Review Article

"The pathogenesis of hepatic fibrosis: basic facts and clinical challenges"—assessment of liver fibrosis: a narrative review

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Background and Objective: Liver fibrosis is the key pathogenic feature for progression of chronic liver diseases. To identify patients at risk, assessment of liver fibrosis is an important diagnostic procedure, which needs to be performed as accurate as possible.

Methods: A literature search in PubMed was performed using "assessment of liver fibrosis", "liver fibrosis" and "non-invasive tests for liver fibrosis" as key words. Novel data and well-known milestone publications (from origin until December 31, 2021) were included in this review. Only publications in English were considered for inclusion.

Key Content and Findings: This narrative review discusses liver biopsy and histopathological assessment as gold standard. Beside molecular agents which can be visualized through histology we outline scoring systems related to different etiologies of liver fibrosis. Moreover, it critically discusses serum biomarker non-invasive tests (sNIT) including well-established tests like the ELF®test and FibroTest® and public sNITs, namely APRI, FIB-4 and NFS, but also novel algorithms. Furthermore, imaging-related non-invasive tests (iNIT), mostly based on ultrasound and MRI techniques which more recently have emerged are included in this review. Beside technical considerations and limitations, the clinical relevance will be presented through all tests.

Conclusions: Liver fibrosis assessment helps to stratify patients' care and may save health care resources.

Keywords: Liver fibrosis; histological fibrosis score; imaging techniques; biomarkers

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Introduction

The progression of chronic liver disease (CLD), irrespective of etiology, encompasses parenchymal injury, inflammatory processes and liver fibrogenesis (1). Liver fibrosis, which is defined as excessive accumulation of extracellular matrix (ECM) proteins, ranges from mild pericellular fibrosis in early stages to cirrhosis, which is considered to be the common end-stage of any liver disease (2,3). Liver fibrosis is the determinant of disease progression, major liver-related adverse events and the risk of hepatocellular carcinoma (4). The underlying pathogenic mechanisms differ among etiologies (4). The main cell type involved in fibrogenesis are hepatic stellate cells (HSC), storing

vitamin-A in a quiescent state in healthy livers and transform into a proliferative, fibrogenic activated state during liver injury (5). While in chronic viral hepatitis damage-associated molecular patterns and host's antiviral response are triggering HSC activation, apoptosis-related interleukin and chemokine release mainly provokes HSC activation in alcoholic liver disease (4,6-9). On the other hand, in non-alcoholic steatohepatitis (NASH), lipotoxicity caused by metabolites of saturated fatty acids, damages hepatocytes and result in oxidative stress (10). The latter generates an inflammatory trigger, resulting again in HSC activation. Furthermore, insulin resistance, nutritional factors and genetic factors contribute to liver injury, which is summarized in the so-called "multiple hit"-theory (11).

Thus far, there is no Food and Drug Association (FDA)-approved antifibrotic agent, so prevention is the key strategy. Prevention of liver fibrosis is mostly achieved by an early diagnosis and treatment of the underlying liver disease. When patients are aware of their chronic liver disease, it can either be treated (e.g., metabolic or cholestatic liver disease) or halted by lifestyle changes which mainly applies for alcoholic liver disease. The clinical relevance of fibrosis prevention is explained by its consequences, which is portal hypertension, a condition caused by fibrosis-dependent increased intrahepatic resistance (12). After exceeding a specific pressure gradient between the portal vein and systemic venous circulation, usually 10 mmHg, we speak of clinically significant portal hypertension, which is associated with an increased risk of complications like ascites, varices and hypersplenism (12,13).

Liver fibrosis is usually classified into five stages depending on the quantity: F0—no fibrosis; F1—mild fibrosis, pericellular collagen deposits; F2—moderate fibrosis, beginning bridging fibrosis; F3—severe fibrosis, defined as presence of numerous bridges and septa; F4—cirrhosis according to the most used scores, which will be discussed more detailed in the histology section (14,15). In this review, significant fibrosis is considered as F2 and above (F ≥2), whereas advanced fibrosis is defined as at least strong fibrosis (F ≥3). This is of high importance when evaluating assessment methods for liver fibrosis.

