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Review

Modernizing metabolic dysfunction-associated steatotic liver disease diagnostics: the progressive shift from liver biopsy to noninvasive techniques

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***Abstract*:** Metabolic dysfunction-associated steatotic liver disease (MASLD) is a growing public health concern worldwide. Liver biopsy is the gold standard for diagnosing and staging MASLD, but it is invasive and carries associated risks. In recent years, there has been significant progress in developing noninvasive techniques for evaluation. This review article discusses briefly current available noninvasive assessments and the various liver biopsy techniques available for MASLD, including invasive techniques such as transjugular and transcutaneous needle biopsy, intraoperative/laparoscopic biopsy, and the evolving role of endoscopic ultrasound-guided biopsy. In addition to discussing the various biopsy techniques, we review the current state of knowledge on the histopathologic evaluation of MASLD, including the various scoring systems used to grade and stage the disease. We also explore current and alternative modalities for histopathologic evaluation, such as whole slide imaging and the utility of immunohistochemistry. Overall, this review article provides a comprehensive overview of the progress in liver biopsy techniques for MASLD and compares invasive and noninvasive modalities. However, beyond clinical trials, the practical application of liver biopsy may be limited, as ongoing advancements in noninvasive fibrosis assessments are expected to more effectively identify candidates for MASLD treatment in real-world settings.

Plain language summary

Modernizing metabolic dysfunction-associated steatotic liver disease diagnostics: the progressive shift from liver biopsy to non-invasive techniques

Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a condition where fat builds up in the liver, causing inflammation and scarring. This problem is becoming more common worldwide and can lead to serious health issues like chronic liver disease, liver failure, and liver cancer. Doctors can use a method called a liver biopsy to check if a patient has a liver problem like MASLD. However, this method can be a bit risky because it involves inserting a needle into the liver to get a sample. Although doctors can also rely on blood work and different medical imaging approaches to assess the severity of liver disease, specifically MASLD, these options aren't completely accurate. Therefore, there is still a need for a liver biopsy. This article explores various methods and techniques that doctors can use to perform a liver biopsy. It explains how the sample taken with the needle can be analyzed under a microscope to help guide the management of patients with MASLD. We hope this review will be useful for doctors and researchers in the field of gastroenterology and hepatology.

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Introduction

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously referred to as non-alcoholic fatty liver disease (NAFLD), has emerged as a substantial global public health concern, affecting over 30% of the population worldwide, leading to a notable increase in resource utilization.[1] Recently, guiding authorities in the field of hepatology have been advocating to abandon the term NAFLD due to concerns regarding stigmatization and a desire to better capture the disease's true etiology. Steatotic liver disease and MASLD have emerged as accepted nomenclature. The formal definition of MASLD is defined as a steatotic liver condition occurring in the presence of at least one of five cardiometabolic risk factors, coupled with reported alcohol intake below 140 g per week in females and 210 g per week in males.[2]

Hepatic steatosis is identified by the buildup of fat within the liver, with a minimum of 5% of the liver by mass being affected. The severity of fat accumulation can be categorized into different grades: grade 0 (indicating a healthy state, less than 5% fat), grade 1 (mild steatosis, 5%–33% fat), grade 2 (moderate steatosis, 34%–66% fat), and grade 3 (severe steatosis, greater than 66% fat).[3] Steatosis results from increased deposition of fatty acids and associated lipogenesis, leading to the accumulation of high levels of triacylglycerols within the liver. The process of steatosis in itself typically has a low risk of advanced fibrosis development estimated at 0.5%–1.0% over 1–2 decades.[4] Metabolic dysfunction-associated steatohepatitis (MASH) is delineated as a pathological state wherein hepatic steatosis coincides with inflammation-induced injury, manifesting as hepatocyte ballooning degeneration and the concomitant existence of Mallory–Denk bodies.[3] In MASH, clinical progression to advanced fibrosis has been observed to accelerate, with the advancement of a stage of fibrosis occurring over a 7-year period, in contrast to a 14-year span for MASLD.[5]

A liver biopsy offers an extensive assessment of architectural abnormalities and can assist with the quantification of inflammation and associated hepatocellular damage in patients with MASLD, along with assessing the extent of underlying fibrosis.[6] Liver biopsy is typically considered when there is a need to confirm advanced fibrosis, discordance in noninvasive tests, clinical trial endpoints, or concerns when alternative etiologies of liver disease are suspected.[7,8] Given the performance of a liver biopsy is invasive and not appropriate for disease screening indications, numerous noninvasive tests have been validated in MASLD to aid in the staging and detection of clinically significant fibrosis which we will also briefly discuss in this review. However, it is important to emphasize that biopsy-confirmed fibrosis has consistently proven to be a strong predictor of liver-related morbidity and mortality and as such, currently remains the gold standard in clinical research.[6,9]

In this review, we will briefly review the current indications for liver biopsy, technique, and associated safety. We will also correlate liver biopsy results with noninvasive diagnostic tests and their role in assessing disease severity. Finally, we will explore evolving histopathological techniques and applications in the future context of MASLD management.

The historical perspective of liver biopsy

Although the role of liver biopsy has changed over the last 65 years since its widespread clinical use, it remains a highly specific tool that provides valuable information for clinical and research purposes. The first liver biopsy was described by Paul Ehrlich in 1883, this was done by a transcutaneous aspiration method to assess glycogen stores in the liver for diabetic patients.[10,11] Its first application for the diagnosis of patients with cirrhosis was published by Schüpfer in 1907, this was expanded by Bingel in 1923.[11] Liver biopsy did not come into widespread clinical use until 1958 when Menghini[12] described the transcutaneous "one-second needle biopsy of the liver" which has now stood the test of time. Since then, obtaining a liver biopsy has been modified with regard to

the approach, needle type, and the utilization of diagnostic imaging.[11]

Due to the risk of hemorrhage, which is one of the feared complications of transcutaneous liver biopsies, and especially since liver biopsies are often performed in patients with coagulopathies, the transvenous approach was developed. This was first described in an experimental model by Dotter in 1964, but clinically performed for the first time in 1967 by Hanafee.[13,14] However, there was concern that transvenous liver biopsy samples were suboptimal compared to those done percutaneously because of their smaller and more fragmented size.[14] In the last 20 years, the advent of automatic cutting-type needles and the improvement of device systems have mitigated that concern as transvenous samples are now comparable to those obtained via the percutaneous technique.[14] Furthermore, in the 1960s, laparoscopy was another technique used for the assessment of liver disease, but its utility significantly declined over the next two decades due to improvements in serological testing.[11] However, in recent years, laparoscopic liver biopsy has started to gain attention due to the development of minimally invasive mini-laparoscopy. This allows for the use of optical instruments with a diameter of less than or equal to 2 mm through a single puncture.[11] The advantages of a minimally invasive mini-laparoscopic approach for a liver biopsy include the ability for a macroscopic assessment of the liver while targeting the area of interest and not being limited by ascitic fluid as it can be evacuated prior to the biopsy.[11]

While the indications and techniques for liver biopsy have changed drastically over the past 20 years due to effective treatments and improved noninvasive biomarkers, there will always be an important role in obtaining a liver biopsy. The future will likely leverage the emergence of better technologies for histologic evaluation combined with the application of machine learning algorithms and artificial intelligence (AI) to augment the diagnosis and prognosis of liver disease.[10]

Conventional techniques/approaches to liver biopsy (review re-organized)

Percutaneous biopsy

Percutaneous liver biopsy is the most commonly used approach. The procedure is performed with the patient in the supine position, and the liver's location is identified through a percussion or ultrasound-based approach, with the liver usually identified between the sixth and ninth intercostal spaces.

