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Review Article

Noninvasive Fibrosis Assessment in Chronic Hepatitis C Infection: An Update

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Abstract

Liver biopsy is historically the gold standard for liver fibrosis assessment of chronic hepatitis C patients. However, with the introduction and validation of noninvasive tests (NITs) to evaluate advanced fibrosis, and the direct-acting antiviral agents for treatment of chronic hepatitis C virus (HCV), the role of NITs have become even more complex. There is now need for longitudinal monitoring and elucidation of cutoff values for prediction of liver-related complication after sustained virological response. The aim of this report is to provide a critical overview of the various NITs available for the assessment of liver fibrosis in HCV patients.

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Keywords: Hepatitis C virus; Fibrosis; Noninvasive serum fibrosis markers; Transient elastography; Shear wave elastography; Magnetic resonance elastography; Steatohepatitis.

Abbreviations: A2M, Alpha-Macroglobulin; ADRES, After DAAs Recommendation for Surveillance Score; AFP, Alpha Fetoprotein; ALBI, Albumin-Bilirubin Score; ALT, Alanine Aminotransferase; APRI, Aspartate Aminotransferase to Platelet Ratio Index; AST, Aspartate Aminotransferase; AUROC, Area Under the Receiver Operating Characteristic; cACLD, Compensated Advanced Chronic Liver Disease; CLD, Chronic Liver Disease; CSPH, Clinically Significant Portal Hypertension; DAA, Direct-Acting Antiviral; EASL, European Association for the Study of the Liver; ECM, Extracellular Matrix; eLIFT, Easy Liver Fibrosis Test; Fib-4, Fibrosis-4 Index; FMVCTE, Fibrometer Vibration-Controlled Transient Elastography; GES, General Evaluation Score; HA, Hyaluronic Acid; HCC, Hepatocellular Carcinoma; HCV, Hepatitis C Virus; LB, Liver Biopsy; LSM, Liver Stiffness Measurement; miRNA, MicroRNA; MRE, Magnetic Resonance Elastography; MR, Magnetic Resonance; NAFLD, Nonalcoholic Fatty Liver Disorders; NIT, Noninvasive Test; PRO-C3, Pro-Peptide of Type III Collagen; pSWE, Point Shear Wave Elastography; RPR, Red Cell Distribution Width (RDW) To Platelet Ratio; SSM, Spleen Stiffness Measurement; SVR, Sustained Virological Response; SVR24, Sustained Virological Response at 24 Weeks; SWE, Shear Wave Elastography; TE, Transient Elastography; VCTE, Vibration-Controlled Transient Elastography; γGT, Gamma-Glutamyltransferase.

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Introduction

Hepatitis C virus (HCV) infection is one of the more prevalent and wide-spread causes of liver-related morbidity and mortality worldwide.[1] Although the prevalence of chronic hepatitis C has decreased by half in the last 30 years,[2] it still represents a major health problem due to the burden of chronic liver disease (CLD), and lack of access to treatment and complications.[3] The global prevalence of HCV infection has been estimated to be 1.0%, corresponding to about 71.1 million individuals.[4]

With the availability of direct-acting antiviral (DAA) agents, high rates of sustained virological response (SVR) are now commonplace. However, evaluation of the degree of liver fibrosis present and the consequent liver function is important to establish prior to institution of DAA therapy. This is important for several reasons, determining the (1) risk of progression of liver disease, (2) urgency and timing of treatment, (3) patient tolerability to DAA treatment (4) DAA plus ribavirin treatment, and (5) monitoring of fibrosis with time. Most liver society guidelines recommend inclusion of evaluation of the stage of liver fibrosis during the initial evaluation and monitoring those who are unable or unwilling to be treated.[5] Percutaneous liver biopsy (LB) has traditionally been regarded as the gold standard for evaluation of structural alterations in CLDs.[6] Histopathological analysis alone does not provide insight into dynamic changes during the fibrogenesis process. In the past two decades, various noninvasive approaches have been introduced and used for liver fibrosis staging. This review focuses on various noninvasive tests (NITs) for assessment of liver fibrosis, distinguishing physical methods from serological ones, and critically evaluating the various options and issues regarding most appropriate uses, availability, and cost.