In order to estimate the individual risk of CLD patients, assessment of liver fibrosis needs to be performed in routine clinical practice, what remains challenging. This review aims to summarize and evaluate available diagnostic procedures to assess liver fibrosis and its surrogates. The different methods will be critically discussed with regard to their rationale, availability, technical considerations and accuracy in the following paragraphs. This review may help hepatologists to choose to appropriate diagnostic tool and informs about their advantages and disadvantages. Liver biopsy is the gold standard of liver fibrosis assessment. We therefore discuss different histological scoring systems, which need to be attentive to the underlying etiology of CLD. Later, we will first outline biomarkers, which are commonly used for detection and grading of liver fibrosis. Many attempts were dared to calculate non-invasive liver fibrosis scores, to predict fibrosis based on standard laboratory findings. These scores will be reviewed in the third paragraph. And finally, in the previous decade, assessment of liver fibrosis through radiological examinations has markedly improved due to technical progress, which even challenges liver biopsy as reference method. Elastographic methods have already decreased the requirement of a liver biopsy, and cross-sectional imaging techniques are establishing themselves in the field of liver fibrosis assessment. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dmr.amegroups.com/article/view/10.21037/dmr-22-9/rc).

Methods

In order to review the available techniques for assessment of liver fibrosis, a literature search using the NIH National Library of Medicine PubMed was performed. All literature published until December 31st, 2022 was eligible for further consideration. Open and closed access publication were both included in this review. Literature which was published in languages other than English were excluded. The search strategy summery is displayed in *Table 1*.

Histological assessment of liver fibrosis

Systematic histopathological studies of morphological aspects of liver fibrosis have been contributing to our today's knowledge of the evolution of liver fibrosis in various chronic inflammatory conditions of the liver. Fibrosis develops in a situation of ongoing inflammation with the imbalance of extracellular matrix production (ECM) and reduction (16). Activation of hepatic stellate cells and portal fibroblasts induces their myofibroblastic proliferation accompanied by the deposition of connective tissue, which results in the picture of parenchymal and portal fibrosis (6). While in the normal liver the space of Disse consists of collagens type IV and laminin, these are replaced by collagens type I and III during the process of

Table 1 The search strategy summary

Items	Specification
Date of search	November 15th, 2021, May 1st 2022 for review
Databases and other sources searched	PubMed
Search terms used	"Assessment of liver fibrosis", "liver fibrosis" and "non-invasive tests for liver fibrosis"
Timeframe	From origin to December 31st, 2021
Inclusion and exclusion criteria	All published studies in English were eligible
Selection process	Selection was performed by the authors and conducted independently

parenchymal fibrosis (17).

In portal tract fibrosis activated myofibroblasts and smooth muscle cells are capable of ECM production in the setting of chronic hepatitis. In chronic bile duct inflammation the source of ECM production are peribiliary fibroblasts, inducing the pattern of biliary fibrosis. The fibrosis contains abundant type I and III collagen and reticulin fibers, elastic fibers are indicators of a several months duration of the process.

For the precise histological assessment of liver fibrosis, stains for connective tissue like Masson trichrome, Elastica van Gieson, Sirius red, Orcein (18), Victoria blue, and Reticulin stains are applied, which demarcate the accumulation of connective tissue. The semiquantitative analysis of fibrosis (staging) is reported within a fibrosis score, as a reproducible predictor for disease progression and clinical outcome.

The Knodell Score was one of the early reported scoring systems for chronic hepatitis which was published in 1981 (19). In contrast to the traditional nomenclature of that time, portal based fibrosis was reported in a numerical scoring system from 0 to 4 in a reproducible and objective form. Other scoring systems and modifications followed (Ishak Score, Desmet Score, Metavir) (20-22). These scoring systems have been shown to be a prerequisite for histology-based treatment stratification, monitoring of the clinical course and outcome in chronic viral and non-viral hepatitis (23).

Taking into account, that non-alcoholic fatty liver disease (NAFLD) displays different fibrosis patterns than chronic hepatitis, special scoring systems for NAFLD were developed by Kleiner *et al.* (24) and Bedossa *et al.* in 2014 (25). The early stages of fibrosis describe mild and moderate pericellular in the absence of portal fibrosis. Histopathological staging of fibrosis in NAFLD, which is still the gold standard, revealed to be a strong predictor of mortality and time to develop severe liver disease in biopsy proven NAFLD (26).

As in NAFLD, histology of alcoholic fatty liver disease (AFLD) is characterized by steatosis, ballooning, inflammation and fibrosis. However, in contrast to NAFLD, most patients have severe fibrosis or cirrhosis at the first presentation (27). A specific scoring system for AFLD has been published by Lackner *et al.* (28). Liver fibrosis is staged into stage 0–3, while stage 4 (cirrhosis) is subdivided into stage 4a, 4b and 4c (cirrhosis with thin septa, broad septa and very broad septa). It was shown, that the scoring system is a prognostically relevant method for the histological assessment of fibrosis in AFLD (28).

For chronic biliary diseases different histopathological scoring systems are available like Ludwig Score (29) and Nakanuma Score (30). For primary biliary cholangitis, which are applicable for primary sclerosing cholangitis with a prognostic value (31).