Ultrasound confirmation is often done to ensure the correct site, especially when percussion is uncertain. For non-targeted liver biopsies, ultrasound or CT imaging is recommended to be completed within 3 months of the procedure to help guide the biopsy.[15] A randomized study demonstrated the superior safety of percutaneous liver biopsy with ultrasound assistance compared to a blind biopsy approach.[16] Since this study, imaging-guided liver biopsy is defined as the current standard of care. Image guidance with real-time ultrasound (US), computed tomography (CT), or magnetic resonance imaging (MRI) is also required in the biopsy of focal liver lesions to accurately localize the biopsy.[17]

An adequate liver biopsy specimen size must be obtained to minimize sampling errors. Techniques vary on the optimization of the liver biopsy sample. Longer sample sizes and the number of visualized complete portal tracts correlate with the accuracy of the obtained sample.[18] It is crucial to obtain an intact tissue sample of adequate size to display the liver's lobular structure and several portal tracts.[19,20] Recent guidelines have suggested biopsy adequacy should include at least 11 portal tracts visualized, which typically correlates with a minimum 20 mm core obtained using a 16-gauge needle.[15] Some studies have explored the use of smaller gauge needles, like an 18-gauge needle, for liver biopsies with the suggestion that a smaller needle gauge might lead to fewer complications. However, research has shown that 16-gauge needles offer better sample quality with a comparable rate of complications.[21,22] Three types of needles can be used for percutaneous liver biopsy: suction needles, cutting needles, and automated spring-loaded cutting needles. Generally, automated cutting needles are chosen due to their ease of use.[23] Only one needle pass is recommended unless the first sample is inadequate, given that more needle passes are known to correlate with complications associated with liver biopsy.[24,25]

Transvenous biopsy

Transvenous liver biopsy is preferred in patients with coagulopathy or ascites. The transvenous route potentially avoids the breach of the liver capsule and reduces the risk of bleeding. The transvenous approach also allows for concurrent hepatic venography or hepatic vein pressure gradient (HVPG) measurements to be performed. The procedure is performed in an angiography suite with real-time fluoroscopic guidance. Continuous cardiac monitoring is used as a safety measure to assess for ventricular arrhythmias due to the catheter crossing the right atrium. It is typically performed under moderate sedation. If possible, any coagulation disorders should be corrected and anticoagulants discontinued prior to the procedure.[26] Imaging should be performed prior to the procedure to ensure that the patient's anatomy is amenable to transvenous biopsy.[15]

Transjugular biopsy is the preferred transvenous approach; however, the transfemoral approach is also used on occasion.[27] In the case of transjugular biopsy, the patient is positioned supine with their head turned away from the venous access site. The right internal jugular vein is typically preferred. After lidocaine is infiltrated, intravenous access is established using the Seldinger technique under ultrasound guidance.

A guidewire is inserted into the inferior vena cava, followed by a transjugular vascular sheath. The catheter is then usually advanced into the right hepatic vein. A venogram is obtained to visualize the venous anatomy, and hepatic venous pressure gradients can be measured if desired. A guidewire is then inserted into the hepatic vein, allowing the catheter to access the hepatic circulation. An 18- or 19-G automated cutting-type biopsy needle is typically used, an aspiration needle may also be used.[28] The biopsy needle is advanced through the catheter, rotated toward the liver parenchyma, and used to obtain tissue samples. Due to smaller and more fragmented specimens compared with the percutaneous approach, several needle passes are usually required.[29] Biopsy specimens should be at least 15 mm long to obtain an adequate sample for analysis.[14]

In addition, the transvenous liver biopsy approach facilitates the calculation of the HVPG. This involves placing an occlusive balloon within the cannulated hepatic vein and inflating it to obtain both wedged and free pressures. The HVPG is determined as the difference between these two pressures.[14] The HVPG calculation remains the gold standard for the measurement of portal hypertension. An HVPG measurement greater than 5 mm Hg is abnormal and a value $\geqslant$10 mm Hg is diagnosed as clinically significant portal hypertension.[30] An increasing HVPG measurement is known to correlate with the risk of decompensation in patients with MASLD.[31] The importance of HVPG measurements can also have therapeutic implications. Beta-blockers, known to reduce elevated HVPG, have recently been shown to prevent decompensation in liver disease when initiated in asymptomatic patients diagnosed with clinically significant portal hypertension, assessed via transvenous HVPG calculation.[32]

Laparoscopic biopsy

Laparoscopic liver biopsy is a less commonly used technique in patients with coagulopathy when a transjugular liver biopsy is not available or has failed. It may also be used when a lesional biopsy is required in cases of severe coagulopathy. This approach provides direct liver visualization and allows for coagulation of the biopsy site for immediate hemostasis. Laparoscopic biopsy is carried out in an operating room, with cross-sectional imaging required beforehand to rule out anatomical contraindications.

Minilaparoscopy provides an alternative to standard laparoscopy using a minimally invasive approach and can be performed under conscious sedation. This approach uses optical instruments of less than 2 mm in diameter.[11] A sheathed needle is used to both create a pneumoperitoneum and introduce the optical instrument through a single puncture near the umbilicus. After a macroscopic assessment of the liver surface, a biopsy can be performed. Ascitic fluid can also be drained if present. While less invasive, minilaparoscopy has limitations including reduced visualization compared with standard laparoscopy due to the small diameter of the optical instrument.

Studies suggest that laparoscopy and minilaparoscopy are effective in diagnosing liver cirrhosis, providing a higher sensitivity compared to percutaneous biopsy.[33,34] Minilaparoscopy is feasible even in patients with coagulation disorders.[35] Potential complications include sedation-related risks, accidental vascular injection of nitrous

Table 1. Summary table of conventional liver biopsy techniques.

	Percutaneous biopsy	Transvenous biopsy	Laparoscopic biopsy
Imaging guidance	Not required, however ultrasound-assistance or guidance can be used For lesion biopsies, real-time imaging guidance with US, CT, or MRI is required	Real-time fluoroscopy	Cross-sectional imaging prior to biopsy
Needle size, biopsy length, number of needle passes	16-Gauge (ideal), at least 20 mm in length One needle pass is preferred unless the inadequate sample	18- or 19-G, at least 15 mm in length At least 2–3 needle passes	Not specified
Tissue sample size	Minimum 20 mm	Minimum 15 mm	Not specified
Complications	Hemorrhage, pneumothorax, puncture of other organs (gallbladder, colon, kidney), bile leak, death	Ventricular arrhythmias, hemorrhage, pneumothorax, death	Bleeding, intestinal perforation, vascular injection of nitrous oxide, death

Source: Behrens and Ferral,[14] Neuberger et al.,[15] Rockey et al.,[41] and Patel et al.[42]
MRI, magnetic resonance imaging.

oxide, visceral injury, and hemorrhage. Reports of liver biopsies using standard and minilaparoscopy techniques demonstrate low rates of hemorrhage, overall complications, and mortality.[36–38]

Furthermore, performing laparoscopic liver biopsy during major bariatric surgery can safely provide histologic information about the liver. Recent studies have demonstrated safety, though increased operative time as a consequence of operative liver biopsy at the time of major bariatric surgery.[39] However, routine liver biopsy during major bariatric surgery remains controversial. While it can identify high rates of advanced fibrosis when performed routinely,[39] proponents caution against routine biopsies due to the lack of current standard therapeutic interventions for MASLD. Furthermore, major bariatric surgery can independently improve MASLD and associated fibrosis, and routine liver biopsy, a static investigation obtained before intervention, will not be utilized to monitor disease improvement.[40]

Please refer to Table 1 for a summary and comparison of current liver biopsy techniques.

