NFS has adequate discriminatory ability in predicting the presence of advanced fibrosis. Values of the NFS below −1.455 have been shown to exclude advanced fibrosis with high accuracy. This means that if a patient has an NFS value below this threshold, there is a high probability that they do not have advanced fibrosis.

On the other hand, values of the NFS above 0.676 offer an improved PPV, which means that if a patient has an NFS value above this threshold, there is an increased probability that they have advanced fibrosis [39].

While FIB-4 was used as a non-invasive tool for the diagnosis of MASH, it is now extensively applied for initial diagnosis of liver fibrosis in MASLD [40]. A FIB-4 score below 1.30 has been shown to effectively rule out advanced fibrosis in MASLD [41]. This means that if a patient's FIB-4 score is below 1.30, there is a high probability that they do not have advanced fibrosis. On the other hand, a FIB-4 score above 2.67 (or in some studies, above 3.25) has been identified as an indicator of advanced fibrosis [41]. This suggests that if a patient's FIB-4 score exceeds this threshold, there is an increased likelihood that they do have advanced fibrosis.

FIB-4 is valuable test in the primary care setting, as it can rule out the presence of advanced fibrosis with an excellent negative predictive value in the context of the absence of additional metabolic comorbidities. However, it is worth mentioning that cut-off values for FIB-4 may vary slightly across different studies or populations [42].

Table 3 reports blood biomarkers of liver fibrosis – including PRO—C3, WFAþ-M2BP, ELF Score, NASH Fibro Test, FibroMeter Virus

Table 3
Blood biomarkers and imaging for diagnosis of liver fibrosis in MASH.

Blood biomarkers	Mechanism	Assay	AUROC	Sensitivity	Specificity	References	Validations studies				Monitoring	Limitations
							AUROC	Sensitivity	Specificity	References		
Cleaved N-terminal propeptide of type III collagen PRO-C3	Extracellular matrix protein fragments released into the circulation during fibrogenesis	ELISA	0.74 for F3–F4	57 %	84 %	48 cross sectional study	0.73	56 %	84 %	29 Meta-analysis	Modest-to-moderate correlation with changes in histological fibrosis in clinical trials	Most data from MASLD
Wisteria floribunda agglutinin positive-M2BP (WFAþ-M2BP)	WFAþ-M2BP secreted from the liver during fibrosis progression	Lectin-antibody sandwich ELISA	0.82 for F3–F4	75 %	79.4 %	43 cross-sectional study	No validation studies				Insufficient data	Expensive; not widely available
Test Enhanced Liver Fibrosis (ELF score)	3 markers of fibrosis: PIIINP, hyaluronic acid, TIMP-1	ELISA	0.74 for F3–F4	71.4 %	74.1 %	49 cross-sectional study	0.83	73 %	80 %	44 meta-analysis	Associated with liver-related outcomes; monitoring role to be determined	Less useful for early fibrosis; expensive; not widely available
FibroMeter NAFLD	GGT, total bilirubin, a2 macroglobulin, apolipoprotein AI, haptoglobin, cholesterol, AST, ALT, triglycerides, and fasting glucose	Routine analyses	0.77 for F3–F4	20.9 %	96.5 %	50 cross-sectional study	0.82	65 %	86 %	45 meta-analysis	Correlates with fibrosis improvement after treatments for chronic viral hepatitis	Less useful for early fibrosis; expensive; most data from viral hepatitis
Hepascore	Bilirubin, gamma-glutamyltransferase, hyaluronic acid, alpha(2)-macroglobulin, age, and sex	Routine analyses ELISA	0.81 for F3–F4	67.4	76.1 %	45 meta-analysis	0.76	63.9 %	92.6 %	51 cross-sectional study	Associated with liver-related outcomes; monitoring role to be determined	Not widely available
RAB14	Waist circumference, plasma glucose, HDL-cholesterol, ALT	Flow cytometry	Fibrosis yes/not 0.96 0.80 for F3	100 % 80 %	95.8 % 81 %	33 Discovery cohort	0.99	99 %	89.6 %	33 validation cohort	Can monitor the effects of bariatric/metabolic surgery	Flow cytometry is not widely available

Imaging	Mechanism	Assay	AUROC	Specificity	Sensitivity	References	Validations studies				Monitoring	Limitations
							AUROC	Sensitivity	Specificity	References		
Transient elastography (Fibroscan)	Measurements are expressed as a velocity Measured in kPa	Ultrasound machine	0.80 for F2-F3	70.4 %	66.6 %	47 meta-analysis	0.85	79.1 %	90.1 %	52 cross sectional study	Can monitor the effects of MASH interventions	Cannot be used in patients with abdominal ascites; less precise in obesity
Vibration-controlled transient elastography	Measured in kPa	Ultrasound machine	0.83 for F=>3	86 %	75 %	53 cross-sectional study	0.94	70 %	93 %	42 meta-analysis	Can monitor the effects of MASH interventions	
Point shear wave elastography	Measured in m/s	Ultrasound machine	0.86 for F2-F4	69 %	85 %	25 Meta-analysis	0.84	92.9 %	83.1 %	54 cross-sectional study	Can monitor the effects of MASH interventions	
2D-Shear wave elastography	Shear wave propagation velocity is proportional to tissue elasticity Measured in kPa	Ultrasound machine	0.75 for F2-F4	71 %	67 %	25 Meta-analysis	0.91	100 %	77 %	55 cross-sectional study	Can monitor the effects of MASH interventions	Confounded by congestive heart failure, active hepatitis, biliary, severe obesity.
Magnetic resonance imaging	MRI Measured in kPa	MRI	0.91 for F2-F4	78 %	89 %	25 Meta-analysis	0.93	89.3 %	72.2 %	56 cross-sectional study	Can monitor the effects of MASH interventions	Expensive, not widely available

second generation, and FibroMeter NAFLD, and RAB14 – along with their AUC, sensitivity, specificity, and limitations [29,30,43–56].

While there are numerous studies evaluating the diagnostic performance of individual biomarkers, direct comparisons between different proprietary biomarkers are scarce in the literature.

However, some conference reports and preliminary studies suggest that the currently available direct biomarkers only marginally outperform or show similar performance to the FIB-4 score when targeting the presence of advanced fibrosis. This indicates that the added benefit of some proprietary biomarkers over FIB-4 may be modest.