Currently available NITs

There are two fundamentally different but complementary, noninvasive approaches in the assessment of liver fibrosis: physical and serological ones.[7–9] The physical approach is based on liver stiffness measurement (known as LSM) by ultrasound or magnetic resonance. These methods rely on intrinsic characteristics of the liver parenchyma. On the other

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Table 1. Interpreting LSM using TE techniques in patients with viral hepatitis and NAFLD

LSM	Recommendations
<5 kPa	NORMAL
7–10 kPa	Follow-up on a case-by-case basis for any alterations that would indicate the development of cACLD
<10 kPa	EXCLUDES cACLD if no additional recognized clinical or imaging symptoms exist
10–15 kPa	SUGGESTIVE OF cACLD: + platelet count ≥150×10^9/L rules out CSPH (sensitivity and NPV >90%)
15–20 kPa	Highly suggestive of cACLD: + platelet count >150×10^9/L avoid endoscopy; + platelet count <110×10^9/L suggestive of CSPH (risk of at least 60%; ANTICIPATE model)
>20 kPa	RULES IN cACLD→liver disease specialist→LSM every 12 months to monitor changes
20–25 kPa	+ platelet count <150×10^9/L→suggestive of CSPH (risk of at least 60%; ANTICIPATE model)
≥25 kPa	RULES IN CSPH (specificity and PPV>90%)

Rule-of-five for LSM by TE (10–15–20–25 kPa) recommendation adopted from Baveno VII consensus. cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; LSM, liver stiffness measurement; NPV, negative predictive value; PPV, positive predictive value.

hand, serological methods depend on quantification of several serum biomarkers.

Noninvasive physical methods

Noninvasive physical methods encompass ultrasound-based elastography and magnetic resonance elastography (MRE).[9] Ultrasound elastography quantifies and displays differences in biomechanical responses of tissues against shear deformation in response to ultrasonic impulses applied by the instrument to the liver parenchyma. Direct measurement of the shear wave propagation speed based on which the LSM is calculated through elastic modules (indirect measurement).[10]

Transient elastography

The most used physical modality for evaluation of liver fibrosis is transient elastography (TE, Fibroscan, Echosens, Paris, France).[11] Numerous studies and meta-analyses have reported better accuracy of TE for cirrhosis than for fibrosis assessment and mean area under the receiver operating characteristic (AUROC) curve values of 0.94 and 0.84, respectively.[12] A meta-analysis by Friedrich-Rust *et al*.[12] included 50 relevant publications including abstracts rather than individual data. The main limitation was the lack of multivariate analysis. TE also performed better at ruling out rather than diagnosing cirrhosis based on a high specificity of >90% and negative predictive value (NPV) of >90%.[7,13,14]

Several meta-analysis and the European Association for the Study of the Liver (EASL) clinical practice guidelines on NITs for evaluation of liver disease severity and prognosis 2021 update state that LSM is strongly correlated with meta-analysis of histological data in viral hepatitis (METAVIR) fibrosis stages.[14] Proposed cutoff values for cirrhosis vary depending on the prevalence of cirrhosis among study populations.[15] This method showed excellent inter- and intraobserver agreement.[16] Although this comparative study included 46 patients with a total of 534 measurements, consistency of evaluation might have been an issue as each patient was examined by only two of a total of four researchers. On the other hand, it identified the specific parameters needed to produce a repeatable LSM by analyzing the impact of several factors on LSM reproducibility. In comparative studies, TE outperformed serological tests, especially for the diagnosis of cirrhosis but with lower applicability.[17] A prospective 5-year analysis based on a study with more than 13,000 examinations found that LSM values were not interpretable in nearly one in five cases (18.4%). The inclusion of patients with various CLDs, not only HCV, and examination by seven different researchers with different experience were limitations of this study.

TE is currently the most useful tool for accurate identification of a subset of HCV patients with an increased risk of complications. Baveno IV consensus conference in 2015 proposed term compensated advanced (cA)CLD to emphasize the importance of early detection of asymptomatic HCV patients with severe fibrosis or liver cirrhosis at risk of clinically significant portal hypertension (CSPH).[18] Suggested dual cutoff values for TE exclusion and diagnosis of cACLD (<7 and >12 kPa respectively) with improved overall accuracy.[19] This multicenter validation study included real-world data from more than 5,600 patients in 10 European liver centers, using LB as reference standard. Despite some limitations, the outcomes improved the overall predictive ability of TE for determining the existence of cACLD. Regarding the liver-related complications, You *et al*.[20] reported excellent diagnostic performance for CSPH prediction using cutoffs of 13.6–18 kPa. These parameters were better for screening and monitoring than cutoffs of 20–25 kPa according to the Baveno VII and European Association for the Study of the Liver–EASL clinical guidelines 2021 update.[5,21] This meta-analysis used systematic review techniques and reported the findings using the Preferred Reporting Items for Systematic Guidelines for Reviews and Meta-Analyses (commonly known as PRISMA). As various cutoff values of LSM were established among included studies, to clarify threshold effect and between-study heterogeneity, this meta-analysis performed bivariate meta-regression analysis on summary estimates of diagnostic accuracy. Study limitations included a relatively small number of studies with only virus-related CLD and usage TE as reference standard.