Endoscopic ultrasound-guided liver biopsy

In addition to the conventional methods of liver biopsy that are performed when tissue sampling is required, endoscopic ultrasound-guided liver biopsy (EUS-LB) stands as a more recent and evolving technique. It has gained large interest given its benefit of enabling image-guided bilobar liver biopsy that theoretically improves the diagnostic accuracy and safety of the procedure, as well as efficiency when combined with other endoscopic procedures.

EUS-LB for the diagnosis of benign liver disease was first reported in 2007, where 21 patients underwent transgastric EUS-LB via a 19-gauge spring-loaded TruCut needle.[43] The technique was shown to be safe and feasible but was limited by smaller tissue samples and inadequate histological assessment. In 2012, one study introduced the use of a 19-gauge fine-needle aspiration (FNA) needle for EUS-LB which showed improved histological adequacy.[44] These results accelerated the development of the technique over the next few years.

EUS linear echoendoscope is used during EUS-LB with endosonographic image and Doppler to obtain accurate visualization of the anatomical and vascular structures. Typically, the left lobe of the liver is accessed from the gastroesophageal junction, whereas the right lobe is accessed from the duodenal bulb. Various needle types, needle preparations, and tissue acquisition techniques have been used to achieve adequate tissue yield, which is a liver core of 20 mm in length and containing at least 10 portal tracts.

Commonly used needles include 19-G Tru-Cut needles, 19-G flexible FNA needles, and

fine-needle biopsy (FNB) needles including Franseen needles, and reverse-bevel with tissue trap. FNB needles have been shown to have improved tissue adequacy when compared to Tru-Cut needles.[45] They also have generally been shown to be superior to FNA needles in obtaining adequate tissue samples. In a prospective randomized study, FNB provided better tissue sample adequacy compared to FNA needles with longer liver specimens and more complete portal triads.[46] Notably, the diagnostic yield is most optimized with a 19-G Franseen needle, while other needle types such as a fork-tip or ProCore reverse bevel have been demonstrated to be inferior.[47,48]

Needle preparation and biopsy techniques are also pertinent to obtaining adequate tissue samples. Multiple biopsy techniques have also been applied, including the dry suction technique where negative pressure is applied via a dry syringe to obtain samples, and the wet suction technique where a saline-flushed syringe is used instead.[49] The latter was associated with less tissue fragmentation. Priming the needle with heparin prior to wet suction, a method known as the wet heparin approach, yielded longer samples with minimal fragmentation and the highest number of complete portal triads when compared to the dry technique.[50]

In comparison to conventional liver biopsy methods of percutaneous or transjugular entry, EUS-LB is equivalent in terms of specimen adequacy.[51,52] However, EUS-LB has limitations and complications, which will factor into its acceptance as a primary modality for liver biopsy. The technical specifications of needle sizes, brands, and types that are conducive for EUS-LB are quite limited and there is yet to be a consensus on the types of needles that are most suited for EUS-LB.[46,47]

Furthermore, specific needle preparation methods and biopsy techniques are necessary to ensure adequate biopsy yield with EUS-LB. For example, the wet approach requires priming of the needle with heparin followed by saline, to improve yield in the number of actuations obtained.[50,53] Further nuances are needed in sample processing as these biopsies have a higher risk of iatrogenic fragmentation as compared to biopsies obtained by conventional biopsy methods. They cannot be handled on tissue gauze, but need a careful transfer, by flushing, into a liquid medium.[49]

The advantages of EUS-LB over other liver biopsy modalities include access to both liver lobes, shorter recovery time, and improved cost-efficiency when the esophagogastroduodenoscopy is done for additional investigations or therapeutic purposes. A recent meta-analysis assessed the incidence of complications associated with different liver biopsy modalities (percutaneous liver biopsy, transvenous liver biopsy, and EUS-LB) and concluded that there was no statistically significant difference among the adverse events between these modalities.[54]

Complications and safety associated with the performance of liver biopsy

The predominantly favored current techniques for liver biopsy are percutaneous or transvenous approaches. The biopsy approach is chosen based on patient factors, including the presence of ascites, elevated body mass index (BMI), severe coagulopathy, or thrombocytopenia, and whether there is a simultaneous need for hemodynamic evaluation or calculation of the hepatic venous portal gradient.[15]

Written, informed consent is required prior to biopsy, including the risks and benefits of the procedure and whether alternative diagnostic tools are appropriate. The risks of liver biopsy include the following:

(a) Bleeding: including intraabdominal, intrahepatic, biopsy (venous or percutaneous) site occurs in up to 10% with severe bleeding occurring in less than 2%. Risk factors for bleeding from a percutaneous liver biopsy include older age, comorbidities, coagulation dysfunction, and indication for liver biopsy.[15,55]
(b) Pain: most common complication of liver biopsy, occurring in 30%–50% of patients. The pain is typically mild and resolves within hours of the procedure.[56]
(c) Mortality associated with liver biopsy is less than 1 in 1000.[57]
(d) Other: rare risk of infection, injury to other organs.
(e) Other (specific to transjugular approach): cardiac arrhythmias, neck hematoma, pneumothorax, transient Horner's syndrome, fistula tract formation.[15]

Liver biopsies are typically outpatient procedures and do not necessitate admission for monitoring.

Patients should lay on their side and be observed for 3 h post-procedure to ensure bleeding cessation. After a biopsy, patients should be advised to seek medical attention if bleeding occurs from the biopsy site, the biopsy site appears erythematous or inflamed, if febrile, or if the pain at the biopsy site does not resolve in a few days despite analgesia.[58]

There are numerous contraindications to liver biopsy which vary depending on the approach. Contraindications to percutaneous liver biopsy include significant irreversible coagulopathy or thrombocytopenia, pharmacologic anticoagulation use, suspected hemangioma, vascular tumor, echinococcal cyst, inability to identify the site, elevated BMI, or infection in nearby pleural or abdominal cavities. Transvenous biopsy approaches may be more suitable if the patient will not accept blood products and could be at an increased risk of a bleeding complication. There are a few specific contraindications to the transvenous approach, including lack of suitable venous access or if the biopsy is targeting a focal lesion. Overall, both approaches require a cooperative patient.[14,15,41]

Certain patient populations should receive specialized care when receiving a liver biopsy. Patients with chronic renal failure on hemodialysis are at risk for increased bleeding as a consequence of platelet dysfunction in the setting of uremia. Ideally, patients on hemodialysis should have a liver biopsy on the day after dialysis if possible. There is some evidence for the administration of desmopressin immediately prior to the procedure despite the coagulation parameters; however, the true benefit of this practice is unclear.[59] Patients with primary disorders of hemostasis may have an increased risk of bleeding requiring preventative measures. There should be consultation with an expert in coagulation disorders prior to proceeding. Patients with amyloidosis are at increased risk of bleeding due to factor X deficiency and amyloid infiltration into blood vessel walls. The diagnosis of amyloidosis should be established from biopsies of other sites, including fat pads, kidneys, bone marrow, colon, or small bowel.[60] Table 2 summarizes and compares the safety of percutaneous and transvenous liver biopsy approaches.