The wider adoption of these proprietary biomarkers may be limited due to factors such as limited availability and higher cost compared to established non-invasive scoring systems like FIB-4. These practical considerations, including accessibility and cost-effectiveness, can influence their use in routine clinical practice.

The most widely adopted elastography technique for assessing liver stiffness is transient elastography, which is performed using FibroScan. FibroScan measures liver stiffness by quantifying the velocity of an induced shear wave as a surrogate of hepatic fibrosis. It is an ultrasound-based technique that is non-invasive and provides quick results.

Another ultrasound-based elastography technique is point shear wave elastography, also known as acoustic radiation force impulse (ARFI), or pSWE. This technique uses focused ultrasound beams to generate shear waves and measures their velocity to assess liver stiffness.

Two-dimensional shear wave elastography (2D-SWE) is another ultrasound-based technique that provides quantitative measurements of liver stiffness by analysing shear wave propagation.

In addition to ultrasound-based techniques, magnetic resonance elastography is an MRI-based method for assessing liver stiffness. MRE combines magnetic resonance imaging with mechanical waves to measure tissue stiffness. These elastography techniques offer non-invasive alternatives to liver biopsy for assessing liver fibrosis. They provide quantitative measurements of liver stiffness, which can correlate with fibrosis severity. These techniques are increasingly being used in clinical practice to assess fibrosis in patients with liver diseases, including MASLD. The accuracy and limitations of these imaging techniques are reported in Table 3 [29,43,48–56].

Each elastography technique has its advantages and limitations, and the choice of a technique may depend on factors such as availability, expertise, and patient-specific factors. Although one of the most promising and innovative techniques is MRE, real-time elastography Fibroscan is easy to perform and widely used. Eddowes et al. [23] found Youden cutoff values for F ≥ F2 of 8.2 kPa, for F ≥ F3 of 9.7 kPa, and for F=F4 of 13.6 kPa, respectively.

In the case of a patient of moderate or high risk for fibrosis, a sequential evaluation of the patient is performed along with imaging modalities (transient elastography, MRE) or other non-invasive tests (ELF) for further risk stratification.

Ras-related protein (RAB14), measured in circulating monocytes, in an algorithm including waist circumference, age, plasma glucose, high-density lipoprotein (HDL) cholesterol and ALT, performed very well in diagnosing the presence/absence of liver fibrosis [30]. The AUC was 0.96 (CI: 0.87–1) in the discovery cohort with sensitivity and specificity of 100 % and 95.8 %, while in the validation cohort it showed an AUC of 0.99 (CI 0.98–1), sensitivity of 99.0 % and specificity 89.6 % in the validation cohort [33].

RAB14 was also a valuable biomarker for diagnosing liver fibrosis stages, F ≤ 1, F = 2 and F = 3. The model's accuracy was about 70 % [33] with values similar to elastography.

3. Lifestyle modification and medications for MASH treatment

Weight loss is generally recommended in people with MASLD/MASH, but the effect of dieting is generally poor as shown in the DIE-TFITS Randomized Clinical Trial (RCT) [57]. In fact, the average 12-month weight loss was 5.3 kg (CI, 4.7–5.9 kg) for a low-fat diet and 6.0 kg (CI, 5.5–6.6 kg) for a low-carbohydrate diet [57]. Moreover, there are currently no specific surgical or pharmacologic interventions for MASH and/or liver fibrosis approved by FDA and/or EMA.

It has been shown that at least 10 % weight loss is necessary to achieve significant rates of MASH resolution [58]. Novel anti-obesity medications, such as semaglutide and tirzepatide, can induce a 12–17 % weight reduction [59,60]. However, although semaglutide administration was associated with MASH resolution without worsening of fibrosis in 59 % of people versus 17 % in the placebo group there were no significant differences in improvement of fibrosis [61].

Though a large number of drugs have been tested or are currently under scrutiny, only few phase 3 RCTs with histological endpoints are expected to be completed before the end of 2024, such as aramchol, resmetiron, obeticholic acid, and lanifibranor. Completion of RCT for semaglutide is expected in May 2028.

Aramchol (Galmed Pharmaceuticals) is a partial inhibitor of hepatic stearoyl-CoA desaturase (SCD1) that acts on both liver steatosis and fibrosis. The phase 2b RCT conducted in 247 patients did not meet the primary endpoint of hepatic triglyceride reduction at 52 weeks measured by MRS with aramchol 600 mg (95 % CI −6.4 to 0.2, p = 0.0655) [62]. NASH resolution without worsening of fibrosis in liver biopsy was not significantly different between aramchol 600 mg and placebo (OR 4.74; 95 % CI: 0.1–22.7; p = 0.051). Moreover, this drug did not improve liver fibrosis by one stage or more without worsening of steatohepatitis (OR 1.88; CI: 0.7–5.0; p = 0.21). In other words, the results of this trial were negative.

Resmetirom (Madrigal Pharmaceuticals) is a selective agonist of the thyroid hormone receptor–beta (THR-β) controlling triglyceride and cholesterol pathways in the liver.

Patients receiving resmetirom 80 mg orally once a day (n = 78) showed a significant reduction of hepatic fat assessed by MRI-PDFF versus placebo (n = 38) both at week 12 and week 36 [63]. However, the number of patients achieving histological reduction of at least 1 point of fibrosis without worsening of NASH did not differ from placebo.

Obeticholic acid (Ocaliva, Intercept Pharmaceuticals) is a semisynthetic bile acid binding to the farnesoid X receptor (FXR), a central hepatic regulator of inflammation, fibrosis, and glucose metabolism.

Forty-five percent of 110 patients receiving 25 mg daily of obeticholic acid had a 2-point or greater improvement in NAS without worsening of fibrosis as compared with 21 % of 109 patients in the placebo group (RR 1.9, 95 % CI 1.3–2·8; p = 0·0002) [64]. However, pruritus intense or widespread was reported in 23 % of the patients with obeticholic acid [64].

Lanifibranor (Inventiva) is an agonist of the three isoforms of PPAR alpha, delta and gamma with anti-inflammatory and anti-fibrotic effects. The primary end-point of the phase 2b trial [65] was the percentage of patients who had a decrease of at least 2 points in the SAF-A score without worsening of fibrosis. The primary end-point was reached in 55 % of the patients receiving a daily dose of 1200-mg versus 33 % in the placebo group (95 % CI, 1.2 to 2.3; p = 0.007).

