Non-invasive testing and risk-stratification in patients with MASLD

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Abstract

The development and validation of non-invasive fibrosis tests (NITs) has changed clinical practice in Hepatology over the last 15 years. Metabolic associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the most prevalent liver disease in western countries, with up to a third of the unselected adult population affected. In this article, we review the use of NITs in the diagnosis and staging of MASLD. We discuss their use in the diagnosis steatosis, steatohepatitis and fibrosis and critically evaluate recently published data. These NITs include a variety of approaches, such as serum markers like FIB-4, pro-C3 and ELF, imaging techniques like Fibroscan® and MRE, and combined scores like Agile 3+ and Agile 4, offering a range of options for healthcare providers. Furthermore, these non-invasive tests also serve as valuable prognostic tools, allowing for better risk assessment and improved patient management, particularly in predicting liver-related events and overall mortality.

Introduction

Hepatic steatosis denotes the excessive accumulation of fat within liver cells. In June 2023, an international expert consensus panel introduced the term steatotic liver disease (SLD), serving as an inclusive term encompassing all the different aetiologies of hepatic steatosis, such as metabolic, alcohol-related, drug-induced and cryptogenic.[1]

Of particular interest is metabolic-dysfunction associated steatotic liver disease (MASLD) – formerly known as non-alcoholic fatty liver disease (NAFLD) – that is currently the most prevalent liver disease globally, affecting approximately 30% of the world population.[2] To correctly define the MASLD, hepatic steatosis must be identified by imaging or biopsy, and the patient should be affected by at least one cardiometabolic risk factor [1].

MASLD can progress over time causing inflammation in the liver tissue (metabolic-associated steatohepatitis, MASH), liver fibrosis, and ultimately liver cirrhosis. Early detection and management of MASLD can help prevent the progression to more severe liver diseases and improve overall liver health. Liver fibrosis is the most important prognostic factor for liver-related events in MASLD.[3] Advanced fibrosis (≥F3), also recognized as compensated advanced chronic liver disease (cACLD) according to latest Baveno VII consensus[4] is an independent risk factor for development of both liver related events and non-liver related morbidity and mortality.[5, 6] The presence of steatosis in isolation is not associated with an increased risk of liver-related events [7].

Currently, histological assessment through a liver biopsy, remains the gold standard for **the** diagnosis of MASH, which is characterized by a combination of steatosis, lobular or portal inflammation and hepatocellular ballooning, and for staging of liver fibrosis. However, liver biopsy has its limitations, including invasiveness, risk of complications, sampling variability, and the potential for patient reluctance due to its invasive nature.[8, 9] Furthermore, a biopsy length of at least 25 mm is recommended for accurate staging, which is not always attenable.[10]

The increasing prevalence of MASLD and the previously cited limits of liver biopsy led to the development of several non-invasive tests (NITs) for accurate staging and risk stratification. These include NITs for diagnosing steatosis, steatohepatitis and for staging liver fibrosis. NITs that were initially developed for staging fibrosis, are also increasingly used to determine liver-related prognosis. NITs can be broadly categorized in serum tests, imaging techniques and scores that combine the two.[11]

Table 1 summarizes the performances and cut-off values of the main NITs for the diagnosis of steatosis, steatohepatitis, and hepatic fibrosis.

Non-invasive diagnosis of liver steatosis

Hepatic steatosis can be diagnosed and quantified using various methods. Usually, the medical history and physical examination rise the suspicion of excessive liver fat accumulation and different risk factors should be taken into account: obesity/overweight, T2DM, alcohol consumption, sedentary lifestyle and sub-optimal diet.
Many radiological imaging techniques, such as abdominal ultrasounds (US), abdominal computed tomography (CT) scans, or magnetic resonance imaging (MRI), can be used to non-invasively identify liver steatosis.[12]

US is often the first non-invasive test performed, as it can assess the size and structure of the liver, and detect the presence of fatty deposits, by identifying hyper-echogenicity of the liver in comparison with the renal parenchyma. It is a simple, non-invasive technique, generally faster and less expensive than other radiological imaging methods, but it is operator-dependent.
Liver US gives a subjective evaluation of hepatic steatosis, with good sensitivity and specificity in detecting moderate to severe levels of steatosis (about 85% and 94%, respectively).[13] However, US is subject to various limitations, including the inherent subjectivity of the criteria employed to distinguish between fatty and normal liver, as well as the absence of well-defined sonographic parameters to categorize varying degrees of steatosis. Notably, the sensitivity and specificity of B-mode sonography decrease as body mass index (BMI) rises. Steatosis by US can be missed if it is less than 20%. [14]