Prior to the biopsy, a review of medications is required. Medications that can prolong bleeding time including antiplatelets, (NSAIDs), anticoagulants, and alternative therapies (Ginkgo biloba and fish oil) should be held prior to the procedure if feasible. Antiplatelet agents, particularly P2Y12 inhibitors, should be discontinued at least 7 days before a procedure. However, discontinuing aspirin (ASA) can be controversial, and the decision to discontinue or continue it depends on the operator's discretion regarding the bleeding risk. A recent study reported a minor increase of 0.2% in the incidence of bleeding complications for patients who remained on ASA.[61] Despite the lack of specific guidelines on whether to continue or stop ASA, approximately 75% of operators choose to withhold ASA before a liver biopsy.[62] Operators who decide to discontinue ASA must weigh the potential increased risk of stroke or cardiovascular events that could result from stopping the medication.[61] Anticoagulants such as warfarin should be held 5 days prior with an international normalized ratio (INR) prior to the procedure and novel direct oral anticoagulant (DOAC) agents should be held 2 days prior to the procedure. In patients with renal disease, DOACs should be held for a longer duration to ensure clearance.[14,15] Recommendations for discontinuation intervals are summarized in Table 3.

In patients with cirrhosis, common hemostasis measures such as INR, platelet count, and fibrinogen levels are often altered due to liver dysfunction, which impacts the synthesis of coagulation factors and leads to thrombocytopenia. However, these parameters only reflect one aspect of hemostasis and do not account for the compensatory mechanisms in cirrhosis, such as elevated von Willebrand factor and the balancing of pro- and anticoagulant factors, leading to a concept of "rebalanced hemostasis."[63,64] Regardless, coagulation parameters are typically measured prior to the procedure, including a complete blood count and INR. More recent evidence has demonstrated that elevated INR in chronic liver disease does not truly reflect bleeding risk. This population has a concomitant risk of thrombosis and bleeding risk from reduced synthesis of procoagulant proteins and number of platelets. INR is sensitive to fibrinogen and factors 2, 5, 7, and 10 and does not accurately test hemostatic balance in patients with liver disease. A Cochrane review concluded that there is uncertainty about the safety and utility of prophylactic fresh frozen plasma (FFP) use.[65] The Society of Interventional Radiology guidelines suggest the INR threshold for performing procedures is <2.5 in those with

Table 2. Comparison of the safety of performing percutaneous and transvenous liver biopsy approaches.

Percutaneous liver biopsy		Transvenous liver biopsy	
Absolute	**Relative**	**Absolute**	**Relative**
Inability to cooperate with the procedure	Elevated BMI	Nil specific	Significant coagulopathy or thrombocytopenia (correct with blood products)
Significant coagulopathy or thrombocytopenia	Hemophilia		Lack of suitable venous access
	Infection within the right pleural cavity		Biopsy targeted to a focal lesion
Suspected hemangioma, vascular tumor, echinococcal cyst	Infection below right hemidiaphragm		
Inability to identify adequate site by percussion and/or ultrasound	Amyloidosis (high bleeding risk)		
Extrahepatic biliary obstruction	Ascites		
Patient refusal to accept blood transfusion	Nonsteroidal anti-inflammatory drugs [NSAID] (including ASA) use within last 7–10 days—operator dependent see Section "Complications and safety associated with the performance of liver biopsy"		

Source: Behrens and Ferral,[14] Neuberger et al.,[15] and Rockey et al.[41]
ASA, aspirin; BMI, body mass index.

chronic liver disease compared to ⩽1.5–1.8 for the general population.[42] There is a paucity of data to indicate the *safe* platelet count prior to liver biopsy; however, common practice is to transfuse platelets if the count is $<50 \times 10^9$/L.[41] Pre-emptive viscoelastic testing may help guide bleeding risk estimation in the future, but further trials are currently required; currently, conventional liver biopsy guidelines and safety protocols should be followed.[63,64]

Once the tissue is obtained, wedge or core biopsies should be placed into a formalin-containing sterile container for planned histopathological analysis. Any specific local practices to handle samples from patients with known risk of infection (hepatitis, HIV, tuberculosis) should be followed. If the diagnostician is assessing for Wilson's disease or hemochromatosis, a separate core of tissue must be obtained to estimate copper or iron content, respectively.[44]

Advances in histopathological analysis

Histopathological features of MASLD/MASH

Histopathological analysis remains the gold standard for diagnosing and staging MASLD. Upon histological examination, the prominent features of MASLD/MASH include centrilobular zone 3 predominance, steatosis, inflammation, ballooning degeneration, and the concurrent assessment of fibrosis severity.[66,67] Other suggestive findings would be acidophil bodies, iron deposition, vacuolated nuclei, Mallory–Denk bodies, and cytoplasmic megamitochondria in ballooned hepatocytes.[68]

The grading of disease activity and staging of fibrosis can be challenging histologically in MASLD.[67] The METAVIR system, originally developed for chronic viral hepatitis C in 1993, was one of the first formalized histologic scoring systems applied to liver histopathology.[69] This

Table 3. Recommendation interval for medication discontinuation prior to liver biopsy.

Drug	Dose	Stop	Notes
Clopidogrel, prasugrel, ticagrelor	–	7 days	If cannot be delayed, stop clopidogrel and consider the transvenous approach
ASA	–	3–7 days	If urgent, biopsy can be continued at clinicians' discretion
Dual antiplatelet	–	Consider if biopsy can be delayed or if clopidogrel can be stopped. Always continue ASA	
Dipyridamole	–	Omit on the day of the biopsy	
Low molecular weight heparin	Prophylaxis	12 h before the procedure	
	Therapeutic	24 h before the procedure	
Warfarin	Therapeutic	5 days before the procedure	Point of care INR should be tested to ensure within the target
Direct oral anticoagulants	–	Omit for 2 days before the procedure	Omit for longer if renal impairment or on dabigatran

Source: Behrens and Ferral[14] and Neuberger et al.[15]
ASA, aspirin; INR, international normalized ratio.

scoring system has also been increasingly applied in MASLD as well as most other causes of chronic liver diseases. It grades viral activity (A0–A3) and categorizes fibrosis into stages (F0–F4) based on the extension of fibrosis—providing valuable prognostic information. Its adoption and spread underscore the importance of standardized fibrosis assessment across liver diseases.[68,69]

In 1999, Brunt et al.[70] introduced one of the earliest histologic scoring systems for MASLD, which included features such as steatosis, hepatocyte ballooning, portal inflammation, and lobular inflammation to grade steatosis levels and formalize fibrosis patterns for staging. Following this work in 2002, the National Institute of Diabetes & Digestive & Kidney Diseases sponsored the multicenter NASH clinical research network (NASH CRN) and validated a histological scoring system, the NAFLD activity score (NAS), to assess the full spectrum of histological changes in adult and pediatric MASLD. The NAS focuses on features like steatosis, lobular inflammation, and ballooning, and excludes fibrosis due to its irreversible nature in disease activity assessment.[71] NAS is currently the most well-validated and applied histopathologic scoring system and has transformed the way pathologists quantify disease activity in clinical trials.[72]