4. Efficacy of metabolic surgery in MASH regression and in liver fibrosis improvement

About 30 % weight loss can be achieved with metabolic surgery even in the long-term [55] together with remission of type 2 diabetes [66–71].

In observational studies, metabolic surgery was able to determine MASH regression and net improvement of liver fibrosis. Lassailly et al. [72] reported resolution of MASH in 84 % of liver biopsies from 180 patients with severe obesity at 5-year follow-up, associated with 70 % improvement of liver fibrosis. Similar findings were also reported in another small observational study including 66 subjects [73].

In our recent RCT [74], the primary endpoint of MASH regression without worsening of fibrosis was achieved in 70.1 % and 69.6 % of

participants who underwent Roux-en-Y Gastric Bypass (RYGB) and Sleeve Gastrectomy (SG) respectively, as compared with 18.7 % in patients under standard of care associated with dieting and physical exercise (p < 0.0001). Improvement of fibrosis of at least one stage without worsening of MASH was instead detected in 79 % and 76 % of participants in the RYGB and SG groups, respectively, while it was observed in 49 % of people in the medical arm (p = 0.006). Interestingly, RYGB and SG had similar results on MASH and liver fibrosis although RYGB was associated with a higher diabetes remission rate and a greater improvement of insulin sensitivity and lipid profile than SG [74]. Indeed, metabolic surgery was more effective than lifestyle interventions and best medical care in the treatment of MASH [74]. These findings further support the use of metabolic surgery in people with metabolic diseases. MASH should be considered as an important factor in decision making around prioritization of surgery in people with obesity and type 2 diabetes.

5. Liver cirrhosis surgical and drug treatment

Although liver cirrhosis was generally regarded as the irreversible stage of both viral and metabolic chronic liver disease, at least in animal models the fibrosis septs rapidly disappear once the hepatic insult is cessed [75,76]. However, regenerative nodules make the cirrhotic process irreversible [77].

A clinical study in 15 patients with liver biopsy before and after therapy demonstrated that viral liver cirrhosis can reverse in 1/3 of the patients after 5-year treatment with adefovir [78].

A retrospective study on 27 patients with liver cirrhosis Child A who underwent either RYGB or SG showed that while 2 patients had a worsening of liver function, resulting in a higher Child–Pugh score, 3 were removed from the waiting list for a liver transplantation [79].

There is a series of RCT targeting metabolically-induced liver cirrhosis.

FALCON 2 (NCT03486912) was a phase 2b RCT assessing the efficacy of Pegbelfermin (10, 20, or 40 mg) versus placebo in biopsy-proven MASH and stage 4 fibrosis. It did not meet its primary endpoint [80].

Patients with MASH and compensated cirrhosis received once a week semaglutide 2.4 mg or placebo with randomization 2:1. Semaglutide was ineffective in improving liver fibrosis versus placebo [81].

Aldafermin is currently being studied in a phase 2b trial in patients with compensated MASH cirrhosis.

MAESTRO-NASHOUTCOMES (NCT05500222) is an ongoing phase 3 RCT in patients with MASH cirrhosis with primary endpoint all-cause mortality and liver decompensation events.

Another ongoing trial is ALPINE 4 study (NCT04210245) that evaluates the safety and efficacy of aldafermin in patients with MASH and compensated cirrhosis.

6. Cost-effectiveness of metabolic surgery in people with obesity and MASH

The cost-effectiveness of metabolic surgery or lifestyle intervention for the treatment of MASH in people with obesity or overweight was studied by Klebanoff et al. [82]. They demonstrated that metabolic surgery is cost-effective for patients with obesity and any stage of liver fibrosis, from F0 to F3. The incremental cost-effectiveness ratio (ICER) for performing metabolic versus reserving surgery only for patients with histological F3 were $48,836/quality-adjusted life years (QALYs), $24,949/QALY and $19,222/QALY, for mild, moderate and severe obesity, respectively. In contrast, extending surgery to people with overweight was not cost-effective unless advanced fibrosis (F3) was present [82].

7. Mechanism of action of metabolic surgery in MASH resolution

It is acknowledged that metabolic surgery improves glycaemic control through early amelioration of hepatic insulin sensitivity and later improvement of peripheral insulin sensitivity [83–86]. Improved insulin sensitivity might contribute to histological MASH resolution or improvement through reduction of hepatocyte de novo lipogenesis and amelioration of mitochondrial fatty acid β-oxidation [87]. Furthermore post-prandial insulin secretion increases as a consequence of the rise in glucagon-like peptide 1 (GLP-1) secretion [88]. GLP-1 has also beneficial gene-regulatory effects on fatty acid oxidation and insulin sensitivity in hepatocytes [89].

An important role in MASH resolution is played by the bypass of the upper gut. We have shown that the bypass of the upper gut improves insulin sensitivity while when glucose is delivered directly into the jejunum insulin resistance is elicited [91]. Following a high-fat, high-sugar diet, the mammalian intestinal epithelial cells produce heat shock proteins (HSPs), HSP70 and GRP78 [91]. Via the portal venous system, these HSPs are transported to the liver where they activate toll-like receptor 4 (TLR4) signalling and trigger endoplasmic reticulum (ER) stress [91]. HSP70 and GRP78 cause insulin resistance and MASH associated with liver fibrosis in rodents, while the bypass of the upper gut reverses their effects [92].

8. Liquid biopsy and penetrance of metabolic surgery

The incidence of MASLD in the general population is difficult to estimate, however a study in Hong Kong using serial proton-MR spectroscopy showed an incidence of 3.7 per 100 person-years [93]. This figure is much worse in people with type 2 diabetes, who have an incidence of MASLD of 52 % in 3 years, as demonstrated by serial transient elastography [94].

Individuals with NAS scores of 4 or higher and fibrosis stages 2 or higher are at a higher risk of progressing to cirrhosis. These patients require close monitoring, lifestyle modifications, and potential pharmacological interventions to slow down or halt the progression of liver disease. Identifying candidates for treatment at this stage allows healthcare providers to implement targeted interventions, such as weight loss programs, dietary changes, exercise regimens, medications, medical devices, or surgery, to manage MASH and prevent further liver damage. Early intervention may help reduce the risk of complications and improve long-term outcomes for individuals with MASH.

A histological feature typical of MASH is the presence of hepatocellular ballooning. Hepatocellular ballooning refers to the swelling and degeneration of hepatocytes with the presence of Mallory-Denk bodies or similar structures [95].