According to the most recent Baveno VII guidelines, high-risk varices could be ruled out in individuals with an LSM<20 kPa and a platelet count of >150×10^9/L, avoiding upper gastrointestinal endoscopy.[21] Proposed rule of five for LSM by TE (5–10–15–20–25 kPa) could be implemented to indicate successively increasing relative risks of decompensation and liver-related mortality (Table 1). The rule was applied in two-dimensional (2D) shear wave elastography (SWE) in the form of a rule-of-four, following the Baveno VII guidelines (Fig. 1).

Decreases in LSM after SVR have been reported,[22,23] but it is still uncertain how much is due to reduced inflammation and how much due to regression of fibrosis.[24] One study determined baseline LSM to be an independent predictor of fibrosis regression. One year after achieving SVR, LSM was used to accurately predict the presence of advanced fibrosis and CSPH (AUROC, 0.902 and 0.888).[22] However, the percentage of LSM reduction was not satisfactory in prediction of fibrosis regression with an AUROC of 0.65.[22] The use of

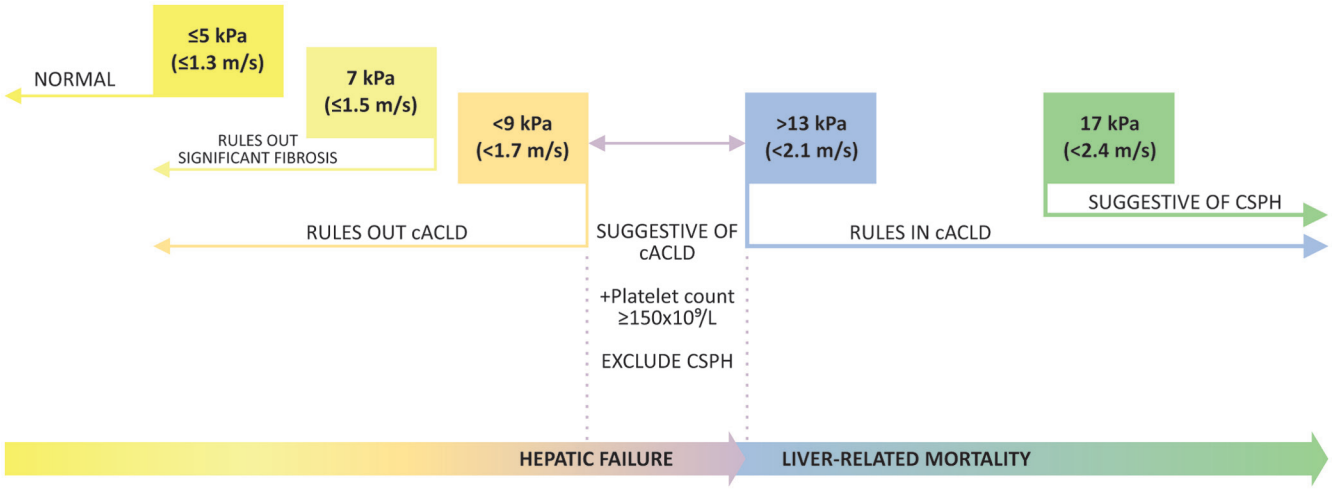

Fig. 1. Schematic presentation of rule of four for LSM by SWE in viral hepatitis and NAFLD - recommendation adopted from Baveno VII consensus. cACLD, compensated advanced chronic liver disease; CSPH, clinically significant portal hypertension; LSM, liver stiffness measurement; NAFLD, nonalcoholic fatty liver disease; SWE, shear wave elastography.

LB and semiquantitative techniques to characterize fibrosis regression, sample bias, and intra- and interobserver discrepancies were the primary drawbacks.