Recently, the Fatty Liver Inhibition of Progression Pathology Consortium developed an algorithm called the Fatty Liver Inhibition of Progression based on Steatosis, Activity (including ballooning degeneration and lobular inflammation), and Fibrosis (SAF score).[73] Originally designed for morbidly obese NAFLD patients, the SAF score has been validated in those with NAFLD and metabolic syndrome.[74] Unlike the NAS, the SAF score distinguishes between steatosis and necro-inflammation, each assessed on a scale from 0 to 3 or 4, respectively. The diagnosis of MASH requires the presence of steatosis alongside at least grade 1 activity (ballooning and lobular inflammation). Unlike the NAS score, MASLD with advanced fibrosis but without necro-inflammation would still be classified as "significant disease" according to the SAF score.[75]

Pathologists assess disease activity in MASLD using steatosis, lobular inflammation, and ballooning degeneration. Steatosis shows the highest reproducibility among pathologists, while agreement on lobular inflammation and ballooning

degeneration is generally less consistent.[76] The ballooning phenomenon, specifically has been previously identified as a critical indicator in the progression of MASH, signifying a higher risk of severe disease outcomes.[77] Both the NAS and SAF scoring methods evaluate hepatocyte ballooning on a 3-point scale (0–2), with subtle differences in their definitions that may result in variations in interpretation and application.[78] Due to significant variability among expert hepatopathologists in assessing hepatocyte ballooning, AI-trained models are increasingly being utilized to improve and standardize grading.[78,79] However, no current AI model has been established as the gold standard for pathologic analysis, and controversy persists in this field.

Despite these limitations, many current MASH clinical trials continue to rely on a single-reader approach, which may introduce temporal bias and intra-reader variability.[80] Acknowledging these limitations, published strategies to try to reduce variability in liver histology interpretation include implementing standardized procedures for slide processing, providing clear guidelines for biopsy interpretation during the study, enhancing pathologists' training before and during studies, and recommending the involvement of at least two pathologists to review each slide, with a third pathologist included if there is disagreement.[81]

Utility of stains and immunohistochemistry

Several studies have also been trying to utilize histopathological stains and immunohistochemistry (IHC) to assess and aid in predicting the severity and prognosis of patients with MASLD, as well as determining the pathogenesis and potential targets for treatments.

Histopathological assessment of liver biopsies is largely done using hematoxylin and eosin (H&E) stain. However, supplementary staining techniques can be employed to discern characteristics that may not be readily observable in an H&E stain. For instance, Masson's trichrome stain has been used to aid pathologists in evaluating the early features of perisinusoidal fibrosis in the setting of MASH/steatohepatitis, by visualizing the early disposition of excess connective tissue.[82]

IHC has an expanding role in the histological assessment of MASLD and MASH. In 2014, a study demonstrated the value of immunohistochemical staining for pathologists to diagnose MASH accurately, using special stains to detect Leukocyte Common Antigen and CD68 which are specific to intralobular inflammation, as well as detecting hepatocellular ballooning using immunohistochemical staining for cytokeratin 8, 18, and ubiquitin.[68] Furthermore, as it is currently hypothesized that hepatotoxicity in MASLD is related to the process dysregulation in the lipid metabolism in the context of lipophagy blockage, a study conducted on liver biopsies from 59 patients using IHC analysis, revealed that some markers—such as p62/SQSTM1 proteins—can be used to predict fibrosis progression in MASLD.[83] In another separate pilot study in 2020, researchers have proposed the use of an IHC assay to detect a specific protein adduct (4-HNE) that is associated with lipid peroxidation which can aid in detecting patients with MASLD and also aid in assessing response to certain antioxidant treatments, that is, Vitamin E.[84] Lastly, IHC presents significant potential for researchers to explore and develop targeted therapies; therefore, numerous studies using immunostaining on animal models have pinpointed certain proteins, such as MiR-122 and MiR-378, which exhibit expression in the context of MASLD-related liver fibrosis. These findings highlight their potential as therapeutic targets in the context of human treatment.[85,86]

Digital pathology and AI in histopathology of MASLD/MASH

Technological advances in pathology have revolutionized the field, particularly whole slide imaging, a technology that digitizes histopathological slides and provides the ability to analyze an entire tissue section.[10] It also enables pathologists to view and analyze tissue samples remotely, increasing the global database of pathological specimens, facilitating collaboration, and improving diagnostic accuracy.[87] Importantly, digital pathology provides detailed quantitative data on fibrosis dynamics, shedding new light on the regression of fibrosis in MASH following treatment.[88] However, despite its great potential benefits, it still has some challenges and limitations. The cost of high-definition scanners, the availability of confidential virtual clouds and storage, and the requirements of staff training are definitely some of the barriers. In addition, regulatory issues might still be raised regarding storing and accessing these digital slides.[89]

AI plays a pivotal role in enhancing the capabilities of digital pathology in MASLD analysis.

Machine learning algorithms can identify subtle histopathological features that might escape the human eye, providing a more comprehensive assessment.[90] AI-driven tools can also predict disease progression and identify patients at risk of developing severe liver disease.[91] Furthermore, the integration of AI and digital pathology has the potential to automate the quantification of fibrosis, thus reducing subjectivity and variability in histological assessment. This streamlined approach not only improves diagnostic consistency but also facilitates the monitoring of treatment response, particularly in MASH.[91,92]

In addition, some AI-based systems have been introduced to address the limitations of the NAS scoring system in diagnosing clinical MASH. The GENESIS system and the qFIBS system utilize AI-driven automation, employing advanced second-harmonic-generation/two-photon excitation fluorescence techniques. This technical approach facilitates the quantification of fibrosis, ballooning, steatosis, and inflammation in patients diagnosed with MASH. Notably, these systems demonstrated robust correlations with NASH CRN scores, effectively distinguishing between various histological disease stages.[93,94] The recent introduction of the popular PathAI, a machine learning-based system, has further emphasized the role of AI for improved quantification of liver histology in MASH and could improve disease understanding and lead to an increase in treatment options.[95] AI applications in MASLD have further extended to subtyping using topological data analysis methodology. Moreover, fully automated software for inflammation, steatosis, ballooning, and fibrosis quantification has shown superior sensitivity compared to semiquantitative scoring systems in liver biopsy specimens. The role of AI holds promise in revolutionizing MASLD histological analysis.[79]

Utility of liver biopsy in MASLD

Liver biopsy continues to serve as the gold standard against which noninvasive (both serologic and imaging) methods are evaluated for the assessment of these features. Given drawbacks such as cost or the risk of morbidity and mortality, liver biopsy is rarely performed in the routine management of patients with MASLD. A survey of academic gastroenterologists and hepatologists in the US reveals that while liver biopsy is recommended as the gold standard for the diagnosis of MASLD, less than 25% of respondents will routinely proceed with completion of liver biopsy. Practitioners will instead rely on serial noninvasive tests or assessments to monitor for progressive disease. Of note, previous studies have cautioned that relying solely on noninvasive markers may result in the underdiagnosis of MASLD and associated fibrosis.[96] However, proponents have argued that since there are currently limited approved therapeutic options for the management of MASLD beyond routine lifestyle recommendations, the utility of liver biopsy for population-level risk stratification is likely inappropriate.[66,97] New noninvasive tests/assessments, such as fibroscan-aspartate aminotransferase (FAST), magnetic resonance elastography (MRE) combined with FIB-4 (MEFIB), MRI-aspartate aminotransferase (MAST), or corrected T1 (cT1), are now being positioned to identify "at-risk" MASH (defined as NAS $\geq$4 and fibrosis stage 2 or higher).[6,98,99] Please refer to the section "Advances in noninvasive fibrosis assessment: Implications for clinical practice" for further information on noninvasive fibrosis assessments.