With upcoming treatment options for chronic liver diseases, the histopathological assessment of liver fibrosis regression is an important issue. Histopathological criteria of fibrosis regression have been described and can be employed for the monitoring of treatment effects like in chronic hepatitis C (32).

Non-invasive tests for liver fibrosis

Serum biomarker-based non-invasive tests for liver fibrosis

Rationale

Serum biomarker-based non-invasive liver fibrosis tests (sNITs) are calculated based on serum biomarker levels which are correlating with the degree of fibrosis. Due to its easy and low risk access, many studies investigated

the ability in fibrosis assessment. Since many sNITs are based on standard laboratory values, they can be assessed repetitively in fibrosis assessment, if necessary. Therefore, sNITs are well accepted and widely used in the community, especially those with high accuracy.

Indications

The most investigated, commercially available tests are the FibroTest™ (LabCorp, Burlington, NC, USA) and the Enhanced Liver Fibrosis (ELF™) test (Siemens, Munich, Germany). FibroTest™ was validated against liver biopsy in patients with chronic hepatitis C, indicating similarity in prediction of 5-year survival compared to liver biopsy (33). For hepatitis B, it showed moderate results in assessment of significant fibrosis (AUROC =0.77) but this could be improved if serum AST-levels and HIV-status were combined with the score (AUROC =0.9) (34). For NAFLD and alcoholic liver disease (ALD), a meta-analysis showed that FibroTest™ had a good accuracy adjusted for etiology specific fibrosis stage spectrum (NAFLD: AUROC =0.84, ALD: AUROC =0.85) (35).

The ELF™ test was validated in numerous studies. A meta-analysis of nine studies revealed that independent of etiology, ELF™ tests accuracy for significant and advanced fibrosis as well as cirrhosis was good (0.87≤ AUROC ≤0.88). The ELF™ test is excellent in identifying patients with advanced liver fibrosis in ALD patients (AUROC =0.92), but not showing superiority to the FibroTest™ (36).

AST-to platelet index (APRI) is a non-patented sNIT and was firstly introduced by Wai *et al.* in 2003 who constructed a simple algorithm to predict significant fibrosis (ISHAK ≥3). With this ratio, significant fibrosis was predicted correctly in 51% and cirrhosis in 81% of patients (AUROC =0.88 for significant fibrosis, AUROC =0.94 for cirrhosis respectively) (37). This index was later validated in other cohorts with various etiologies and is mainly considered for quickly ruling out cirrhosis, but not valuable for predicting earlier stages of fibrosis (38).

Moreover, Sterling *et al.* developed another easy to calculate score which comprises two more variables (age and ALT beside AST and platelet count) and was named Fibrosis-4 Index (FIB-4) (39). It was initially designed in hepatitis C/HIV coinfected patients, but later validated for hepatitis B, NAFLD and ALD (40,41). A small study by Miyata *et al.* demonstrated that FIB-4 could be used as a monitoring parameter for patients treated with methotrexate (42). However, in elderly people (age ≥65 years) with NAFLD, specificity of FIB-4 was very low, with the need of adjusted thresholds (43). Overall, FIB-4 showed adequate accuracies for NAFLD patients according to a meta-analysis by Xiao *et al.* (44). Here, summary AUROCs for detection of significant fibrosis, advanced fibrosis and cirrhosis were 0.73, 0.84 and 0.85 respectively, which was superior to other NITs except for significant fibrosis in which APRI performed better (AUROC =0.76).

Beside these two NITs which are most frequently used in daily clinical practice, many others have been developed for NAFLD, like NAFLD Fibrosis Score (NFS) (45) and BARD score (46), which overall only show, if any, minor superiority to APRI and FIB-4 (44). Very recently, novel scores are emerging, including new biomarkers with standard laboratory finding, which can further improve AUROC. Daniels *et al.* developed a new algorithm including age, presence of diabetes, platelet count and Pro-Collagen 3 ("ADAPT" algorithm) with an AUROC of 0.86 for predicting advanced fibrosis in NAFLD patients and 0.88 for advanced fibrosis in ALD patients, respectively (47,48). More models with both standard laboratory findings and novel biomarkers will likely come up in the near future to better address specific clinical scenarios and better predict fibrosis in order to stratify patients and further reduce the need of liver biopsies. The calculation algorithms for the above-mentioned scores can be found in *Table 2*. The cutoffs for the two most abundant etiologies of chronic liver disease, ALD and NAFLD can be obtained in *Table 3*.