Fibroscan® is a non-invasive medical device designed to perform liver stiffness measurement (LSM) by vibration-controlled transient elastography (VCTE). Fibroscan® can be equipped with a module called controlled attenuation parameter (CAP), based on an algorithm which, during the execution of the exam, automatically provides a rather precise estimate of any excess fat identified in the liver tissue[15]. More in detail, CAP was found to correctly identify patients with steatosis with:

- an AUROC of 0.87 (95% CI 0.82–0.92) for steatosis ≥S1,
- an AUROC of 0.77 (95% CI 0.71–0.82) for steatosis ≥S2,
- an AUROC of 0.70 (95% CI 0.64–0.75) for steatosis S3 [16]

A CT or MRI scan can provide more detailed information about the liver and help diagnose the presence of cirrhosis or portal hypertension. In CT scans, steatosis reduces liver attenuation, measured by Hounsfield Units (HU), causing hypodense liver. Enhanced CT has a limited role in diagnosing steatosis, due to contrast injection rate and timing affecting liver attenuation, while unenhanced CT could qualitatively assess steatosis, using the spleen as a reference organ: spleen attenuation is about 8–10 HU lower than the liver in healthy individuals.[17] A retrospective study revealed that hepatic venous phase-normalized liver attenuation with spleen could aid steatosis assessment in unacceptable liver donation candidates with moderate to severe steatosis, potentially averting needless biopsies.[18]

The MRI-estimated proton density fat fraction (MRI-PDFF) represents the gold standard for the assessment and quantification of liver steatosis. In fact, MRI-PDFF has proven to be highly accurate in quantifying hepatic steatosis, but also very precise and reproducible, over other invasive (liver biopsy) and non-invasive assessment techniques.[19]

Blood tests can help assess liver function and detect elevated liver enzymes, which might indicate liver damage in the context of a steatotic liver. Common tests include: full blood count; liver blood tests, such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST), released into the blood when liver cells are damaged, total bilirubin, and gamma-glutamyl transferase (GGT), which may arise in certain liver diseases, including hepatic steatosis; gluco-lipid profile, including fasting blood sugar, triglycerides, and cholesterol level; apolipoprotein A1; alpha-2-microglobulin, haptoglobin. Normal liver blood tests do not exclude the presence of MASLD, MASH or fibrosis [20].

Some scores were created and validated using different blood tests often associated with demographic characteristics, to diagnose steatosis. These include:

- Fatty Liver Index (FLI): a simple algorithm based on BMI, waist circumference, triglycerides, and GGT, created using US as reference. FLI can detect the presence of steatosis with high accuracy (AUROC 0.85; 95%CI 0.82–0.89) but not the severity of the steatosis.[8, 21] FLI has undergone external validation and correlates with cardiovascular, liver and cancer-related mortality, as well as reduced insulin sensitivity, risk of T2DM, accelerated atherosclerosis, and cardiovascular risk.[22]
- NAFLD Liver Fat Score (LFS): it was developed using magnetic resonance spectroscopy as gold standard, but not directly compared to hepatic histology. It is based on AST, ALT, fasting insulin level, presence of T2DM and metabolic syndrome. LFS detect the presence of

steatosis (but not the severity grade) with high accuracy (AUROC 0.80; 95% CI 0.69-0.88). Like FLI, LFS has been external-validated, and also correlated with insulin resistance and T2DM.[22]

- Hepatic Steatosis Index (HSI): it was created using ALT, AST, BMI, T2DM, gender, and US as reference; the AUROC of HSI for detecting steatosis is 0.81 (95% CI 0.801–0.824).[23]
- SteatoTest-2: constructed using a combination of the 6 components of FibroTest-ActiTest (GGT, total bilirubin, alpha-2-macroglobulin, apolipoprotein A1, haptoglobin, ALT) plus BMI, serum cholesterol, triglycerides, and glucose adjusted for age and gender; the reference standard was biopsy and the AUROC 0.78 for any grade of steatosis.[24]

The above tests are better suited for large epidemiological studies rather than for use in individual patients due to their sub-optimal specificity.

Non-invasive diagnosis of MASH and fibrotic-MASH

Distinguishing between MASLD and MASH poses significant clinical and diagnostic challenges. The evolving understanding of these conditions necessitates accurate diagnostic methods to guide appropriate interventions. Existing non-invasive markers do not have adequate diagnostic accuracy, therefore accurate diagnosis still relies on a liver biopsy. Among various biomarkers, cytokeratin-18 (CK-18) is the most widely studied candidate, however it remains inadequate for reliable clinical use, underscoring the need for further research for appropriate NITs.[25, 26]

The entity of “fibrotic MASH” which is defined as MASH with significant liver fibrosis (stage 2 or higher) has emerged as a diagnosis of clinical significance, as this defines the population that is currently enrolled in clinical trials and is also associated with a higher risk of liver-related events. Several scores have been developed for this which are outlined below.