Recent guidelines pertaining to the management of MASLD have tried to clarify the role of liver biopsy in routine clinical decision-making. In cases where cirrhosis is suspected based on noninvasive tests, clinical data, or radiological findings, it is reasonable to institute a cirrhosis-based management strategy without completion of a liver biopsy. Nevertheless, guidelines suggest the consideration of liver biopsy in scenarios where noninvasive tests indicate indeterminate degrees of fibrosis ($\geq$F2) when noninvasive tests suggest "at-risk" MASH, or when noninvasive test results yield inconclusive findings.[6,100] Additional recommendations suggest that liver biopsy can be considered when aminotransferases remain persistently elevated for a duration exceeding 6 months, or in the presence of indications suggestive of additional or alternative diagnostic considerations.[6] Figure 1 illustrates an approach to assess a patient with MASLD and outlines management considerations that may necessitate a liver biopsy.

Liver biopsy finds its most robust utility in the context of clinical research, particularly in its capacity to assist with the identification of histological endpoints in therapeutic intervention trials. A recognized knowledge deficit exists in the field concerning the validation of noninvasive assessments for monitoring the progression of

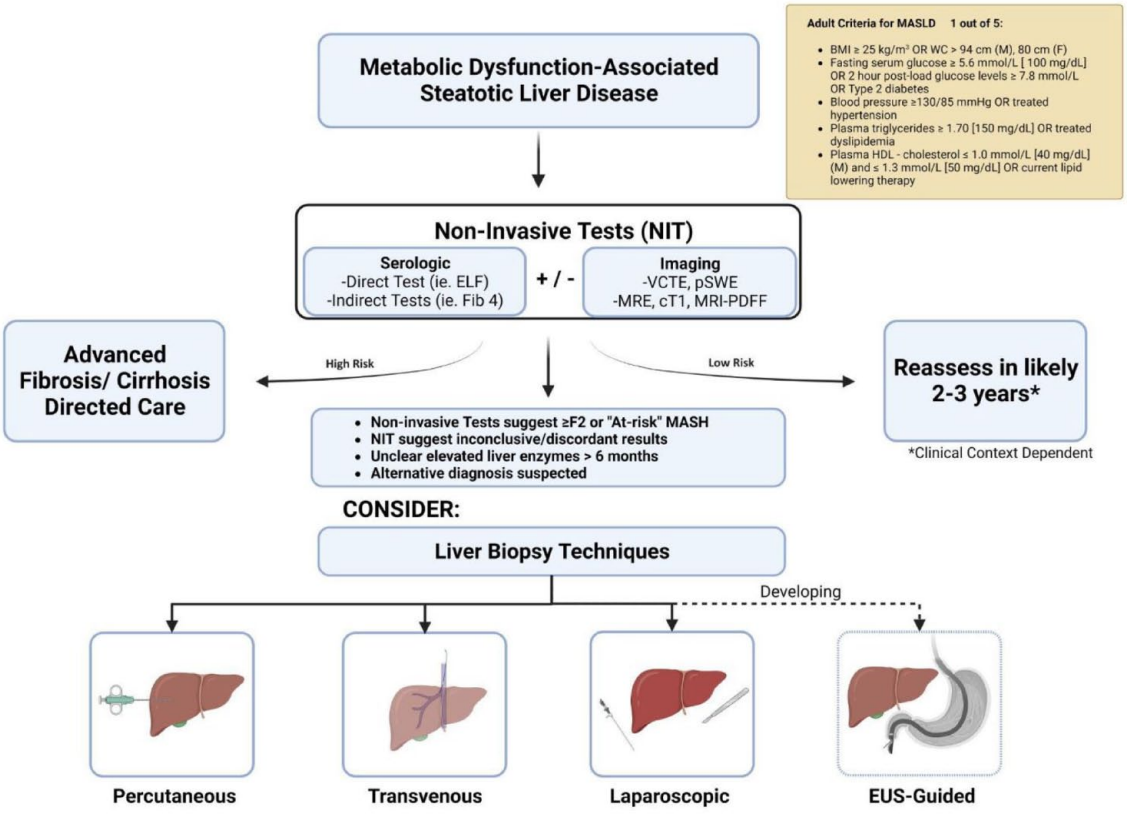

Figure 1. An approach to assessing a patient with MASLD and clinical considerations that may necessitate a liver biopsy.
MASLD, metabolic dysfunction-associated steatotic liver disease.

MASH/MASLD in addition to the ability of noninvasive assessments to accurately prognosticate disease.[101] Following guidance Food and Drug Administration and American Association for the Study of Liver Diseases (AASLD)/ European Association for the Study of the Liver (EASL), patients typically enrolled in phase II and phase III trials depend upon a definite histologic diagnosis of MASH and identification of "at-risk" status with a NAS ⩾4.[101–103] Most trials typically enroll patients with biopsy-confirmed F2 or F3 fibrosis, and recognizing the potential for variability in interpretation, they often involve central pathologist review or consensus by a pathology adjudication committee comprising at least two pathologists to ensure precise scoring.[66] Histologic outcomes are typically used as surrogate endpoints as liver-related mortality correlates with increasing fibrosis; particularly evident in cirrhosis (F4), while even F3 and F2 stages show significantly elevated mortality compared to minimal or absent fibrosis (F1 or F0).[104] Consequently, histological examination becomes crucial for identifying patients with MASH at the F2 and F3 stages, where potential trial interventions could demonstrate clinical benefits. This underscores the importance of liver biopsy in clinical research, as opposed to relying solely on noninvasive assessments.

A reliance on histological analysis has to also be cautioned when strictly applied to the development of clinical drug trials in MASLD. Recent studies have suggested significant inter-reader variability that can impact clinical trial critical endpoints such as steatosis, fibrosis, and the diagnosis of MASH.[80,105] A simulation assessing the suboptimal reliability of histopathological analysis on patient inclusion revealed significant impacts on the misclassification of fibrosis stages and the perceived treatment efficacy, potentially reducing study power from over 90% to as low as 40%.[80] This reliability issue may markedly affect the robustness of pharmaceutical studies in MASLD.[106] Moreover, the variability of liver biopsy samples presents a challenge because histologic MASLD lesions can be unevenly distributed

within the liver, rendering one or two biopsy specimens potentially non-representative.[107]

Post-treatment liver biopsies in MASLD trials aim to demonstrate ⩾1 stage improvement in fibrosis, but due to the gradual nature of fibrosis reversal and the static nature of current pathological staging, detecting subtle changes within 48–72 weeks may be challenging, highlighting the need for a more dynamic understanding of disease progression beyond traditional scar assessments.[106] As a result, new trials have begun to incorporate noninvasive fibrosis assessments to capture dynamic changes in disease activity.[108,109]

Advances in noninvasive fibrosis assessment: Implications for clinical practice

In clinical practice, the utilization of standard liver biopsy as the primary means of identifying underlying fibrosis is neither practical nor appropriate, particularly in light of the prevailing prevalence of MASLD. This judgment is largely predicated on the significant risks of morbidity and mortality associated with liver biopsy techniques.[41] Consequently, a critical need has emerged for effective patient triage to liver biopsy, a task that may be facilitated by noninvasive assessments.