Technical considerations

SNITs can be divided in commercially available test, which can be obtained only in validated laboratories resulting in very well reliable and validated results, but are therefore intricate and costly (62,63). Contrary to these tests, non-patented NITs can be calculated by standard laboratory findings and are therefore an easy, cheap and helpful diagnostic tool.

Limitations

Commercially available NITs perform well in fibrosis prediction, but pricing and logistics hinder its wide use in routine practice.

Imaging-based non-invasive tests for liver fibrosis

Rationale

B-mode abdominal ultrasound is a widely-available

Table 2 Non-invasive methods to assess liver fibrosis

NIT	Pro	Con	Ref.
ELF: 2.278 + 0.851 ln(HA) + 0.751 ln(PIIINP) + 0.394 ln(TIMP-1)	Best validation among sNITs	Can be obtained in validated laboratories only	(49)
FibroTest: Calculation based on alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, gamma-glutamyl transpeptidase, total bilirubin, and ALT	Good accuracy in alcoholic and non-alcoholic fatty liver disease	Can be obtained in validated laboratories only	(33)
APRI: [(AST level/ULN)]/Platelet counts (10^9/L)	Very easy calculation algorithm	Poor performance for intermediate fibrosis stages	(37)
FIB-4: (Age × AST)/[Platelets × √(ALT)]	Best accuracy among non-commercial sNITs	Less reliable in patients below 35 and above 65 years	(39)
VCTE: It relies on a probe that includes both the vibrator and the transducer. Low frequency vibration of small amplitude is transmitted to the tissue what induces an elastic shear wave. Simultaneously, pulse-echo ultrasonic acquisitions are performed to detect the propagation of the shear wave and measure its velocity, which is directly related to liver stiffness	most validation studies performed, steatosis assessment included	No simultaneous control picture in B-mode, Disturbed by obesity and/or ascites	(50,51)
P-SWE: It can be performed with standard ultrasound machines. It uses acoustic radiation force impulse which generates shear waves which velocity can be determined and translated in tissue stiffness	No extra machine needed, B-mode guided	One-dimensional, pending validation due to various commercially available machines	(52,53)
2D-SWE: Acoustic radiation force impulses at different depths generate a mechanical impulse that causes shear waves to propagate vertical to the incident ultrasound axis. The propagation can be reconstructed into a real-time color map of the shear wave front in m/s or kPa	Additional to PSWE: Larger region of interest, not hindered by visceral fat or ascites	Pending validation due to various commercially available machines	(54,55)
MRE: It combines a phase-contrast pulse sequence with motion-encoding gradients to encode tissue motion, which is applied by low frequency vibrations of an external driver. With postprocessing, color wave images and stiffness maps, also known as elastograms, are generated that show stiffness of liver tissue in kPa	Whole liver measurement, accurate in early stages of liver fibrosis	Time consuming, expensive, radiological expertise required	(56)

Description of serum-derived and imaging-derived non-invasive tests for liver fibrosis assessment alongside with main pros and cons. NIT, non-invasive test; Ref, references; sNITs, serum biomarker-based non-invasive tests; ELF, enhanced liver fibrosis; HA, hyaluronic adic; PIINP, amino terminal propeptide of type III procollagen; TIMP, tissue inhibitor of matrix metalloproteinase; VCTE, vibration-controlled transient elastography; APRI, AST-to-platelet-ratio index; AST, aspartate aminotransferase; ULN, upper limit of the normal; P-SWE, point shear-wave elastography; 2D-SWE, two-dimensional shear-wave elastography; FIB-4, fibrosis-4 test; ALT, alanine aminotransferase; MRE, magnetic resonance elastography.

Table 3 Cutoffs for sNITs

Test	Etiology	Significant fibrosis	Advanced fibrosis	Cirrhosis	Ref
APRI	Hepatitis C	>0.7	>1.0	>1.0	(57)
APRI	NAFLD	>0.42	>0.98	n.a.	(58,59)
FIB-4	Hepatitis C	>1.45	>3.25	n.a.	(60)
FIB-4	NAFLD	>1.12	>2.67	n.a.	(58,61)

Validated cutoffs for fibrosis classification for most commonly used sNITs. For alcoholic liver disease, the same cutoffs are used as for NAFLD. APRI, AST-to-platelet-ratio index; NAFLD, non-alcoholic fatty liver disease; FIB-4, fibrosis 4 test; n.a., not applicable; Ref, references.