The most prevalent liver-related event in individuals with cACLD who have achieved SVR with DAA is the development of hepatocellular carcinoma (HCC). LSM by TE and albumin levels were shown to be beneficial for stratifying cACLD patients in a study by Pons *et al*.[25] Individuals with LSM≥20 kPa at follow-up or those with LSM between 10–20 kPa and albumin levels <4.4 g/dL were at the highest risk for developing HCC. Despite some limitations, LSM was conducted a year after DAA treatment ended, study results are applicable to patients in whom a valid and reproducible LSM could be acquired, and lack of an external cohort. Additional studies confirmed the above results implying that post-SVR LSM>20 kPa may have additional predictive significance for HCC and other negative outcomes.[26,27]

Ji *et al*.[28] showed that pretreatment older age (55 years), nonalcoholic fatty liver disorders (NAFLDs), higher alpha fetoprotein (AFP) levels (20 ng/mL), greater LSM (14.6 kPa), and diabetes mellitus were linked to the development of HCC. It included a large cohort of patients over a long period in two canters, under the control of a single principal investigator, using TE as the reference standard as a limitation. It emphasized that the present one-size-fits-all surveillance guidelines may require additional adjustment and that chronic HCV patients with DAAs induced SVR and NAFLD may also benefit from more extensive screening.

In conclusion, TE is recommended as the first-line assessment for the severity of liver fibrosis in patients with chronic HCV infection. Results of LSM≥7.1 kPa are equivalent to significant fibrosis ≥F2, and LSM≥9.5 kPa are equivalent to advanced fibrosis ≥F3.[10] The inability to see and avoid large vessels and masses at the site of measurement, the need for recalibration of the spring in the device at 6–12 months intervals (depending on the type of probe), reduced applicability in cases of obesity, and the inability to use it in patients with ascites are weaknesses.[29] Applicability for monitoring fibrosis regression after HCV eradication is limited since it is difficult to determine whether the post-SVR LSM decline is due to a decrease in liver fibrosis or a resolution of hepatic inflammation.[30] Consequently, routine use post-SVR is still not recommended according to the EASL guidelines, 2021 update.[5] However, the same guidelines state that for patients with cACLD prior to anti-HCV therapy, LSM post-SVR could be helpful to stratify the residual risk of hepatic complications (HCC and CSPH).[5,21,29] The cutoffs determined in viremic individuals should not be utilized in HCV patients who have received effective antiviral therapy since in these patients a rapid LSM decline has been determined already during treatment with continued improvement post-treatment, probably related to a reduction in liver inflammation.[31]

Point (p-)SWE using acoustic radiation force impulse elastography (ARFI) quantification

In this modality, shear-waves are induced using high-intensity acoustic pulses of short duration.[10] The operator can select the depth and the optimal placement for the region of interest. Compared with TE, this method has better applicability, since its success rate is significantly higher (97.1% vs. 93.6%, $p<0.001$), in obese patients and those with ascites.[32] A meta-analysis by Bota *et al*.[6] determined the inability to obtain reliable measurements 6.6% for TE and 2.1% for p-SWE. Furthermore, it demonstrated equivalent diagnostic accuracy in comparison to TE for significant fibrosis and cirrhosis. Differentiation of mild (F1) and moderate (F2) degrees of fibrosis was found to be less reliable because of the substantial overlap between LSM.[6] Additionally, a notable limitation is the narrow range of p-SWE results (0.5–4.4 m/s) which limits the definition of cutoff values between individual stages of fibrosis.[7,10] Currently, the major weakness is the lack of validation. Also, other techniques have shown similar or even greater efficacy.[11,33] Recently, this technique had been used to measure the spleen stiffness measurement (SSM). Knop *et al*.[34] did not detect significant changes of SSM during 3 years of follow-up. As SSM is considered as a noninvasive marker of the severity of PH, the authors concluded that hepatic fibrosis regressed after HCV elimination. However, PH can still exist in people with severe fibrosis with the consequent clinically significant risk of developing HCC. Thus, SSM could be used to evaluate CSPH with further validation of the optimal cut offs using TE (SSM<21 kPa and SSM>50 kPa, respectively) as well as utilizing p-SWE and 2D SWE according to the Baveno VII consensus.[21]

A new predictive model for the post-SVR HCC stratification

risk was proposed in 2021 by Dajti at al. based on post-treatment values of LSM and SSM.[35] Standard monitoring using ultrasound and biochemical markers should be carried out in individuals at intermediate risk for HCC (LSM-SVR24 10–20 kPa and SSM-SVR24<42 kPa) once every 6 months, however it can be avoided in patients at low risk (LSM-SVR24<10 kPa). A more extensive follow-up using computed tomography (CT) or magnetic resonance (MR) imaging coupled with ultrasound may be beneficial for people at high risk for developing HCC (LSM-SVR24>20 kPa or SSM-SVR24>42 kPa). Limitations include a retrospective design, a relatively small sample of 140 patients followed for 2 years.[35]

2D SWE

2D SWE is performed in real time with the possibility of simultaneous B-mode analysis of the structural changes of the liver parenchyma and additional assessment of focal liver lesions and PH related complications.[36] A pilot study by Ferraioli *et al*.[37] showed better accuracy of SWE than TE in assessing significant fibrosis with no major differences between SWE and TE for advanced fibrosis (0.98 and 0.96, respectively) and cirrhosis (0.98 and 0.96, respectively). It was suggested that 2D SWE could be used as the TE to evaluate advanced fibrosis and cirrhosis with more accurate assessment of significant fibrosis. This was a single-center comparative study involving a relatively small number of European patients with a low prevalence of obesity, which is a major technical limitation of TE.