Newsome et al developed and validated a score known as FAST (FibroScan-AST), which combines LSM, CAP, and AST levels. The score uses two cut-offs, one for ruling out and one for diagnosing fibrotic MASH, and has an AUROC of 0.85 (95% CI 0.83-0.87).[27]

MAST is an MRI-serum-based score designed to identify patients with fibrotic MASH, developed using data from MRI-PDFF, MR elastography (MRE), and a blood test for AST. MAST score demonstrated high accuracy in identifying Fibrotic MASH patients, with an AUC of 0.93 (95% CI

0.88-0.97) in the validation cohort. Two specific cut-off points were identified: at the 90%-sensitivity cut-off of 0.165, the test showed a specificity of 72.2%, while at the 90%-specificity threshold of 0.242, the test exhibited a sensitivity of 75.0%. About 17.6% of the patients in the validation cohort fell in the "grey-zone".[28]

The Fibrotic NASH Index (FNI) is a non-invasive scoring system, proposed in 2023, and designed to identify fibrotic MASH in MASLD patients. FNI is based on AST, HDL, and HbA1c. It showed good performances in validation cohorts with AUROC ranging from 0.80 to 0.95. Using a rule-out cut-off of 0.10, FNI demonstrated high sensitivity and NPV, meaning it accurately identified individuals without fibrotic MASH, reducing the need for referral to liver specialists.[29]

NIS2+™ is a novel two-biomarker-test corrected for sex, consisting of miR-34a-5p and YKL-40. It was recently proposed to identify individuals at risk of developing MASH. In the derivation cohort, NIS2+™ exhibited an AUROC of 0.81 for detecting at-risk MASH. Moreover, it consistently maintained high and stable AUROC values (ranging from 0.780 to 0.807), when utilized for identifying at-risk F3, MASH and F>=2.[30] Importantly, the diagnostic accuracy of NIS2+™ is maintained in people >65 years old.[31]

The MACK-3 score, based on 3 biomarkers (AST, homeostasis model assessment [HOMA], and CK-18), was recently validated in an international multicentric study (AUROC=0.79), suggesting that it could be a valuable tool in improving patient selection for MASH therapeutic trials.[32]

All the above scores are suboptimal if used on their own for the accurate diagnosis of fibrotic MASH. It is still uncertain if the presence of fibrotic MASH (rather than fibrosis alone) will be required for treatment eligibility once pharmacotherapy becomes available. The concomitant use of two or more the above tests (with a liver biopsy if discordant) needs to be tested in future studies. At the moment, the utility of these scores is to better select patients for screening biopsies for participation in clinical trials.

NITs for fibrosis assessment

Serum Markers

Serum markers can be categorized as either indirect or direct. Indirect serum markers comprise combinations of routine laboratory parameters along with demographic factors such as BMI, age or

the presence of diabetes. Among indirect serum biomarkers the most extensively validated and commonly used are Fibrosis 4 (FIB-4) and NAFLD Fibrosis Score (NFS) for advanced fibrosis. Their ability to discriminate advanced fibrosis is good with an AUROC among 0.7 and 0.8 according to different multicentric studies and meta-analysis.[33-36] These biomarkers have dual cut-offs, whith a low cut-off to rule out and a high cut-off to diagnose advanced fibrosis. Their main use is to rule out advanced fibrosis, at <-1.455 for NFS and <1.3 for FIB-4. It has been suggested that the low cut-offs should be higher in patients >65 years old at 2.0 for FIB-4 and 0.12 for NFS. [37, 38] FIB-4 underestimates fibrosis in patients <35 years old and has decreased diagnostic accuracy, even after cut-off adjustments, in those >65 years.

Since the calculation of both these scores is based on simple parameters that can be easily obtained with a clinical evaluation and routine blood tests, their use is encouraged in primary care clinics and diabetology clinics to identify patients at higher risk of advanced fibrosis that should be referred to secondary care/hepatology clinics.[9, 39, 40]

Other simple markers, such as the Aspartate Aminotransferase to Platelet Ratio Index (APRI) [41] and the BARD score [42] are not used routinely in clinical practice.

Direct serum biomarkers related to the fibrogenic process and extracellular matrix turnover have been proposed and validated for staging of liver fibrosis. The Enhanced Liver Fibrosis (ELF) test is based on the measurement of three serum biomarkers involved in matrix turnover: tissue inhibitor of metalloproteinase-1, hyaluronic acid and N-terminal procollagen III peptide.[43] ELF has a good diagnostic performance for diagnosis of advanced fibrosis (AUROC ≥0.8)[34, 44] with a cut-off of >9.8 being highly sensitive for advanced fibrosis[45, 46] although the presence of extra-hepatic inflammatory or fibrotic diseases could lead to false positive results.[47]

ELF has become increasingly available in laboratories in recent years, especially in Western countries, but remains expensive when applied to the general population. A two-step approach of FIB-4 followed by ELF allows the test to be used only in the population with indeterminate FIB-4 results. This approach reduces costs and improve the selection of patients for secondary care referral.[48, 49]