frequently used diagnostic tool, which can be used for detection of advanced liver fibrosis or cirrhosis but it fails in detection of liver fibrosis. However, the Vibration-controlled transient elastography (VCTE), mostly performed with FibroScan® (Echosens, Paris, France) is currently the most abundantly investigated non-invasive tool for liver fibrosis measurement which is approved by the FDA. Cross-sectional imaging, like computed tomography (CT) or magnetic resonance imaging (MRI), allows for morphological assessment of liver parenchyma and has been applied for a long time for the detection of cirrhotic liver features in clinical routine. Morphological signs of liver cirrhosis on CT or MRI includes an increased nodularity of liver surface, heterogeneous liver parenchyma, a caudate lobe enlargement, alterations in right-to-left-lobe-volume ratio, an expanded gallbladder fossa or a posterior notch sign (64,65). Other hallmarks and complications of liver cirrhosis, like portosystemic collaterals, splenomegaly and ascites can also be visualized using standard cross-sectional imaging.

Indications

VCTE was firstly introduced for fibrosis staging in chronic hepatitis C, meta-analyses have demonstrated that TE can measure the degree of fibrosis irrespective of etiology (66,67). However, excellent diagnostic accuracy (AUROC >90%) is reached only if cirrhosis is of interest. For detection of significant fibrosis, defined by METAVIR F2 or F3 since most studies were performed in viral hepatitis patients, diagnostic accuracy is good ranging between 84–89% (68). Moreover, TE can be used for longitudinal studies, as demonstrated in two studies which investigated fibrosis reversal in hepatitis C patients after successful treatment (69,70).

More recently, techniques were developed that only use ultrasound waves to detect fibrosis and are included in conventional ultrasound devices: point shear wave elastography (P-SWE) and 2D-shear wave elastography (2D-SWE). Several studies compared P-SWE to TE, and showed accurate results for the diagnosis of cirrhosis, but worse result for earlier stages of fibrosis (AUC =0.76 for significant fibrosis) (71,72). P-SWE and 2D-SWE are reliable and reproducible techniques. This was demonstrated in healthy volunteers, where similar results were observed between different examiners. However, absolute values must not be used interchangeably (73). In contrast to P-SWE, 2D-SWE provides a larger region of interest, adjustable by the examiner and allows real-time measurement (68). Due to the adjustable region of interest, in specific conditions like post-hepatectomy patients, 2D-SWE might be more accurate which was shown in a recent study (74). A large, multicenter study with 1,827 patients included, could demonstrate that patients with a liver 2D-SWE ≥20 kPa and a Model of End-Stage Liver Disease (MELD)-score ≥10 have a higher 2-year mortality and a risk of hepatic decompensation, requiring a tighter follow-up visit intervals (75). Finally, there is evidence emerging 2D-SWE seem to be superior compared with TE, with an increase of AUROC between 1.4% and 12.8% depending on fibrosis stage and underlying etiology, according to a large multi-center analysis with 1,134 patients (76). Taken this together, further studies are needed to better validate shear wave elastography, which is challenged through the numerous different commercially available machines, which renders inter-device comparability difficult. However, the algorithm predicting decompensation and outcome based on liver stiffness ≥20 kPa and MELD ≥10 holds true for different devices and etiologies (75). Example images of ultrasound-based non-invasive tests (TE, P-SWE and 2D-SWE) are provided in *Figure 1*.

The diagnostic accuracy of standard cross-sectional imagining for the detection liver cirrhosis is moderate ranging from 68% to 72% (65). For the detection pre-cirrhotic fibrosis and fibrosis stage classification morphological assessment alone is not useful. Nevertheless, different more advanced CT and MRI techniques are capable to quantify hepatic fibrosis in a pre-cirrhotic stage and provide, in contrast to TE, SWE, or biopsy, information of fibrosis distribution in the entire liver parenchyma (56). The wider spatial resolution of cross-sectional imaging techniques could help to identify most appropriate sites for liver biopsy or lead to a disease prognostication that is more accurate (77).

Especially, delayed dual-energy CT, deep learning algorithms, MR elastography (MRE), diffusion-weighted MRI, and MRI mapping techniques can detect liver fibrosis in a pre-cirrhotic stage with a good diagnostic performance (78-80).