Another study showed better SWE applicability in patients with ascites compared to TE with the similar diagnostic performance for the diagnosis of cirrhosis.[38] A shortcoming of the study was that it included individuals with a variety of chronic liver disorders, not just chronic HCV patients. A meta-analysis confirmed the results and concluded that 2D SWE was not inferior to TE and provided at least equivalent results regarding the diagnostic accuracy for fibrosis staging and cirrhosis diagnosing.[39]

Although, other 2D SWEs are available on the market, these conclusions, particularly the cutoffs, only accounted for 2D SWE based on Supersonic shear imaging (Aixplorer). Another meta-analysis suggested that SWE could guide clinicians in monitoring of fibrosis dynamics in real time.[40] A study by Tada *et al*.[41] demonstrated a significant decrease in LSM at the end of DAA treatment and 24 weeks later, primarily in patients with progressive liver fibrosis. It assessed successive LSM while taking necroinflammatory activity into account. The retrospective nature of this study and the relatively limited number of chronic HCV patients who achieved SVR are major weaknesses (210 out of 288 patients).

Regarding predicting the risk of complications, 24 months after achieving SVR (SVR 24), LSM≥11 kPa was independently associated with the risk of HCC development (RR=28.71).[42] Despite the limitations of this Japanese single-center retrospective study with relatively short follow-up, it has been proven that LSM at SVR24 offers a clear evaluation of liver fibrosis since inflammation improved. Thus, 2D SWE was effective not just for longitudinal monitoring of LSM but also in screening for HCC after SVR (Fig. 1). Validation and clear definition of quality criteria are mandatory before its routine clinical application, especially for SSM.[5,21]

Overall, all physical methods have a high accuracy in ruling out cirrhosis. The literature suggests that the accuracy of TE and p-SWE are equivalent, but p-SWE is more reliable than TE and 2D SWE is more accurate than TE. Regarding post-SVR follow-up, in an update to the Society of Radiologists in the Ultrasound Liver Elastography Consensus Statement, Barr *et al*.[31] recommended that instead of using absolute data, the delta change in LSM over time in the same patient using the same equipment with the baseline LSM obtained after viral eradication or suppression should be analyzed. When the delta change was more than 10%, clinically significant differences could be considered.

The main limitations of ultrasound-based elastography include several physiological and anthropometric factors (obesity, waist circumference, narrow intercostal spaces, distance between skin and liver capsule), ascites, and limited operator experience.[17] Additionally, TE and all SWE methods can be complicated by hepatic inflammation indicated by aspartate aminotransferase (AST) and/or alanine transaminase (ALT) elevation >5 times the upper reference limit), hepatic congestion, extrahepatic cholestasis, acute hepatitis, and infiltrative liver disease.[10] All the above-mentioned confounding factors must be excluded to avoid overestimation of liver fibrosis. In these cases, a statement indicating the results "may overestimate the degree of fibrosis" should be included in the report. In case of alcoholic hepatitis, LSM decreases following 1–4 weeks of abstinence.

MRE

MRE is a USA Food and Drug Administration-approved phase contrast 2D gradient recalled echo (2D GRE MRE) sequence used to display propagation of shear wave in the liver.[43] When comparing data across published papers, it is crucial to notice the difference between MRE and ultrasound elastography (G=1/3E in TE).[10] In comparison with TE, MRE allows imaging of the entire liver with the possibility to detect focal liver lesions (such as HCC) and liver-related complications using standard MR protocols during the same session.[44] The MRE studies are unaffected by conditions such as obesity, ascites, and hepatodiaphragmatic interposition of the bowel loops which restrict the use of ultrasound elastography[44] but are limited in iron overload/hemochromatosis.[44]