The recent EASL and AASLD guidelines recommend that patients at risk of MASLD with high FIB-4 or NFS and patients with intermediate FIB-4 or NFS and high Fibroscan® (>8 KPa) or ELF (>9.8 for EASL, >7.7 for AASLD) should be referred to secondary care for further evaluation.[9, 39]

Pro-C3 is a direct biomarker of type-III collagen formation and has been proposed and validated as NIT for liver fibrosis assessment either alone or in algorithms (ADAPT).[50, 51] They showed a good performance for diagnosis of advanced fibrosis in patients with MASLD with an AUROC of 0.75

(Pro-C3) and 0.81 (ADAPT) in a recent multicentric study.[34] Currently, its cost and low availability render it seldomly used in routine clinical practice.

Liver Elastography

In addition to serum biomarkers and scoring systems, LSM is closely associated with liver fibrosis and can be determined using various US and magnetic resonance elastography (MRE) techniques.[52] US elastography methods include Vibration Controlled Transient Elastography (VCTE or Fibroscan®), acoustic radiation force impulse (ARFI), point shear wave elastography (p-SWE), and two-dimensional shear wave elastography (2D-SWE).[53] However, it must always be kept in mind that other characteristics of the liver tissue can significantly influence its rigidity and limit the diagnostic accuracy of elastography. Liver tissue stiffness could be potentially influenced by steatosis, necro-inflammation, postprandial hepatic hyperaemia, thickness of subcutaneous tissue, cholestasis and increased central venous pressure.[54-57]

VCTE is the first-developed, most validated and extensively used US-based elastography method worldwide. It measures liver stiffness by assessing the speed of shear wave propagation through the hepatic tissue. According to two recent meta-analysis, VCTE has an excellent diagnostic accuracy for diagnosis of advanced fibrosis and cirrhosis in MASLD patients with AUROCs close to 0.90 [58, 59]. VCTE (and other US elastography methods) are less accurate in diagnosing significant or lesser degrees of fibrosis [60]. Although data have shown that the presence of steatosis can increase liver stiffness values[61], this was not confirmed in a prospective study of patients with suspected NAFLD.[62]

A limitation of VCTE is that there are no established cut-offs for the diagnosis of advanced fibrosis and cirrhosis. When a single cut-off is used, VCTE is better in excluding that ruling in advanced fibrosis or cirrhosis, also due to the relatively low prevalence of these stages. To overcome this, a dual cut-off strategy has been proposed by the Baveno VI and VII - a VCTE cut-off of <10 kPa to rule out and a cut-off of >15 kPa to diagnose cACLD.[4, 63] A recent real-world validation of these thresholds revealed that the optimal cut-offs for diagnosing cACLD in patients with MASLD are <8 kPa (with a sensitivity >90%) and >12 kPa (with a specificity of 88.2%).[64]

pSWE is a method that can assess the elastic characteristics of the liver in real-time B mode US examination using ARFI technology.[65] pSWE is based on the quantification of shear wave travelling speed, which is directly reliant on viscoelastic properties of the parenchyma.[52, 66] Compared with VCTE, ARFI showed similar capability to detect liver cirrhosis (AUROC 0.87 and

0.85 for VCTE and ARFI, respectively) with a lower failure rate.[67] Recent meta-analyses set as good the pSWE accuracy for detecting significant fibrosis (≥2) with a pooled sensitivity of 80.2% and a specificity of 85.2%.[68] Diagnostic performance of pSWE for advanced fibrosis (F≥3) was still good with AUROC of 0.94.[69]

2D-SWE uses real time conventional B-mode imaging to produce a two-dimensional quantitative map of liver tissue stiffness. The operator can specify of the region of interest where liver stiffness is measured. This allows the measurement of stiffness to be oriented towards a specific area of the liver but on the other hand it has the disadvantage of increasing intra- and inter-observer variability.[70] A recent meta-analysis showed that the diagnostic performance of 2D-SWE for various fibrosis stages was good-to-optimal with AUROCs of 0.855, 0.928 and 0.917 for ≥F2, ≥F3 and cirrhosis, respectively. Proposed cut-offs were 7.1 kPa for significant fibrosis (≥F2), 9.2 kPa for advanced fibrosis (≥F3) and 13.0 kPa for cirrhosis (F4).[71]

Some studies have compared the diagnostic performance of fibrosis of different US elastography techniques. No significant differences were highlighted in terms of performance although VCTE remains the most validated technique and therefore still the most recommended by international guidelines.[9, 39, 72, 73]