The distinction between MASLD with a relatively benign prognosis and MASH with a considerably worse prognosis is important because MASH has the potential to progress to advanced liver diseases such as fibrosis, cirrhosis, and even hepatocellular carcinoma [8,9]. Hepatocellular ballooning is considered a key histological feature that helps differentiate between these two conditions. Its presence indicates a higher likelihood of MASH and a more severe form of MASLD.

Hepatocellular ballooning is indeed a critical component of various morphological grading and staging systems for MASLD. The presence and degree of hepatocellular ballooning, along with other features like inflammation and fibrosis, are used to determine the stage of the disease and guide clinical management [95].

When it comes to clinical studies evaluating novel therapeutic strategies for MASLD, researchers often focus on patients who have a histologically confirmed diagnosis of MASH. This selection criterion ensures that the study population includes individuals with more advanced liver disease and a higher risk of progression, which is crucial for assessing the efficacy of the new treatments. Since MASH is associated with worse outcomes compared to simple steatosis, targeting patients with confirmed MASH in clinical trials helps identifying interventions that can effectively address the more severe form of the disease.

It is worth noting that while liver biopsy remains the gold standard

for diagnosing and grading MASLD, efforts are being made to develop non-invasive methods for assessing disease severity, such as imaging techniques and blood-based biomarkers. These methods aim to reduce the need for invasive procedures like liver biopsies while still providing accurate information about the extent of liver damage and the presence of features like hepatocellular ballooning. The diagnosis of MASH based on histological features can be challenging, and hepatocellular ballooning is one of the key features that contribute to this diagnostic difficulty. Ballooning hepatocytes exhibit a range of morphological changes in their cytoplasm and contents, which can make their reliable recognition difficult, even for experienced pathologists. This can lead to variability in interpretation between different observers [96]. Therefore, a liquid biopsy that provides not only a yes or no response in terms of presence or absence of MASH and/or liver fibrosis, but which can grade the severity of MASH and stage the level of fibrosis is of outmost importance permitting to identify people in need of treatment for their hepatic disease.

We have shown with class 1 evidence that metabolic surgery determines MASH regression without worsening of fibrosis in two-thirds of the patients and amelioration of at least 1 stage of liver fibrosis in 76–79 % of cases [63]. Therefore, the use of a liquid biopsy such as monocyte PLIN2 and RAB14 analysis with the potential to replace invasive liver biopsy-based histology for the diagnosis of MASH and liver fibrosis can provide an early diagnosis of at-risk MASH and enable the implementation of surgical intervention in eligible patients.

Despite several pharmaceutical companies have been conducting clinical trials focused on various targets – including metabolic pathways, inflammation, and fibrosis – to evaluate potential therapies for MASH, there are no MASH therapies approved by FDA and/or EMA. Given the low compliance to long-term dieting and the lack of available pharmacological treatments, metabolic surgery currently remains the only possible therapy for MASH [63].

In 2012 the Centers for Medicare & Medicaid Services (CMS) released the National Coverage Determination (NCD) regarding metabolic surgery for the treatment of morbid obesity [97]. The NCD outlines the criteria for Medicare coverage of metabolic surgical procedures for eligible beneficiaries with severe obesity and associated comorbidities. According to the NCD, Medicare beneficiaries who have a body mass index equal to or >35 together with at least one comorbidity that is directly related to obesity, such as type 2 diabetes, refractory hypertension, refractory hyperlipidemia, obesity-induced cardiomyopathy, clinically significant obstructive sleep apnea, obesity-related hypoventilation, pseudotumor cerebri, severe arthropathy of spine and/or weight-bearing joints or hepatic steatosis without prior evidence of active inflammation, may be eligible for coverage of metabolic surgery: This means that even the presence of MASLD in people with BMI ≥ 35 kg/m^2 makes these patients eligible for metabolic surgery.

For eligible beneficiaries meeting these criteria, the following surgical procedures were deemed reasonable and necessary under the 2012 NCD [97]:

- Open and Laparoscopic Roux-en-Y Gastric Bypass.
- Laparoscopic Adjustable Gastric Banding.
- Open and Laparoscopic Biliopancreatic Diversion with Duodenal Switch.

At least in the US, sleeve gastrectomy is not considered among the procedures that can be performed with Medicare coverage. Since SG performs equally well as RYGB [63] for MASH treatment, hopefully SG might be considered in the future for the treatment of MASH.

Should the liquid biopsy increase dramatically the diagnosis of MASLD/MASH, then all patients with a BMI ≥ 35 kg/m^2 and MASLD/MASH would be eligible for metabolic surgery increasing exponentially the penetration of metabolic surgery.

At the moment, in fact, the AASLD Practice Guidance for MASLD do not include metabolic surgery as a specific indication for treating MASLD or MASH, but rather it reiterates that the surgical option should be reserved for those patients who already "meet criteria for metabolic weight loss" [98].

9. Brief overview of complications of metabolic surgery and its cost-effectiveness in MASH

The 90-day mortality rate of metabolic surgery is 0.1–0.3 % depending on the type of operation while the complication rate accounts to 7–10 % and includes upper gastrointestinal haemorrhage, leaks, stenosis, regurgitation, obstruction, and severe reflux [99].

Cost-effectiveness of metabolic surgery was recently analysed in patients with MASH and liver fibrosis stages from F0 to F3 [82]. Quality-adjusted life years (QALYs) varied from $48,836/QALY to $24,949/QALY and to $19,222/QALY for patients with mild, moderate, or severe obesity independently of the severity of liver fibrosis. The authors found that metabolic surgery was both effective and cost-effective for people with MASH and obesity, regardless of fibrosis stages [82].

10. Conclusions

Metabolic surgery has been shown to regress MASH and liver fibrosis and is superior to lifestyle modification as best medical care. Liquid biopsies can substantially increase MASH diagnoses and consequently enhance the penetration of metabolic surgery that is a cost-effective therapy for MASH independently of the severity of liver fibrosis. Yet, there are no established criteria for metabolic surgery, which is often prescribed on a first-come-first-served basis. Therefore, it is imperative to develop clear, evidence-based criteria for qualifying patients for metabolic surgery, prioritizing those with diagnosed MASH, to ensure a more effective and equitable allocation of this limited treatment resource.