A meta-analysis of diagnostic performance of MRE in 2015 encompassing 12 studies with 697 patients with CLD (47.1% HCV patients) showed excellent overall diagnostic accuracy of MRE for discriminating advanced fibrosis (F≥3) and cirrhosis (F≥4) with the AUROC of 0.93 and 0.92, respectively. MRE performed well for diagnosis of significant (F≥2) and any fibrosis (F≥1) with an AUROC of 0.84–0.88. The optimal cutoff values of MRE for diagnosis of any, significant, advanced fibrosis and cirrhosis were 3.45, 3.66, 4.11 and 4.71 kPa, respectively.[45] Comparative studies of TE and p-SWE have demonstrated ambiguous results. According to above-mentioned meta-analysis, the diagnostic performance of MRE was comparable, if not superior, to that of ultrasound-based methods,[45] while several other studies and meta-analyses suggested superior diagnostic accuracy of MRE.[12,46,47]

The literature also suggested a good prognostic value of MRE in risk stratification of clinical progression of cirrhosis in HCV infection.[48,49] With regards to prediction of HCC, LSM by MRE was an autonomous marker for predicting development of HCC[49,50,51] as well as independent predictive factor of early recurrence of treated HCC.[52] Although MRE is an excellent technique for longitudinal follow-up of HCV patients,[53] but its time-consumption and high cost limits its implementation into routine clinical use. Additionally, claustrophobia and the presence of ferromagnetic implants are limitations for any MR examination.

Serological methods

Noninvasive methods based on detection and quantification of various serum biomarkers have been used to obtain the information about the degree of chronic liver injury and to

assess the progression of liver fibrosis.[54] However, none of the existing blood markers are liver-specific, and each have some constraints in diagnostic accuracy.[55] Over the past few years, the diagnostic value of hepatic fibrosis biomarkers has been examined in various studies and reviews.[11,56–58] Serum fibrosis biomarkers are divided into direct biomarkers that reflect the activity of the fibrotic process, and the rate of extracellular matrix turnover, and indirect biomarkers that reflect deterioration of hepatic function.[54]

Direct biomarkers

Direct biomarkers of fibrosis are the liver extracellular matrix (ECM) components derived mostly from hepatic stellate cells during the process of ECM remodeling. They typically reflect deposition (progression of the disease) or removal of ECM (with response to the treatment).[59] Direct markers include (1) collagens, glycoproteins, and polysaccharides, which are associated with ECM deposition; (2) collagenases and their inhibitors, which are markers associated with ECM degradation, and fragments of these serum molecules may serve as biomarker targets in a novel approach called protein fingerprint technology; and (3) cytokine and chemokines, which are markers associated with hepatic fibrosis; and (4) proteomic markers.[60–62]

Hyaluronic acid (HA) is the most studied direct serum marker. In patients with chronic HCV, HA was effective in differentiating between the stages F0/F1 and F2/F3/F4. HA has shown its superiority over almost all direct serum markers. But, in terms of diagnosing the exact stage of fibrosis, results have been inconsistent with only a few studies demonstrating that it could differentiate between them. AUROC for both diagnosis of fibrosis and cirrhosis was about 0.90 for HA compared to 0.75 for procollagen III, N-terminal propeptide (PIIINP).[63] Rewisha *et al*.[64] compared serum levels of HA and fibrosis-4 index (FIB-4) during a 1 year follow-up after DAA therapy and showed a significant decrease in serum levels of HA. However, the diagnosis was based on laboratory and imaging parameters not the serum levels of HA with any reference value. Thus, it is not known whether the regression of fibrosis was due to the direct effect of medications or the elimination of HCV infection.

The European/Enhanced Liver Fibrosis panel (ELF) (Siemens Healthcare, Erlangen, Germany) is a commercial, NIT for staging of liver fibrosis in treatment of both chronic HCV infection and HCV/human immunodeficiency virus co-infection.[65] It consists of a combination of direct biomarkers including HA, PIIINP, and tissue inhibitor of metalloproteinase 1 (TIMP-1). A prospective study on 181 patients showed AUROC of 0.76 for advanced fibrosis with a sensitivity and specificity of 78% and 98%, respectively.[66] The study showed a good result with cutoff values consistent with previously published data. Recent research has suggested several other new indicators that are not currently routinely available to or collected by hepatologists but may be needed.[67,68] The biomarker pro-peptide of type lll collagen (PRO-C3) is one example.[69] The PRO-C3-based fibrosis algorithm uses PRO-C3 as a marker of type III collagen formation, and include age, presence of diabetes, and platelet count. PRO-C3 is a standalone predictor of NAFLD fibrosis stage.