MRE studies the propagation of shear waves in liver tissue converting the wavelength information into tissue stiffness maps.[74, 75] Compared with US elastography techniques**,** MRE offers a panoramic assessment of fibrosis and a superior spatial resolution, exhibiting superior performance in stratifying intermediate stages of fibrosis. However, its high cost and low availability limits its practical application primarily to research purposes.[9, 12] MRE, is reliable for diagnosis of significant fibrosis (F≥2, AUC: 0.88), advanced fibrosis (F≥3, AUC: 0.93) and cirrhosis (AUC: 0.92) and it is not affected by gender, BMI, steatosis and inflammation grade. Proposed cut-offs are 3.66 kPa for significant fibrosis (sensitivity: 79%, specificity: 81%), 4.11 kPa for advanced fibrosis (sensitivity: 85%, specificity: 85%) and 4.71 kPa for liver cirrhosis (sensitivity: 91%, specificity: 81%).[76]

Elastography Scores

The gradual increase in the availability of elastography in secondary care settings, in particular the Fibroscan®, has highlighted some limitations in the reliability of elastography methods. Many co-factors such as age, obesity, liver congestion and significant elevation of liver enzymes have proved to impact on elastography results.[77, 78]

In this scenario, scores combining clinical, laboratory and elastography variables have been proposed to overcome those limitations and to more accurately identify MASLD patients with advanced fibrosis or cirrhosis.

The Agile 3+ proposed by Sanyal et al takes into account LSM (Fibroscan®), platelets, AST, ALT, diagnosis of diabetes age and sex. This score showed a very good diagnostic performance of advanced fibrosis in the European and US validation courts with AUROC>0.85, significantly higher than LSM alone, FIB4 and NFS.[79] Interestingly, Agile3+ superiority compared to LSM alone is not confirmed in diabetic populations, arising questions about the net benefit of using the score in patients with diabetes.[33] The proposed cutoff for the exclusion of advanced fibrosis (0.451) showed a sensitivity >80% in both European and US cohorts, while the proposed cutoff for the diagnosis of cACLD (0.679) showed a specificity >85% in both European and US validation cohorts. The percentage of patients falling into the grey area is small compared to other NITs and is between 8 and 20 % in both European and American validation cohorts.[80-82]

Interestingly, similar results were not evident in Asian cohorts. In a recent Asian multicentre study in a population of 641 patients with MASLD, the AUROC of Agile 3+ for diagnosis of advanced fibrosis was 0.82, and was equal to that of LSM alone. Furthermore, the percentage of patients with an indeterminate result was significantly high for both LSM and Agile3+ (34% and 39%), respectively, compared to the multicentric US and European studies. [83]

The Agile 4 aims to diagnose cirrhosis and consists of the following variables: LSM, platelets, ALT, AST, Gender and T2DM status.[80] It showed a high diagnostic performance for cirrhosis both in European and Nord American validation cohorts with AUC of 0.89 and 0.85 respectively. A dual cutoff strategy uses 0.251 as a rule-out cutoff and 0.565 as a rule-in cutoff. In European validation cohorts this strategy led to a low percentage of patients falling in the gray zone (11-16% depending on different cohorts) whereas in North American validation cohort that percentage was higher (23%).[80, 81]

Jung et al assessed the diagnostic accuracy of combining Magnetic Resonance Elastography (MRE) with FIB-4 (MEFIB score) in identifying candidates with significant fibrosis. When MRE (≥3.3 kPa) was combined with FIB-4 (≥1.6), it produced a clinical-prediction-rule with a PPV of 97.1% for diagnosing significant fibrosis. These findings were validated in the "Japan-NAFLD" cohort, where the MEFIB score had an AUROC of 0.84 (95% CI 0.78-0.89), demonstrating its potential as a non-invasive tool for identifying MASH patients in need of treatment.[84]

Sequential use of NITs for optimized management

International guidelines recommend the use of a two-tier testing in primary care, starting from the FIB4, which is an inexpensive widely available serum test that can rule out with high sensitivity people who do not have advanced fibrosis, before moving to a second tier test.[9, 39] A proposed algorithm for testing patients at risk of MASLD in non-hepatology settings is shown in Figure 1. In the primary care setting, it is necessary to use a test that is easy and inexpensive to calculate or measure, and given the low prevalence of advanced fibrosis, the goal should be to use a cut-off that can reliably exclude disease (high sensitivity and NPV) and refer to secondary care patients at risk of disease.[85] In the secondary care setting, the size of the population to be tested is smaller and the prevalence of cACLD is higher. This allows the use of NITs that have a better diagnostic accuracy but also a higher cost, with the aim of reliably identifying patients with the target condition (high specificity and high PPV) and undertaking the correct clinical and therapeutic management of cACLD. The main limitation of the dual cut-off strategy lies in the fact that a relevant percentage of patients, 10 to 40% depending on the setting and the NIT, have an indeterminate result. This limitation can be partially overcome by using independent NITs in a sequential or simultaneous approach or by performing a liver biopsy.[6, 57, 86]. Ultimately, the widespread use of such testing algorithms in populations at risk of liver disease will require a substantial increase in testing capacity, both in terms of funding and also facilities. The use of population-derived risk scores, such as the LiverRisk score, can potentially refine the patient population that requires testing. [87]

The implementation of a two-tier approaches utilizing NITs is overall a promising strategy in the management of MASLD in primary care, offering cost-effective and clinically efficient pathways for identifying patients at risk of advanced fibrosis and streamlining referrals to secondary care.