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References

[1] Rinella ME, Lazarus JV, Ratziu V, Francque SM, Sanyal AJ, Kanwal F, et al. A multi-society Delphi consensus statement on new fatty liver disease nomenclature. Hepatology 2023 Jun 24. https://doi.org/10.1097/HEP.0000000000000520.

[2] Riazi K, Azhari H, Charette JH, Underwood FE, King JA, Afshar EE, et al. The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2022 Sep;7(9):851–61.

[3] Le MH, Yeo YH, Zou B, Barnet S, Henry L, Cheung R, et al. Forecasted 2040 global prevalence of nonalcoholic fatty liver disease using hierarchical Bayesian approach. Clin Mol Hepatol 2022 Oct;28(4):841–50.

[4] Younossi ZM, Golabi P, de Avila L, Paik JM, Srishord M, Fukui N, et al. The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: a systematic review and meta-analysis. J Hepatol 2019;71(4):793–801.

[5] Quek J, Chan KE, Wong ZY, Tan C, Tan B, Lim WH, et al. Global prevalence of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis in the overweight and obese population: a systematic review and meta-analysis. Lancet Gastroenterol Hepatol 2023 Jan;8(1):20–30.

[6] McPherson S, Hardy T, Henderson E, Burt AD, Day CP, Anstee QM. Evidence of NAFLD progression from steatosis to fibrosing-steatohepatitis using paired biopsies: implications for prognosis and clinical management. J Hepatol 2015;62 (5):1148–55.

[7] Singh S, Khera R, Allen AM, Murad MH, Loomba R. Comparative effectiveness of pharmacological interventions for nonalcoholic steatohepatitis: a systematic review and network meta-analysis. Hepatology 2015;62(5):1417–32.

[8] Chalasani N, Younossi Z, Lavine JE, Charlton M, Cusi K, Rinella M, et al. The diagnosis and management of nonalcoholic fatty liver disease: practice guidance from the American Association for the Study of Liver Diseases. Hepatology 2018; 67:328–57.

[9] Estes C, Anstee QM, Arias-Loste MT, Bantel H, Bellentani S, Caballeria J, et al. Modeling NAFLD disease burden in China, France, Germany, Italy, Japan, Spain, United Kingdom, and United States for the period 2016–2030. J Hepatol 2018;69: 896–904.

[10] Patil R, Sood GK. Non-alcoholic fatty liver disease and cardiovascular risk. World J Gastrointest Pathophysiol 2017;8:51–8.

[11] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.

[12] Kleiner DE, Makhlouf HR. Histology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in adults and children. Clin Liver Dis 2016;20: 293–312.

[13] Friedman SL, Ratziu V, Harrison SA, Abdelmalek MF, Aithal GP, Caballeria J, et al. A randomized, placebo-controlled trial of cenicriviroc for treatment of nonalcoholic steatohepatitis with fibrosis. Hepatology 2018;67:1754–67.

[14] Rasmussen DGK, Anstee QM, Torstenson R, Golding B, Patterson SD, Brass C, et al. NAFLD and NASH biomarker qualification in the LITMUS consortium – lessons learned. J Hepatol 2023 Apr;78(4):852–65.

[15] Anstee QM, Castera L, Loomba R. Impact of non-invasive biomarkers on hepatology practice: past, present and future. J Hepatol 2022 Jun;76(6):1362–78.

[16] Caballería L, Pera G, Arteaga I, Rodríguez L, Alumà A, Morillas RM, et al. High prevalence of liver fibrosis among European adults with unknown liver disease: a population-based study. Clin Gastroenterol Hepatol 2018 Jul;16(7):1138–1145.e5.

[17] Majumdar A, Campos S, Gurusamy K, Pinzani M, Tsochatzis EA. Defining the minimum acceptable diagnostic accuracy of noninvasive fibrosis testing in cirrhosis: a decision analytic modeling study. Hepatology 2020 Feb;71(2):627–42.

[18] NCD Risk Factor Collaboration (NCD-RisC). Trends in adult body-mass index in 200 countries from 1975 to 2014: a pooled analysis of 1698 population-based measurement studies with 19·2 million participants. Lancet 2016 Apr 2;387 (10026):1377–96 [Williamson K, Nimegeer A, Lean M. Rising prevalence of BMI ≥40 kg/m2: A high-demand epidemic needing better documentation. Obes Rev. 2020 Apr;21(4):e12986].

[19] Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. Aliment Pharmacol Ther 2014 Nov;40(10):1209–22.

[20] Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011 Sep 2;54(3):1082–90.

[21] Wildman-Tobriner B, Middleton MM, Moylan CA, Rossi S, Flores O, Chang ZA, et al. Association between magnetic resonance imaging-proton density fat fraction and liver histology features in patients with nonalcoholic fatty liver disease or nonalcoholic steatohepatitis. Gastroenterology 2018 Nov;155(5):1428–1435.e2.

[22] Loomba R, Neuschwander-Tetri BA, Sanyal A, Chalasani N, Diehl AM, Terrault N, et al. Multicenter validation of association between decline in MRI-PDFF and histologic response in NASH. Hepatology 2020 Oct;72(4):1219–29.

[23] Eddowes PJ, Sasso M, Allison M, Tsochatzis E, Anstee QM, Sheridan D, et al. Accuracy of FibroScan controlled attenuation parameter and liver stiffness measurement in assessing steatosis and fibrosis in patients with nonalcoholic fatty liver disease. Gastroenterology 2019 May;156(6):1717–30.

[24] Poynard T, Lassailly G, Diaz E, Clement K, Caïazzo R, Tordjman J, et al. Performance of biomarkers FibroTest, ActiTest, SteatoTest, and NashTest in patients with severe obesity: meta-analysis of individual patient data. PLoS One 2012;7(3):e30325.

[25] Bril F, McPhaul MJ, Caulfield MP, Castille JM, Poynard T, Soldevila-Pico C, et al. Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. J Investig Med 2019 Feb;67(2): 303–11.

[26] Poynard T, Paradis V, Mullaert J, Deckmyn O, Gault N, Marcault E, et al. Prospective external validation of a new non-invasive test for the diagnosis of non-alcoholic steatohepatitis in patients with type 2 diabetes. Aliment Pharmacol Ther 2021 Oct;54(7):952–66.