Dual-energy CT separates two x-ray energy spectra, allowing the differentiation of materials that have different attenuation properties at different energy levels. Dual-energy CT enables assessment of the severity of liver fibrosis by means of contrast-enhanced delayed acquisition with calculation of a normalized iodine concentration of liver parenchyma. In a study of 107 participants with

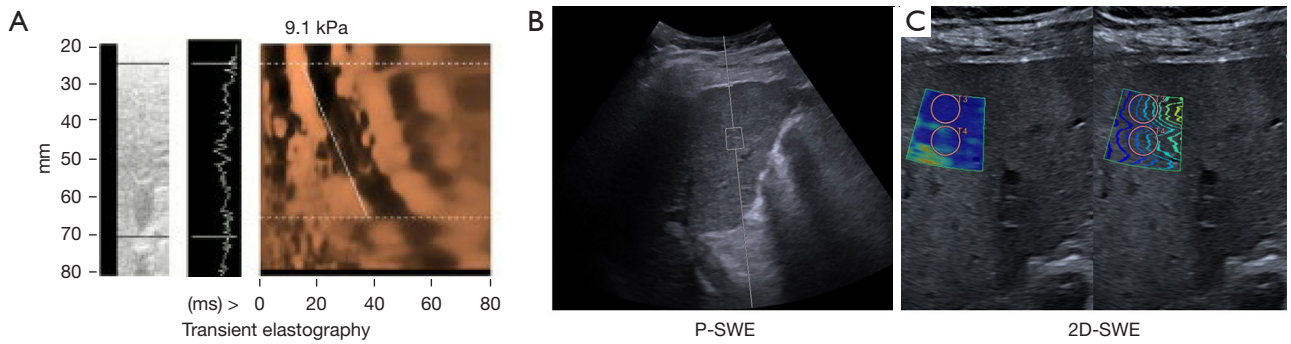

Figure 1 Ultrasound-based non-invasive tests for liver fibrosis. (A) Example of vibration-controlled transient elastography; (B) example of P-SWE; (C) example image of 2D-SWE. P-SWE, point-shear wave elastography; 2D-SWE, 2D-shear wave elastography.

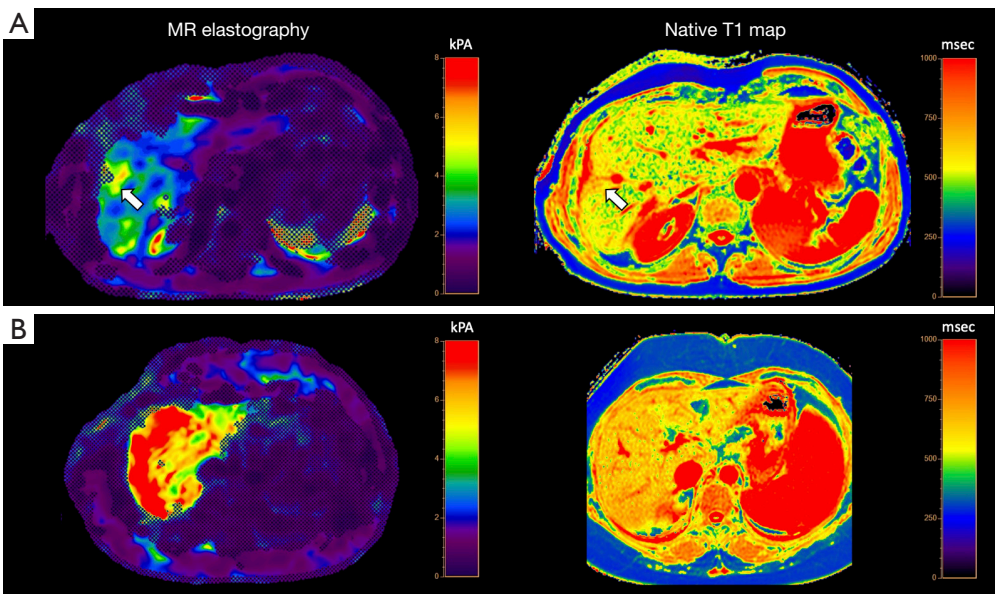

Figure 2 MRE. (A) Liver section with beginning fibrosis; (B) liver section with significant fibrosis. The colors are coding the corresponding liver stiffness in the MRE and the repetition tine in msec in the T1-weighted section. MRE, magnetic resonance elastography.

chronic liver disease, dual-energy CT derived normalized iodine concentration of the right liver lobe achieved an AUC of 0.86 to differentiate F0 from F1–3 and 0.96 to differentiate F0-3 from F4 fibrosis (79).

Deep learning techniques, especially convolutional neural networks (CNNs), have an enormous potential for image segmentation and classification, which also includes the detection of liver fibrosis. With deep learning algorithms, liver cirrhosis detection, even on standard T2-weighted images, can achieve a classification accuracy of up 0.99 (81). Reported AUC values of different CNNs to differentiate between F0 and F1-4 stages range from 0.77 to 0.96, depending on applied imaging modality and technique (82).