In a modelling study, Crossan et al. tested a pathway for triaging patients with NAFLD in primary care using NITs to identify those at risk of advanced fibrosis (AF). The sequential use of NITs, starting with FIB-4 followed by ELF or Fibroscan® for indeterminate results, effectively reduced unnecessary secondary care referrals by 90%, resulting in a 40% cost savings per patient. The proposed two-tier approach accurately identified patients at the highest risk of AF and disease progression, offering an efficient strategy for managing NAFLD in primary care settings.[88]

Srivastava et al. assessed the clinical and cost effectiveness of implementing NITs in primary care for patients with NAFLD, using a similar two-tier model. Their study revealed that the adoption of NITs led to a notable reduction in unnecessary referrals, resulting in substantial cost savings (from 3 to 17%). This underscores the significant benefits of integrating NITs into primary care for effective NAFLD patient management.[89]

NITs for assessment of prognosis in MASLD patients

Liver fibrosis represents the most relevant prognostic factor in patients with MASH, particularly regarding the risk of liver cancer, liver-related events, cardiovascular events, liver transplant and death.[3, 90-93].

The need of histopathological staging of fibrosis for the assessment of the prognosis of patients with MASH is a major limitation as liver biopsy is invasive, expensive and therefore can't be performed on a large population. The use of non-invasive tests as prognostic factors that guide the clinical and therapeutic choices of patients with MASH represents an overcoming of this limit and is strongly encouraged as an area of research by the main scientific societies worldwide.

NFS and FIB-4 showed a good performance for prediction of liver related events (hazard ratio [HR], 2.77 and 1.68, respectively), HCC (HR, 4.2 and 1.67, respectively) and overall mortality (HR, 2.86 and 2.00 respectively) whereas their association with risk of cardiovascular events (HR, 1.46 and 1.24, respectively) and extrahepatic cancers (HR, 1.66 and 1.24, respectively) is lower.[94]

According to a recent meta-analysis, NFS and FIB-4 have the best performance for prediction of all-cause mortality compared with other indirect NITs (APRI and BARD) with a HR>3. Interestingly, NFS appear to be a strong predictor also of cardiovascular-related mortality in this study (HR 3.09), probably due to the fact that NFS includes known cardiovascular risk factors such as T2DM and BMI, not considered in the FIB-4.[95] Changes over time of NFS and FIB-4 are significantly associated with fibrosis progression: for every unit change in FIB-4 or NFS a mean fibrosis stage progression of 0.26 and 0.19 was observed, respectively.[96]

A prognostic role in MASLD patients has also been demonstrated for ELF. Many authors showed that patients with ELF>11.3 have a higher risk (5-fold) of liver related events in patients with MASLD [97].

LSM has been proposed as a reliable prognostic factor in MASLD patients.[98, 99] Boursier et al showed that a prognostic stratification could be achieved according to baseline LSM in terms of overall survival and risk of liver related events.[100] Similar results have been confirmed by other studies and meta-analyses.[101, 102] LSM values above 10-12 kPa are associated with a higher risk of liver related events and a lower overall survival, confirming advanced fibrosis as a main prognostic factor for MASLD. A recent individual patient meta-analysis by Mozes et al showed that the prognostic stratification based on LSM performed as well as the one based on histologically assessed fibrosis. Patients with LSM>20kPa and LSM between 10 and 20 kPa had respectively a 10-fold and 3-fold higher risk of liver related events, HCC occurrence and overall mortality compared with patients with LSM<10kPa.[102]

Agile3+ has been evaluated as a prognostic factor in MASH patients, particularly for prediction of liver related events. Pennisi et al showed that both Agile3+ and LSM has an excellent performance for predicting liver related events at 3, 5 and 8 years (AUROCs> 0.9) with Agile 3+ slightly superior to LSM.[82]

Serra-Burriel et al recently developed the LiverRisk score, a newly developed NIT based on age, sex, and six standard laboratory variables, which is designed to identify patients at risk for future liver-related complications (including mortality, hospitalization, and liver cancer). The LiverRisk score was derived from a large international cohort study, and validated in two prospective cohorts from the general population. It demonstrated high accuracy in predicting liver stiffness, with an AUROC of 0.83 (95% CI: 0.78-0.89). Furthermore, the LiverRisk score effectively stratified individuals into different risk groups for liver-related outcomes. Overall, this score provides a valuable means of identifying individuals at risk for liver-related complications in the general population, potentially enabling more targeted and timely preventative care.[103]

Conclusions

Non-invasive testing and risk stratification in patients with MASLD represent critical improvements in addressing the challenges posed by hepatic steatosis.