[27] Kim TH, Jeong CW, Jun HY, Lee C, Noh S, Kim JE, et al. Accuracy of proton magnetic resonance for diagnosing non-alcoholic steatohepatitis: a meta-analysis. Sci Rep 2019 Oct 18;9(1):15002.

[28] Selvaraj EA, Mózes FE, Jayaswal ANA, Zafarmand MH, Vali Y, Lee JA, et al. Diagnostic accuracy of elastography and magnetic resonance imaging in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2021 Oct;75(4): 770–85.

[29] Contreras D, González-Rocha A, Clark P, Barquera S, Denova-Gutiérrez E. Diagnostic accuracy of blood biomarkers and non-invasive scores for the diagnosis of NAFLD and NASH: systematic review and meta-analysis. Ann Hepatol 2023 Jan–Feb;28(1):100873.

[30] Nielsen MJ, Leeming DJ, Goodman Z, Friedman S, Frederiksen P, Rasmussen DGK, et al. Comparison of ADAPT, FIB-4 and APRI as non-invasive predictors of liver

[31] fibrosis and NASH within the CENTAUR screening population. J Hepatol 2021 Dec; 75(6):1292–300.

[31] Harrison SA, Ratziu V, Boursier J, Francque S, Bedossa P, Majd Z, 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. Lancet Gastroenterol Hepatol 2020 Nov;5(11):970–85.

[32] Harrison SA, Ratziu V, Magnanensi J, Hajji Y, Deledicque S, Majd Z, et al. NIS2+ TM, an optimization of the blood-based biomarker NIS4® technology for the detection of at-risk NASH: a prospective derivation and validation study. J Hepatol 2023 May 8;S0168-8278(23):00318-5.

[33] Angelini G, Panunzi S, Castagneto-Gissey L, Pellicanò F, De Gaetano A, Pompili M, et al. Accurate liquid biopsy for the diagnosis of non-alcoholic steatohepatitis and liver fibrosis. Gut 2023 Feb;72(2):392–403.

[34] Perakakis N, Stefanakis K, Mantzoros CS. The role of omics in the pathophysiology, diagnosis and treatment of non-alcoholic fatty liver disease. Metabolism 2020 Oct; 111S:154320.

[35] Sheth SG, Flamm SL, Gordon FD, Chopra S. AST/ALT ratio predicts cirrhosis in patients with chronic hepatitis C virus infection. Am J Gastroenterol 1998 Jan;93 (1):44–8.

[36] Imperiale TF, Said AT, Cummings OW, Born LJ. Need for validation of clinical decision aids: use of the AST/ALT ratio in predicting cirrhosis in chronic hepatitis C. Am J Gastroenterol 2000 Sep;95(9):2328–32.

[37] McPherson S, Stewart SF, Henderson E, Burt AD, Day CP. Simple non-invasive fibrosis scoring systems can reliably exclude advanced fibrosis in patients with non-alcoholic fatty liver disease. Gut 2010 Sep;59(9):1265–9.

[38] McPherson S, Hardy T, Dufour JF, Petta S, Romero-Gomez M, Allison M, et al. Age as a confounding factor for the accurate non-invasive diagnosis of advanced NAFLD fibrosis. Am J Gastroenterol 2017 May;112(5):740–51.

[39] Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology 2007 Apr;45(4):846–54.

[40] Anstee QM, Lawitz EJ, Alkhouri N, Wong VW, Romero-Gomez M, Okanoue T, et al. Noninvasive tests accurately identify advanced fibrosis due to NASH: baseline data from the STELLAR trials. Hepatology 2019 Nov;70(5):1521–30.

[41] Siddiqui MS, Yamada G, Vuppalanchi R, Van Natta M, Loomba R, Guy C, et al. Diagnostic accuracy of noninvasive fibrosis models to detect change in fibrosis stage. Clin Gastroenterol Hepatol 2019 Aug;17(9):1877–1885.e5.

[42] Blanco-Grau A, Gabriel-Medina P, Rodriguez-Algarra F, Villena Y, Lopez-Martínez R, Augustín S, et al. Assessing liver fibrosis using the FIB4 index in the community setting. Diagnostics 2021 Nov 29;11(12):2236.

[43] Jang TY, Huang CF, Yeh ML, Huang CI, Dai CY, Tsai PC, et al. Serum Wisteria floribunda agglutinin-positive Mac-2-binding protein expression predicts disease severity in nonalcoholic steatohepatitis patients. Kaohsiung J Med Sci 2022 Mar;38 (3):261–7.

[44] Vali Y, Lee J, Boursier J, Spijker R, Löffler J, Verheij J, et al. LITMUS systematic review team(†). Enhanced liver fibrosis test for the non-invasive diagnosis of fibrosis in patients with NAFLD: a systematic review and meta-analysis. J Hepatol 2020 Aug;73(2):252–62.

[45] Van Dijk AM, Vali Y, Mak AL, Lee J, Tushuizen ME, Zafarmand MH, et al. Systematic review with meta-analyses: diagnostic accuracy of FibroMeter tests in patients with non-alcoholic fatty liver disease. J Clin Med 2021 Jun 29;10(13): 2910.

[46] Martinou E, Pericleous M, Stefanova I, Kaur V, Angelidi AM. Diagnostic modalities of non-alcoholic fatty liver disease: from biochemical biomarkers to multi-omics non-invasive approaches. Diagnostics 2022 Feb 4;12(2):407.

[47] Hashemi SA, Alavian SM, Gholami-Fesharaki M. Assessment of transient elastography (FibroScan) for diagnosis of fibrosis in non-alcoholic fatty liver disease: a systematic review and meta-analysis. Caspian J Intern Med 2016 Fall;7 (4):242–52.

[48] Luo Y, Oseini A, Gagnon R, Charles ED, Sidik K, Vincent R, et al. An evaluation of the collagen fragments related to fibrogenesis and fibrolysis in nonalcoholic steatohepatitis. Sci Rep 2018 Aug 17;8(1):12414.

[49] López IC, Aroca FG, Bernal MDF, Mompeán JAL, Bernal ÁB, Martínez AMH, et al. Utility of the ELF test for detecting non-alcoholic fatty liver disease in morbid obese patients with suspicion of non-alcoholic fatty liver disease. Obes Surg 2017 Sep;27(9):2347–53.

[50] Loong TC, Wei JL, Leung JC, Wong GL, Shu SS, Chim AM, et al. Application of the combined FibroMeter vibration-controlled transient elastography algorithm in Chinese patients with non-alcoholic fatty liver disease. J Gastroenterol Hepatol 2017 Jul;32(7):1363–9.