Compared with the AST to platelet ratio index (APRI), FIB-4, and NAFLD fibrosis score (NFS), a PRO-C3-based score accurately classifies patients with NAFLD and advanced fibrosis. However, further details are beyond the scope of this review, as PRO-C3 is still not used in routine clinical practice for fibrosis staging in HVC patients.[70] Other markers under investigation include hepcidin, of adiponectin, leptin, transforming growth factor-beta (TGF-β1), platelet-derived growth factor (PDGF), and especially ferritin, but clinical data are limited.[71]

Indirect biomarkers

Combinations of indirect biomarkers have been reported to have diagnostic value for the assessment of fibrosis in chronic HCV infection.[72] However, their role is currently limited to certain pathological conditions,[54] and certain stages of fibrosis.[55,60,73] By using only two blood parameters, red cell distribution width and platelet ratio, Elmdams *et al*.[74] described a novel noninvasive index – red cell distribution width (RDW) to platelet ratio (RPR) with sensitivities of 83.3% for liver fibrosis and 90% for cirrhosis in patients with chronic HCV. The RPR was not compared with LB as a reference standard but with APRI, FIB-4, and aspartate aminotransferase (AST) to alanine aminotransferase (ALT) ratio (AAR). Despite the above limitations of the study, RPR was found to be a very promising NIT with higher accuracy in detecting significant fibrosis and cirrhosis than APRI, FIB-4, and AAR. APRI is a nonpatented biomarker panel with sensitivity in detecting liver fibrosis of 77–89% and specificity of 72–75%.[75] Diagnostic accuracies of APRI for predicting the liver fibrosis in 1,716 treatment-naïve chronic HCV patients showed that AUROCs for the diagnosis of F≥2, F≥3 and F≥4 were 0.68, 0.68, and 0.70, respectively.[76] The study was retrospective and conducted in a single referral facility with possible interobserver discrepancies.

In a retrospective study, FIB-4 index[77] of treatment-naïve chronic HCV patients, FIB-4 predicted fibrosis with an AUROC of 0.70 for mild significant fibrosis, 0.73 for severe fibrosis, and 0.73 for cirrhosis, respectively.[76] In a study by Maev *et al*.,[78] FIB-4 was validated for use in chronic HCV infection when compared against APRI index and Bonacini index in chronic HCV patients. The sensitivity and specificity of the FIB-4, APRI index and the Bonacini index for the determination of F3–F4 in those patients was 68% and 86%, 79% and 69%, and 81% and 77%, respectively. Papadopoulos *et al*.[79] reported that the combination of APRI and FIB-4 was promising for predicting significant fibrosis while FIB-4 performed well in predicting cirrhosis. Research on progression of liver fibrosis in untreated HCV patients showed that both APRI and FIB-4 had as accuracy rate of 70%.[80] The results were likely affected by multicentric histological analysis. Eradication of HCV was not considered.

The Forns index is complex algorithm of serum tests and clinical features based on age, platelet count, gamma-glutamyltransferase (γGT) and cholesterol levels,[81] while the Fibro index combines platelet count, AST and γGT. Fibro index was compared with APRI and the Forns index and showed a higher median AUROC for significant fibrosis (0.76) and for cirrhosis (0.86) detection.[82] Direct comparisons showed no superiority of Fibro index over APRI.[83] The same was found using the Forns index which showed a very similar performance for both fibrosis (AUROC 0.76) and cirrhosis (AUROC 0.87).[83] Varchetta *et al*.[58] developed new algorithm named SiGAP including serum sialic acid-binding Ig-like lectin 7 (Siglec-7) values, age, γGT and platelet count. This index showed high specificity and sensitivity in predicting liver fibrosis when compared to APRI and FIB-4, but its clinical value as single marker was not superior to APRI and FIB-4.

Combined direct and indirect biomarker tests

With combinations of both sensitive and specific biomarker parameters that can reach up to 95% accuracy, noninvasive biological tests may generally improve diagnostic accuracy and provide better estimation of fibrosis progress.[62,84] Only a few biomarkers (APRI, FibroTest, FibroMeter and HepaScore)

have an AUROC of >90 for both significant liver fibrosis and cirrhosis, making them the first choice for use in HCV fibrosis management.[65] However, serum panels incorporating aminotransferases like APRI, FIB-4, Forns index, and FibroMeter may be falsely positive in acute hepatitis or in patients with hemolysin by FibroTest.[54] A pilot study including children with viral hepatitis suggested that serum soluble Fas antigen (sFas) and monocyte chemoattractant protein 1 (MCP-1) both individually and in combination with APRI, differentiated between mild (F0–1) and significant (F2–3) fibrosis.[85]