Noninvasive assessments, in this context, serve as a preliminary screening tool aimed at gauging the severity of the disease and providing clinicians with valuable insights into the potential need for a liver biopsy with pathologic assessment. This determination informs the clinician as to whether such an invasive procedure would lead to a change in the management plan, thereby justifying its pursuit. There are emerging noninvasive assessments to distinguish MASH from simple steatosis, facilitating the stratification and identification of high-risk MASLD/MASH patient populations who may benefit from intensive lifestyle and pharmacologic interventions.[110]

Noninvasive assessments can be broadly classified into two main categories: serologic tests, encompassing both direct and indirect markers, and imaging techniques.

Serologic tests

Direct markers. Direct markers of fibrosis reflect changes in the extracellular matrix of the liver and reflect the pathogenesis of fibrosis being dynamic and derived from hepatic stellate cells and myofibroblasts. Direct markers are subcategorized into markers associated with matrix deposition (i.e. procollagen, type I and IV collagen levels), matrix degradation (i.e. matrix metalloproteinases, tissue inhibitors of metalloproteinase), and cytokines and chemokines associated with fibrinogenesis or fibrinolysis.[111] Direct markers find limited use in clinical practice as they are associated with extended testing turnaround times, restricted accessibility, mainly within research settings, and high costs.[112]

Enhanced liver fibrosis score. The enhanced liver fibrosis score is a combination score of three different markers of direct fibrosis: Human Procollagen Type II N-Terminal propeptide, Metalloproteinase-1, and hyaluronic acid. A higher score is indicative of increased levels of fibrogenesis. In patients with MASLD, this was shown to have a sensitivity of 89% and specificity of 96% for diagnosis of advanced fibrosis.[113,114]

Indirect markers

Aspartate aminotransferase to platelet ratio. Aspartate aminotransferase to platelet ratio (APRI), a simplified model utilizing measured aspartate aminotransferase (AST) and platelet count, effectively identifies patients with a history of hepatitis C infection and advanced fibrosis. In validation, APRI demonstrated high accuracy, with an area under the curve (AUC) of 0.88 for significant fibrosis and 0.94 for cirrhosis.[115]

Fibrosis-4 index. This score utilizes serologic markers such as alanine aminotransferase (ALT), AST, platelet count, and age to predict at-risk individuals who may potentially benefit from further investigations. The low cost, high negative predictive value (NPV) of 88%–95% when a cutoff of less than 1.30 is used, and ease of calculation makes this score extremely favorable to implement at the level of primary care.[116]

NAFLD fibrosis score. The NAFLD fibrosis score calculates a score after measuring ALT, AST, platelet count, and albumin as well as taking into account patient characteristics including age, BMI, and presence of hyperglycemia, this score was also shown to have a high NPV of 88% and positive predictive value (PPV) of 82% in the validation groups.[117]

Fibrotic NASH index. The fibrotic NASH index (FNI) calculates a score combining AST,

high-density lipoprotein cholesterol, and hemoglobin A1c to detect fibrotic MASH in high-risk individuals. The FNI showed robust predictive accuracy in both derivations (AUC = 0.78) and external validation cohorts (AUC = 0.80–0.95), making it a promising tool for primary healthcare screening.[118,119]

FibroTest. This score was initially developed and validated to predict the level of fibrosis in patients with chronic hepatitis C infections but has since been validated in NAFLD. It combines alpha-2 microglobulin, apolipoprotein A1, gamma-glutamyl transpeptidase, haptoglobin, total bilirubin, and ALT with the age and gender of the patient to generate a score that correlates with the level of fibrosis.[120]

Imaging-based modalities

Vibration-controlled transient elastography ± controlled attenuation parameter. Vibration-controlled transient elastography (VCTE) measures liver stiffness by an ultrasound transducer that is mounted on the axis of a vibrator that transmits vibrations of mild amplitude and low frequency to tissues which, in turn, produces an elastic shear wave, pulse echo then follows the propagation of the elastic shear wave, and measures its velocity which is directly related to the tissue stiffness. When the elastic shear wave is propagated faster, the tissue stiffness is calculated to be higher; these measurements are expressed in kilopascals (kPa).[121,122] During an examination, liver stiffness is assessed within a cylindrical volume measuring roughly 10×40 mm in dimension and situated at a depth of 25–65 mm beneath the skin surface. This volume is at least 100 times larger than a typical biopsy specimen, making it a significantly more representative sample of the liver parenchyma.[123,124]

Machines equipped with VCTE have been enhanced to incorporate the evaluation of hepatic steatosis levels by utilizing the controlled attenuation parameter (CAP). This parameter has demonstrated a correlation with both sensitivity and specificity in diagnosing the presence of steatosis. However, the precision of the established cutoff values for the classification of steatosis stages has raised concerns and warrants further scrutiny.[125]

Point shear wave elastography. The point shear wave elastography technique measures shear wave propagation velocity in the liver parenchyma, offering an accurate and reproducible assessment of liver stiffness without the need for vibratory stimulation. An acoustic radiation force impulse is applied in a region of interest. This technique is versatile, allowing measurements in different liver segments, including those in obese patients, and provides valuable information on liver morphology and vascular structures in addition to stiffness assessment.[124]

Ultrasonography and two-dimensional shear wave elastography. Ultrasound is a widely available, cost-effective, and radiation-free imaging modality that can detect hepatic steatosis based on increased echogenicity of the hepatic parenchyma; however, it is limited by its inability to precisely quantify the degree and severity of steatosis.[126]

Some ultrasound machines offer the integration of two-dimensional shear wave elastography; this technique involves making multiple acoustic radiation force impulse measurements across a large field of view, all in real time, and analyzing mean, maximum, minimum, and standard deviation of shear wave velocity in a single region of interest. This approach allows for the visualization of elastography measurements in real time on a color display as they accumulate. The method involves dragging the acoustic radiation force impulse focus below the acoustic axis to create a shallow-angle cone of shear wave propagation, achieving greater accuracy in detecting early and intermediate stages of fibrosis in patients with chronic liver disease compared to the transient elastography (TE) technique.[124]

Magnetic resonance elastography. Magnetic resonance elastography (MRE) is a diagnostic tool that is used in evaluating chronic liver disease and assesses for the presence of any hepatic steatosis or fibrosis. It utilizes propagating waves that are transmitted through the liver and create microscopic shear displacement that can be imaged by the MR sequence. It can also automatically produce stiffness maps to calculate mean liver stiffness expressed in kPa. In contrast to liver biopsy, it can evaluate larger portions of the liver for fibrosis with better interobserver agreement and has a good correlation with liver biopsy in evaluating many chronic liver diseases.[127]

MRI-derived proton density fat fraction. MRI-derived proton density fat fraction (MRI-PDFF)

is currently being studied as a surrogate for liver biopsy in assessing patients with MASH and their response to treatment, though further studies are needed to confirm its accuracy.[128,129] MRI-PDFF has high accuracy in estimating the fat on all the liver surfaces. It utilizes a technique that measures the proton signal coming from the water and fat in any tissue and eventually using what is called the chemical shift encoded MRI method, it will quantify the amount of fat in the tissue. The PDFF is correlated to histological assessment of hepatic steatosis that is measuring the percentage of cells that contain fat. It has been mainly utilized in the assessment of treatment in MASH patients and is not used to measure other outcomes, such as inflammation or fibrosis. It was found from the previous data that histological response in patients with MASH is achieved once there is a 29% relative reduction in fat on MRI-PDFF.[130]