As the field evolves, the integration of these non-invasive methods promises to revolutionize the diagnostic landscape, facilitating timely interventions and improving outcomes for patients with MASLD.

With the introduction of accurate NITs, patients with MASLD can benefit from accurate diagnoses and risk assessments without the need for invasive liver biopsies. These NITs include a variety of approaches, such as serum markers like FIB-4, pro-C3 and ELF, imaging techniques like Fibroscan® and MRE, and combined scores like Agile 3+ and Agile 4, offering a range of options for healthcare providers. Furthermore, these non-invasive tests also serve as valuable prognostic tools, allowing for better risk assessment and improved patient management, particularly in predicting liver-related events and overall mortality.

Table 1. Main Non-Invasive Tests (NITs) used for diagnosis and staging of steatosis, MASH, MASH with significant fibrosis and advanced fibrosis.

NIT	Use	Cut-off values	AUROC (95% CI)	Sensitivity (%)	Specificity (%)
HEPATIC STEATOSIS					
Ultrasounds (US) [13]	Diagnosis of liver steatosis	NA	0.93 (0.91-0.95)	85	94
Fibroscan® controlled attenuation parameter (CAP) [15, 104]	Diagnosis of liver steatosis grade ≥S1	248 dB/m	0.82 (0.81-0.84)	68.8	82.2
	Diagnosis of liver steatosis grade ≥S2	268 dB/m	0.87 (0.85-0.88)	77.3	81.2
	Diagnosis of liver steatosis grade S3	280 dB/m	0.88 (0.86-0.91)	88.2	77.6
Liver-to-spleen attenuation ratio on non-enhanced phase of computed tomography CT scan [18]	Diagnosis of steatosis presence with single cut-off (best sensitivity and specificity)	1.12	0.87 (0.77-0.96)	81	79
	Rule-in steatosis	0.99		52	100
	Detecting ≥20% steatosis with a single cut-off (best sensitivity and specificity)	0.97	0.88 (0.76-0.99)	75	92
	Rule-in ≥20% steatosis	0.91		63	100

Liver to spleen attenuation ratio on hepatic venous phase of computed tomography (CT) scan [18]	Diagnosis of steatosis presence with single cut-off (best sensitivity and specificity)	0.98 §	0.93 (0.87-0.99)	77	100
	Detecting ≥20% steatosis with a single cut-off (best sensitivity and specificity)	0.94	0.95 (0.88-1.01)	94	90
	Rule-in ≥20% steatosis	0.87		56	100
Magnetic Resonance Imaging with estimated proton density fat fraction (MRI-PDFF)* [19, 105-107]	Discriminating ≥S1	From 8.9 to 6.4%**	0.99	96	100
	Discriminating ≥S2	From 15 to 17.4%**	From 0.83 to 0.95	From 52 to 93	From 81 to 85
	Discriminating ≥S3	From 22.1 to 25%**	From 0.89 to 0.95	From 74 to 93	From 81 to 85
Fatty Liver Index (FLI) [21]	Rule-out steatosis	30	0.85 (0.82-0.89)	87	64
	Rule-in steatosis	60		61	86
NAFLD Liver Fat Score (LFS) [22]	Diagnosis of steatosis ≥5%	0.16	0.80 (0.69–0.88)	65	87
Hepatic Steatosis Index (HIS) [23]	Rule-out steatosis ≥S1	30	0.81 (0.80-0.82	92.5	40.0
	Rule-in steatosis ≥S1	36		46.0	92.4
SteatoTest-2 [24]	Diagnosis of steatosis ≥5% (single cut-off, adjusted for a prevalence of steatosis of 18%)	0.40	0.77 (0.71-0.82); 0.79 (0.73-0.83) ***	79.0	50.0