Among cross-sectional imaging methods, MRE (*Figure 2*) is the best studied MRI-based imaging technique for liver fibrosis assessment and shows a high diagnostic performance for classification of liver fibrosis stages with AUCs of 0.84–0.95 (F ≥1), 0.88–0.98 (F ≥2), 0.93–0.98 (F ≥3), and 0.92–0.99 (F ≥4) (80,83-85). MRE is also useful to differentiate NAFLD from individuals with steatohepatitis, even before onset of fibrosis development (86). However, diagnostic performance of MRE might be limited in inflammatory hepatic disease, iron overload or cardiac congestion (56).

Diffusion-weighted MRI is based upon measuring the random Brownian motion of water molecules. Diffusion of water molecules is restricted in fibrosis and leads to lower apparent diffusion coefficient (ADC) values, which are calculated from at least two *b*-values using a monoexponential model. Although MRE has a significantly higher accuracy than diffusion-weighted MRI for differentiation of different fibrosis stages, future developments in diffusion-weighted MRI including perfusion models might further elevate the diagnostic performance of this technique (80,87).

T1rho elongation was demonstrated to be an effective biomarker for collagen deposition and therefore liver fibrosis in animal models (88). Significant differences of T1rho values were demonstrated between Child-Pugh cirrhosis stages, with AUCs of 0.95–0.98, indicating the ability of MRI techniques in differentiating different stages of cirrhosis (89). However, it should be noted that women show a physiologically decrease of liver T1rho value with increasing age (90).

Hepatic T1 relaxation times and T1-derived extracellular volume fraction (ECV) are also promising quantitative MRI techniques to diagnose liver fibrosis, which can be obtained with T1 mapping sequences (91,92). Hepatic T1 relaxations times are increased in liver fibrosis and allow the calculation of hepatic ECV, which dichotomizes the liver parenchyma into an extracellular and cellular compartment due to an additional measurement of contrast-enhanced hepatic T1 relaxation times (93). Hepatic ECV already showed promising results for the detection of clinically significant fibrosis (F ≥2) in patients with primary sclerosing cholangitis or autoimmune hepatitis (94,95).

Technical considerations

Technical details of the discussed methods are provided in *Table 2*. VCTE is based on both ultrasound and low-frequency waves whose motion is directly correlated with liver elasticity (66). In contrast to VCTE, PSWE and 2D-SWE are not limited to obesity or presence of ascites and provide comparable results to TE (96). Here, an acoustic radiofrequency impulse is produced, which leads to transversely-oriented waves. Propagation velocity can be determined and tissue elasticity can be calculated based on these parameters (97). It has been demonstrated that intra-operator variability is increasing at higher liver stiffness measurements (LSM) and obese patients. It is therefore recommended to perform multiple LSM if body mass index is above 25 kg/m² and the median liver stiffness is measured above 7.1 kPa (98).

Limitations

For VCTE, the measurement depth is between 25–65 mm with the standard probe (68). Thus, in obese patients, TE needs to be performed with a larger probe permitting deeper tissue penetration with comparable results (99,100). As mentioned above, standard cross-sectional imaging techniques have low accuracy in fibrosis determination. Diffusion weighed MRI can detect liver cirrhosis due to its association with the ADC, which is lower in cirrhosis, but fails in differentiating individual stages of fibrosis (101). This can be improved via novel techniques as MRE and diffusion-weighted MRI.

Further tests for liver fibrosis

Rationale

Since performance of liver biopsies is associated with a periprocedural risk of complications, and sNITs and iNITs still comprise a portion of false-negative and false-positive classifications, further research is urgently needed in order to find novel biomarkers for fibrosis assessment. It stands to reason that components of hepatic ECM, which is modified during fibrogenesis, may serve as biomarkers. Furthermore, altered micro RNAs (miR) patterns have been identified during liver fibrosis and are therefore promising biomarkers (102). Giving recent technological advances, including high throughput screening and Omics techniques, new biomarkers are likely to emerge in the near future.

Indications

To be suitable as biomarkers, the molecules of interest should be well-detectable in peripheral blood samples. It was hypothesized that miR, that are downregulated in chronic diseased liver tissue, might be released via exocytotic pathways (103). Importantly, many miR are therefore detectable in peripheral blood samples of patients with fibrosis and are therefore suggested to function as biomarkers (102). As one of the first miRs, miR-122 was proposed to be marker of liver injury, given its down-regulation in liver fibrosis in hepatitis C, NASH and drug-induced liver damage (104-106). Indeed, circulating miR-122 levels are inversely correlated with chronic inflammation in patients with chronic hepatitis C, but are not capable to correctly predict liver fibrosis (107-109). However, in HIV/hepatitis C-coinfected patients, miR-

122 correlated negatively with the hepatic venous pressure gradient, rendering it suitable for identifying portal hypertension (109). In the same cohort, miR-122 and miR-22 were correlating with significant fibrosis according to sNITs (110). In a microarray-based approach in a cohort of 130 chronic hepatitis C patients, the let-7 miR-family showed the best ability to predict liver fibrosis but were not superior to sNITs (111). For liver cirrhosis, two miRs, miR-571 and miR-652, were demonstrated to be differentially altered in serum samples. While miR-571 showed stage-dependent values according to the Child-Pugh classification, miR-652 was dysregulated independent of cirrhosis stage (112).