[51] Bertot LC, Jeffrey GP, de Boer B, Wang Z, Huang Y, Garas G, et al. Comparative accuracy of clinical fibrosis markers, Hepascore and Fibroscan® to detect advanced fibrosis in patients with nonalcoholic fatty liver disease. Dig Dis Sci 2023 Jun;68 (6):2757–67.

[52] Dincses E, Yilmaz Y. Diagnostic usefulness of FibroMeter VCTE for hepatic fibrosis in patients with nonalcoholic fatty liver disease. Eur J Gastroenterol Hepatol 2015; 27:1149–53.

[53] Eilenberg M, Munda P, Stift J, Langer FB, Prager G, Trauner M, Staufer K. Accuracy of non-invasive liver stiffness measurement and steatosis quantification in patients with severe and morbid obesity. Hepatobiliary Surg Nutr 2021 Oct;10(5):610–22. https://doi.org/10.21037/hbsn-20-787.

[54] Joo I, Kim SY, Park HS, Lee ES, Kang HJ, Lee JM. Validation of a new point shear-wave elastography method for noninvasive assessment of liver fibrosis: a prospective multicenter study. Korean J Radiol 2019 Nov;20(11):1527–35.

[55] Jang JK, Lee ES, Seo JW, Kim YR, Kim SY, Cho YY, et al. Two-dimensional shear-wave elastography and US attenuation imaging for nonalcoholic steatohepatitis diagnosis: a cross-sectional, multicenter study. Radiology 2022 Oct;305(1):118–26.

[56] Noureddin M, Truong E, Gornbein JA, Saouaf R, Guindi M, Todo T, et al. MRI-based (MAST) score accurately identifies patients with NASH and significant fibrosis. J Hepatol 2022 Apr;76(4):781–7.

[57] Gardner CD, Trepanowski JF, Del Gobbo LC, Hauser ME, Rigdon J, Ioannidis JPA, et al. Effect of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion: the DIETFITS randomized clinical trial. JAMA 2018 Feb 20;319(7):667–79.

[58] Vilar-Gomez E, Martinez-Perez Y, Calzadilla-Bertot L, Torres-Gonzalez A, Gra-Oramas B, Gonzalez-Fabian L, et al. Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. Gastroenterology 2015;149:367–378.e5.

[59] Wilding JPH, Batterham RL, Calanna S, Davies M, Van Gaal LF, Lingvay I, et al. Once-weekly semaglutide in adults with overweight or obesity. N Engl J Med 2021 Mar 18;384(11):989–1002.

[60] Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, et al. SURMOUNT-1 Investigators. Tirzepatide once weekly for the treatment of obesity. N Engl J Med 2022;387:205–16.

[61] Newsome PN, Buchholtz K, Cusi K, Linder M, Okanoue T, Ratziu V, et al. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. N Engl J Med 2021;384:1113–24.

[62] Ratziu V, de Guevara L, Safadi R, Poordad F, Fuster F, Flores-Figueroa J, et al. Aramchol in patients with nonalcoholic steatohepatitis: a randomized, double-blind, placebo-controlled phase 2b trial. Nat Med 2021 Oct;27(10):1825–35.

[63] Harrison SA, Bashir MR, Guy CD, Zhou R, Moylan CA, Frias JP, et al. Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. Lancet 2019 Nov 30; 394(10213):2012–24.

[64] Neuschwander-Tetri BA, Loomba R, Sanyal AJ, Lavine JE, Van Natta ML, Abdelmalek MF, et al. Farnesoid X nuclear receptor ligand obeticholic acid for non-cirrhotic, non-alcoholic steatohepatitis (FLINT): a multicentre, randomised, placebo-controlled trial. Lancet 2015 Mar 14;385(9972):956–65.

[65] Francque SM, Bedossa P, Ratziu V, Anstee QM, Bugianesi E, Sanyal AJ, et al. A randomized, controlled trial of the pan-PPAR agonist lanifibranor in NASH. N Engl J Med 2021 Oct 21;385(17):1547–58.

[66] Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Leccesi L, et al. Bariatric-metabolic surgery versus conventional medical therapy for type 2 diabetes. N Engl J Med 2012;366:1577–85.

[67] Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Nanni G, et al. Bariatric-bariatric-metabolic surgery versus conventional medical treatment in obese patients with type 2 diabetes: 5 year follow-up of an open-label, single-centre, randomised controlled trial. Lancet 2015;386:964–73.

[68] Schauer PR, Kashyap SR, Wolski K, Brethauer SA, Kirwan JP, Pothier CE, et al. Bariatric-metabolic surgery versus intensive medical therapy in obese patients with diabetes. N Engl J Med 2012;366:1567–76.

[69] Schauer PR, Bhatt DL, Kirwan JP, Wolski K, Brethauer SA, Navaneethan SD, et al. Bariatric-metabolic surgery versus intensive medical therapy for diabetes—3-year outcomes. N Engl J Med 2014;370:2002–13.

[70] Ikramuddin S, Korner J, Lee WJ, Thomas AJ, Connett JE, Bantle JP, et al. Lifestyle intervention and medical management with vs without roux-en-y gastric bypass and control of hemoglobin A1c, LDL cholesterol, and systolic blood pressure at 5 years in the diabetes surgery study. JAMA 2018;319:266–78.

[71] Mingrone G, Panunzi S, De Gaetano A, Guidone C, Iaconelli A, Capristo E, et al. Bariatric-metabolic surgery versus conventional medical therapy in patients with type 2 diabetes: 10-year follow-up of an open-label, single-centre, randomised controlled trial. Lancet 2021;397:293–304.

[72] Lassailly G, Caiazzo R, Ntandja-Wandji LC, Gnemmi V, Baud G, Verkindt H, et al. Bariatric-metabolic surgery provides long-term resolution of nonalcoholic steatohepatitis and regression of fibrosis. Gastroenterology 2020;159:1290–1301. e5.

[73] Pais R, Aron-Wisnewsky J, Bedossa P, Ponnaiah M, Oppert JM, Siksik JM, et al. Persistence of severe liver fibrosis despite substantial weight loss with bariatric-metabolic surgery. Hepatology 2022;76(2):456–68.