Corrected T1. cT1 is an MRI-based test that evaluates for any inflammation or fibrosis and helps in differentiating MASH from MASLD. This test is superior to regular ultrasound or MRI in terms of being able to quantify the degrees of steatosis or fibrosis in the liver and it has been validated against liver biopsy. This MRI technique measures T1 relaxation time, which is a measurement of free water content. It eventually aids in estimating the degrees of steatosis and fibrosis and quantifying iron levels in the liver. A cT1 level of more than 875 ms is associated with a risk of developing decompensating liver disease features, such as ascites and hepatic encephalopathy.[131] Both cT1 and PDFF scores were correlated with the histologic-based NAS, but only cT1 was associated with changes correlating with both the degree of steatosis and fibrosis. A cT1 point difference of 88 ms correlated with a 2-point difference in the value of the NAS.[132]

Evolving methods—the combination of serologic and imaging-based modalities. Many combinations of noninvasive modalities have been validated for assessing MASH. The FAST score is among the initial combination tests, which in calculation combines the CAP and liver stiffness measurements obtained from vibration-controlled elastography with the serologic value for AST. The primary objective of this score is to identify patients with significant fibrosis and a pathological NAS score greater than 4. The test has demonstrated to have an AUC of 79% with a PPV of 85% and sensitivity/specificity of 65% and 92%, respectively.[133] In general, this test has shown favorable outcomes in identifying patients with fibrosis. However, there are instances where some patients may fall into a gray zone where results are inconclusive. In such cases, it is recommended to repeat the FAST score after a reasonable time interval to reassess for fibrosis.[134]

AGILE 3+ is another score to evaluate MASLD patients that includes AST/ALT ratio, platelets count, age, sex, diabetes mellitus, and liver stiffness measurement by VCTE. In previous studies, it has been shown to outperform the fibrosis-4 index (FIB-4) score for detection of advanced fibrosis. It has a demonstrated AUC of 88% in patients with advanced fibrosis due to MASLD.[135] AGILE 4 is another score that was developed to identify patients with MASLD and cirrhosis. This score combines VCTE to estimate liver stiffness with ALT, AST, and platelet count to estimate the degree of fibrosis. Evaluating both scores, the results indicate that the AGILE 4 score exhibited an 85% sensitivity and 95% specificity in predicting cirrhosis. Conversely, AGILE 3+ demonstrated an 85% sensitivity and 90% specificity in favor of rule-in to predict advanced fibrosis.[136]

Another validated score is the MAST score, which is calculated based on the combination of AST, MR elastography, and MRI-PDFF. This score has been shown to surpass the performance of the FAST score, with an AUC of 93% for detecting advanced liver fibrosis. In addition, it boasts a specificity and sensitivity of 90% and 70%, respectively, as well as a PPV of 29.4% and an NPV of 98.1%.[137] Another study demonstrated a sensitivity and specificity of 90% for the MAST score in detecting fibrosis stage greater than METAVIR fibrosis stage 2. The MAST score has proven highly effective in identifying and predicting patients at risk of developing decompensating features, including ascites, hepatic encephalopathy, and hepatocellular carcinoma. This ability allows for a certain degree of prognostication. Moreover, the MAST score exhibited its highest AUC at 0.919 ± 0.042 for each continuous 1 logit unit score increase, outperforming the AUC for the FAST test, which stood at 0.817 (0.768–0.866).[138]

The MEFIB score combines the result of the FIB-4 score and MR elastography liver stiffness measurement.[139] The MEFIB score is considered positive when the MRE score for stiffness is

>3.3 kPa and the FIB-4 score is >1.4 as this cutoff point was found to be able to detect stage 2 fibrosis and above with a high PPV. Additional studies have investigated additional cutoff points, for example, a cutoff point of >5 kPa was found to be associated with cirrhosis, and a cutoff point of >8 kPa was found to have a 20% risk at 1 year of developing decompensating features of chronic liver disease. When the MEFIB score was evaluated, it was found that a positive score had an odds ratio of 19.5 with a p-value < 0.001 for developing hepatic decompensating features compared to negative MEFIB score patients. It was also noted that the incidence of hepatocellular carcinoma was 2.76% and 3.92% in 1 and 3 years, respectively, for patients with a positive MEFIB score compared with <0.01% and 0.15% for patients with a negative MEFIB index.[140] The AUC for detecting liver fibrosis greater than F2 was 92%, indicating strong performance. In addition, it achieved a PPV of 97.1%, further demonstrating its effectiveness in identifying advanced fibrosis.[139]

Multiple pharmacologic agents are being developed to target MASLD liver disease and prevent disease progression. Recently, resmetirom, an oral partial agonist of the thyroid hormone receptor-beta, for the treatment of noncirrhotic MASH with moderate to advanced fibrosis, has been approved based on the results of the MAESTRO-NASH study.[108,141] In addition, Tirzepatide, recently approved for type 2 diabetes mellitus as a dual agonist of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide receptors, has shown benefits in improving MASH-related fibrosis according to recent findings published from the phase II SYNERGY-NASH trial.[109] Both studies utilized liver biopsy to confirm fibrosis for trial inclusion, but noninvasive fibrosis assessments such as MAST, FAST, FNI, and MEFIB scores are anticipated to replace biopsies in real-world settings due to the risks associated with liver biopsy in general population screening for MASLD and advanced fibrosis (F2 or F3). Recent head-to-head trials have shown these noninvasive markers to be sufficiently accurate in identifying patients with MASH and F2–F3 fibrosis, yet the optimal method for selecting candidates for therapeutic intervention remains uncertain, likely necessitating further comparative research.[98,142] Despite liver biopsy remaining the gold standard for trial enrollment, its application may not align with clinical practice in real-world settings.

Conclusion

MASLD is a growing public health concern worldwide, and liver biopsy remains the gold standard for diagnosing and staging the disease. However, liver biopsy is invasive and carries risks including the risk of morbidity and mortality.

This review article has discussed the various liver biopsy techniques available for MASLD including different approaches such as transcutaneous, transvenous, laparoscopic, and the evolving role of EUS-guided biopsy. Currently, there is a push for the research and design of noninvasive modalities to assist in the diagnosis and management of MASLD given liver biopsy is likely an inappropriate modality to screen for disease at a population level. Liver biopsy though still has a very strong role within the field of clinical research where histologic surrogate histopathologic outcomes are known to strongly correlate with desired patient outcomes such as mortality. The current state of knowledge on the histopathologic evaluation of MASLD is ever evolving with various scoring systems used to grade and stage the disease, and we acknowledge that current histopathologic systems and clinical trial endpoints are likely to be revised with the everchanging understanding of the pathophysiology of MASLD and potential future applications of AI. There is an identified need for further research in this area.

In summary, this review article highlights the progress that has been made in liver biopsy techniques for MASLD and the challenges that remain. There are still many challenges to be addressed in the diagnosis and management of MASLD, including the need for more accurate and reliable noninvasive tests, the development of effective treatments, and the implementation of population-based screening programs. Continued research in this area is essential to address these challenges and improve the health outcomes of patients with MASLD.

Declarations

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Consent for publication

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