MASH (NASH)					
Cytokeratin-18 (CK-18) [108]	Diagnosis of NASH/MASH in patients with FIB-4 ≥2.67	260 U/L	0.77	82.7	57.4
	Diagnosis of NASH/MASH in patients with FIB-4 <2.67	260 U/L	0.77	82.4	56.9
FibroScan-AST (FAST) [27]	Rule-out NASH (NASH/MASH + NAS≥4 + F≥2)	0.35	0.85 (0.83-0.87) (pooled external validation cohort).	89	64
	Rule-in NASH (NASH/MASH + NAS≥4 + F≥2)	0.67		92	49
MRI-PDFF-AST (MAST) score [28]	Rule-out fibro-NASH	0.165	0.93 (0.88-0.97)	90.0	72.2
	Rule-in fibro-NASH	0.242		75.0	90.0
Magnetic Resonance Elastography (MRE) with FIB-4 (MEFIB score) [84]	Diagnosis of fibro-NASH F≥2	MRE≥3.3kPa + FIB-4 ≥ 1.6	0.90 (0.85-0.95) (CSD-NAFLD cohort); 0.84 (0.78-0.89) (Japan-NAFLD cohort)	NA	NA
Fibrotic NASH index (FNI) [29]	Rule-out fibro-NASH	≤0.10	0.78 (0.71-0.85) (derivation cohort).	89 (derivation cohort)	37 (derivation cohort)
	Rule-in fibro-NASH	≥0.33	From 0.80 (0.75-0.83) to 0.95 (0.92.-0.98)	52 (derivation cohort)	90 (derivation cohort)

			(validations cohorts).		
Liquid biopsy based on PLIN-2 mean florescence intensity [109]	Diagnosis of NASH (with NAS>3)	NA (score derived from a neural network classifier including PLIN2)	0.98 (0.95-1.00) (derivation cohort)	95 (derivation cohort)	90 (derivation cohort)
Liquid biopsy based on RAB14 mean florescence intensity [109]	Diagnosis of liver fibrosis	NA (score derived from a neural network classifier including RAB14)	0.96 (0.88-1.00) (derivation cohort)	100 (derivation cohort)	95.8 (derivation cohort)
NIS2+™ [30]	Rule-out at-risk NASH	0.4564	0.81 (0.80-0.83)	85	61
	Rule-in at-risk NASH	0.6815		62	85
MACK-3 [32]	Rule-out of fibrotic-NASH	0.135	0.79 (0.77-0.81)	90.8	48.4
	Rule-in of fibrotic-NASH	0.549		48.0	86.4
ADVANCED FIBROSIS (F≥3)					
Fibrosis-4 index (FIB-4) [38]	Rule-out advanced fibrosis	1.30	0.80 (0.77-0.84)	77.8	71.2
	Rule-in advanced fibrosis	3.25		37.3	95.8

NAFLD Fibrosis Score (NFS) [38]	Rule-out advanced fibrosis	-1.455	0.78 (0.75-0.81)	72.9	73.8
	Rule-in advanced fibrosis	0.676		43.1	88.4
Aspartate Aminotransferase to Platelet Ratio Index (APRI) [38]	Rule-out advanced fibrosis	0.5	0.75 (0.72-0.77)	72.9	67.7
	Rule-in advanced fibrosis	1.5		32.9	90.5
BARD score [38]	Diagnosis of advanced fibrosis	2	0.73 (0.71-0.75)	75.2	61.6
Enhanced Liver Fibrosis (ELF) [44]	Diagnosis of advanced fibrosis	9.8	0.948 (0.88-1.00)	86.7	92.5
Pro-C3 [34]	Diagnosis of advanced fibrosis	NA	0·75 (0.70-0.80)	NA	NA
ADAPT [51]	Diagnosis of advanced fibrosis	6.3287	0.87 (0.83-0.91)	NA	NA
Vibration-controlled transient elastography (VCTE) with Fibroscan® [64]	Rule-out advanced fibrosis	7 kPa	0.87 (0.86-0.88)	95.6	48.6
	Rule-in advanced fibrosis	12 kPa		61.1	88.3
Point Shear Wave Elastography (pSWE) [69]	Diagnosis of advanced fibrosis	2.06 m/s	0.94 (0.91-0.96)	90	90
Two-dimensional shear [79]wave elastography (2D-SWE) [71]	Diagnosis of advanced fibrosis	9.2 m/s	0.928	93.1	80.9

Magnetic Resonance Elastography (MRE) [76]	Diagnosis of advanced fibrosis	4.11 kPa	0.94 (0.91-0.98)	89	0.84
Agile 3+ [79]	Rule-out advanced fibrosis	0.451	0.87 (0.85-0.89)	83	75
	Rule-in advanced fibrosis	0.679		61	90
Agile 4 [79]	Rule-out cirrhosis	0.251	0.89 (0.86-0.92)	88	71
	Rule-in cirrhosis	0.565		44	97

Legend:

§: represents the cut-off value that provided a balance between sensitivity and specificity, and yielded a 100% specificity.

*: data presented as single value or range of values, when declared in the original studies included in the cited review.

**: percentage of estimate steatosis in the liver using a generate PDFF map with ranges from 0% to 50%, representative of the fat content in the liver.

***: values varying according to subsets and the prevalence of steatosis, as stated by Authors.

NA: Not-Available

Figure 1: Testing algorithm for patients with diagnosed or suspected MASLD in primary care or endocrinology clinics. This consists of a two-tier sequential testing, starting with a FIB-4 and followed by either Fibroscan or ELF, depending on what is locally available.

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