[74] Verrastro O, Panunzi S, Castagneto-Gissey L, De Gaetano A, Lembo E, Capristo E, et al. Bariatric-metabolic surgery versus lifestyle intervention plus best medical care in non-alcoholic steatohepatitis (BRAVES): a multicentre, open-label, randomised trial. Lancet 2023 May 27;401(10390):1786–97.

[75] Maurice J, Pinzani M. The stratification of cirrhosis. Hepatol Res 2020;50:535–41.

[76] Theise ND, Jia J, Sun Y, Wee A, You H. Progression and regression of fibrosis in viral hepatitis in the treatment era: the Beijing classification. Mod Pathol 2018;31: 1191–200.

[77] Quinn PS, Higginson J. Reversible and irreversible changes in experimental cirrhosis. Am J Pathol 1965;47:354–69.

[78] Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. Gastroenterology 2006 Dec;131(6):1743–51.

[79] Mehdorn AS, Moulla Y, Mehdorn M, Dietrich A, Schönfels W, Becker T, et al. Bariatric surgery in liver cirrhosis. Front Surg 2022 Dec 15;(9):986297.

[80] Abdelmalek MF, Sanyal AJ, Nakajima A, Neuschwander-Tetri BA, Goodman ZD, Lawitz EJ, et al. Pegbelfermin in patients with nonalcoholic steatohepatitis and compensated cirrhosis (FALCON 2): a randomized phase 2b study. Clin Gastroenterol Hepatol 2023 Apr 23;S1542-3565(23):00311-7.

[81] Loomba R, Abdelmalek MF, Armstrong MJ, Jara M, Kjær MS, Krarup N, et al. Semaglutide 2·4 mg once weekly in patients with non-alcoholic steatohepatitis-related cirrhosis: a randomised, placebo-controlled phase 2 trial. Lancet. Gastroenterol Hepatol 2023 Jun;8(6):511–22. https://doi.org/10.1016/S2468-1253(23)00068-7 [Epub 2023 Mar 16. PMID: 36934740].

[82] Klebanoff MJ, Corey KE, Chhatwal J, Kaplan LM, Chung RT, Hur C. Bariatric surgery for nonalcoholic fatty liver disease: a clinical and cost-effectiveness analysis. Hepatology 2017 Apr;65(4):1156–64.

[83] Previti E, Salinari S, Bertuzzi A, Capristo E, Bornstein S, Mingrone G. Glycemic control after metabolic surgery: a Granger causality and graph analysis. Am J Physiol Endocrinol Metab 2017 Nov 1;313(5):E622–30.

[84] Gastaldelli A, Iaconelli A, Gaggini M, Magnone MC, Veneziani A, Rubino F, et al. Short-term effects of laparoscopic adjustable gastric banding versus roux-en-y gastric bypass. Diabetes Care 2016 Nov;39(11):1925–31.

[85] Russo MF, Lembo E, Mari A, Angelini G, Verrastro O, Nanni G, et al. Insulin resistance is central to long-term reversal of histologic nonalcoholic steatohepatitis after metabolic surgery. J Clin Endocrinol Metab 2021 Mar 8;106(3):750–61.

[86] Khan RS, Bril F, Cusi K, Newsome PN. Modulation of insulin resistance in nonalcoholic fatty liver disease. Hepatology 2019 Aug;70(2):711–24.

[87] Laursen TL, Hagemann CA, Wei C, Kazankov K, Thomsen KL, Knop FK, et al. Bariatric surgery in patients with non-alcoholic fatty liver disease - from pathophysiology to clinical effects. World J Hepatol 2019;11(2):138–49 [PMID: 30820265], https://doi.org/10.4254/wjh.v11.i2.138.

[88] Hsiao PJ, Chiou HC, Jiang HJ, Lee MY, Hsieh TJ, Kuo KK. Pioglitazone enhances cytosolic lipolysis, β-oxidation and autophagy to ameliorate hepatic steatosis. Sci Rep 2017 Aug 22;7(1):9030.

[89] Svegliati-Baroni G, Saccomanno S, Rychlicki C, Agostinelli L, De Minicis S, Candelaresi C, et al. Glucagon-like peptide-1 receptor activation stimulates hepatic lipid oxidation and restores hepatic signalling alteration induced by a high-fat diet in nonalcoholic steatohepatitis. Liver Int 2011;31:1285–97.

[91] Angelini G, Salinari S, Castagneto-Gissey L, Bertuzzi A, Casella-Mariolo J, Ahlin S, et al. Small intestinal metabolism is central to whole-body insulin resistance. Gut 2021 Jun;70(6):1098–109.

[92] Angelini G, Castagneto-Gissey L, Salinari S, Bertuzzi A, Anello D, Pradhan M, et al. Upper gut heat shock proteins HSP70 and GRP78 promote insulin resistance, hyperglycemia, and non-alcoholic steatohepatitis. Nat Commun 2022 Dec 13;13 (1):7715.

[93] Wong VW, Wong GL, Yeung DK, Lau TK, Chan CK, Chim AM, et al. Incidence of non-alcoholic fatty liver disease in Hong Kong: a population study with paired proton-magnetic resonance spectroscopy. J Hepatol 2015 Jan;62(1):182–9.

[94] Lee HW, Wong GL, Kwok R, Choi KC, Chan CK, Shu SS, et al. Serial transient elastography examinations to monitor patients with type 2 diabetes: a prospective cohort study. Hepatology 2020 Oct;72(4):1230–41.

[95] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005 Jun;41(6):1313–21.

[96] Younossi ZM, Gramlich T, Liu YC, Matteoni C, Petrelli M, Goldblum J, et al. Nonalcoholic fatty liver disease: assessment of variability in pathologic interpretations. Mod Pathol 1998 Jun;11(6):560–5.

[97] https://www.cms.gov/medicare-coverage-database/view/ncd.aspx?ncdid=57&n cdver=4&.

[98] Rinella ME, Neuschwander-Tetri BA, Siddiqui MS, Abdelmalek MF, Caldwell S, Barb D, et al. AASLD Practice Guidance on the clinical assessment and management of nonalcoholic fatty liver disease. Hepatology 2023 May 1;77(5):1797–835.

[99] Kumbhari V, Cummings DE, Kalloo AN, Schauer PR. AGA clinical practice update on evaluation and management of early complications after bariatric/metabolic surgery: expert review. Clin Gastroenterol Hepatol 2021 Aug;19(8):1531–7.