Beside miRs, components of the ECM have been identified as circulating biomarkers. Circulating collagen fragments are generated during fibrosis-related ECM remodeling and were checked for their ability to assess liver fibrosis (113-115). In particular, collagen peptides are suggested as biomarkers (116-118). Matrix metalloproteinase (MMP) degraded n-terminal propeptide of type III collagen, PRO-C3, was demonstrated to strongly correlate with FIB-4 values (sNIT, see above) and could serve as a fibrosis biomarker, which was proved in a cohort of HIV/Hepatitis C-coinfected patients (116). ECM components are emerging as biomarkers, which are not only assessing fibrosis but can predict patients' outcome. A significant correlation between PRO-C3 and Hepatic venous pressure gradient (HVPG) was shown, providing additional predictive value for portal hypertension (116). Elastin is processed during fibrotic remodeling and its fragments can be detected in hepatic venous blood. It has been demonstrated that elastin fragments are more abundant in Child-Pugh C cirrhotic patients compared to Child-Pugh A and B. Moreover, circulating elastin fragments levels in the hepatic vein might reflect fibrosis remodeling and predict survival, what was investigated in patients receiving transjugular intrahepatic portosystemic stent shunting (TIPS) (119). MMP degraded elastin further might predict fibrosis progression, which was demonstrated in HIV infected patients (120). A model including MMP degraded elastin, PRO C3 and type VI collagen was demonstrated a high correlation with HVPG, which can be used to detect the degree of portal hypertension (121). Indeed, elastin is the most relevant biomarker in terms of prognosis either progression of fibrosis or outcome in CLD patients, probably since it reflects the crosslinking of ECM and therefore a more difficult to reverse process (122). More recently, microfibrillar-associated protein 4 (MFAP-4) was identified as novel biomarker, and validated in patients with liver fibrosis due to chronic hepatitis C and ALD (123,124). Combining MFAP-4 with other sNITs was able to improve the diagnostic accuracy in prediction of advanced fibrosis (123).

Technical considerations

Micro RNAs are small non-coding RNAs which are involved in posttrancriptional gene expression regulation. Micro RNAs have emerged as putative biomarkers due to their high abundance in various body fluids and their molecular stability (125,126). Since there is a lack of an endogenous normalizing control, which can be used for normalization of quantitative miR levels, spiked in of foreign RNA such as *Simian virus-40 RNA* (106,109), *C. elegans* short RNA, or to a short synthetic oligonucleotide (127) are frequently used as technical control and reference for quantification. Studies in patients undergoing TIPS are of great value because the access to blood of the portal vein. In portal vein analysis, hepatic derivation of biomarkers can be proven (128). Advanced sequencing methods (e.g., small RNA sequencing) will allow deeper insights in miR profiles of extracellular vesicles providing further evidence for these novel biomarkers (129).

Limitations

Due to its experimental nature, novel biomarkers are currently not used in the clinics and require further validation. Liver fibrosis is a dynamic process in chronic liver disease. It is noteworthy, that miR abundance can be perturbated because of the injury-dependent replacement of hepatocytes during fibrogenesis, which are the main source of miRs (107). Therefore, the combination of different approaches such as miR analyses and collagen peptide monitoring, and future options such as exosomal classification and non-coding RNA patterns might provide efficient non-invasive diagnostic strategies.

Conclusions

Assessment of liver fibrosis is key in order to stratify patients and plan management and surveillance. Liver biopsy and histological assessment still remains the gold-standard in determining fibrosis despite its inherent limitations. The value of NITs is increasingly acknowledged in daily clinical practice. Among them elastography methods were

extensively investigated and are with AUROCs >0.9 very useful. However, NITs based on serum markers perform similar and do not consist inter-operator variability. Finally, cross-sectional imaging techniques are emerging and provide information on fibrosis distribution since the whole liver is examined. Combination of sNIT and iNIT is probably the best way to stratify patients' risk as outlined in the current Baveno VII guidelines (130) and demonstrated in recent studies (75). Further research is urgently needed to convey between these methods and provide clear clinical algorithms for patients with CLD and suspected liver fibrosis.

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Footnote

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