FibroTest is a panel of biochemical markers including alpha-macroglobulin (A2M), haptoglobin, γGT, age, bilirubin, apolipoprotein A1, used to detect liver fibrosis. In a meta-analysis that included eight studies, Jang *et al*.[86] showed a median AUROC of 0.84 for the diagnosis of advanced liver fibrosis in patients diagnosed with chronic HCV and hepatitis B virus (HBV), alcoholic liver disease, and NAFLD. In most studies and meta-analyses that compared sensitivity, specificity, and AUROC of APRI, FIB-4, AP index, and Forns index, FibroTest demonstrated the best diagnostic performance for detecting advanced fibrosis. Köksal *et al*.[87] concluded that the concurrent use of FibroTest with APRI and/or FIB-4 and FIB-4 with APRI) could provide highly accurate solution for the diagnosis of significant or advanced fibrosis in chronic HCV infection. This prospective observational trial indicated that the specificity of serum biomarkers increases when these are used together.

The FibroMeter test combines platelet count, prothrombin index, AST, A2M, HA, blood urea nitrogen, and age.[88] A study by Calès *et al*.[89] included patients with CLD (including chronic HCV infection), multi-FibroMeter showed overall accuracy in fibrosis staging and diagnosis of cirrhosis superior to classical single-targeted blood tests or TE. The sequential algorithm for fibrosis evaluation (SAFE biopsy)[90] is a stepwise combination of APRI, Forns index, and FibroTest at the time of LB. A study by Sebastiani *et al*.[91] showed a diagnostic accuracy of 94% and 95% for detecting advanced fibrosis and cirrhosis, respectively. The prospective study included two groups of consecutive naïve HCV patients with cACLD using LB as a reference standard. These results for detecting advanced fibrosis and cirrhosis were confirmed three years later with reported diagnostic accuracies of 90.1% and 92.5%, respectively. The same study also indicated that the number of LB preformed was reduced by 50–70%.

Liver outcome score (LOS) is a NIT that includes combination of age and sex with HA, γGT, bilirubin, A2M, platelet count, international normalised ratio (INR), prothrombin time (PT), AST, alkaline phosphatase (ALP), albumin, and creatinine. With AUROC of 0.95, LOS had an excellent predictive accuracy.[92] In accordance with previous studies by Ho *et al*.,[93] the best serum predictor for liver fibrosis in HCV-related HCC patients were FIB-4 and Lok index and for cirrhosis was cirrhosis discriminate score. This study confirmed the fact that current noninvasive biomarkers do not predict histological fibrosis severity in HCC patients.

Combination of noninvasive serological and physical methods

A testing algorithm based on combination of conventional biochemical and serological tests (for example APRI, FIB-4, FibroTest) and LSM (for example TE-FibroScan) may be efficient for detecting the liver fibrosis stage. While serum biomarkers have a higher diagnostic accuracy for detection of severe fibrosis, radiological markers are better for detection of cirrhosis.[94–99]

The combination of FibroTest and TE can be used to avoid LB in a large proportion of HCV patients (94%) as confirmed by Castera *et al*.[100] The AUROC for the diagnosis of liver cirrhosis for FibroTest alone was 0.87 but 0.95 in combination with TE. Based on this methodology, LB was avoided in 140 (77%) out of the 183 individuals. However, 10 (5%) of the patients were overweight or obese and could not be evaluated satisfactorily using this technique, a limitation of the study.

Knop *et al*.[34] compared diagnostic accuracy of noncommercial serum tests with TE in a study on 2 458 HCV patients. Results showed that significant fibrosis and cirrhosis were predicted with moderate accuracy using APRI, Forns index and FIB-4. AUROC, sensitivity, and specificity in discriminating advanced fibrosis were around 0.79, 0.60 and 0.94 by APRI, 0.84, 0.85 and 0.75 by Forns index, and 0.83, 0.66 and 0.95 by FIB-4. Limitations included usage of TE as reference standard and not LB.

Boursier *et al*.[101] created the easy liver fibrosis test (eLIFT), which integrates biomarkers such as age, sex, AST, PT, γGT and platelets. When compared to the other seven fibrosis tests evaluated in the core group (1,946 HCV patients), FibroMeter vibration-controlled (FMVC)TE showed a significantly higher AUROC for the diagnosis of advanced fibrosis and was chosen as the second-line test. These two tests were combined into a new stepwise algorithm called eLIFT-FMVCTE. For identification of advanced fibrosis and cirrhosis, their combined sensitivity was 76.1% and 92.1%, respectively.[102] Further investigation is needed to determine whether serial eLIFT testing can identify patients with worsening prognoses and liver fibrosis progression.[101,103]

Two years later, Boursier proposed another sequential combination with better diagnostic accuracy than other blood fibrosis tests and (VC)TE alone for the detection of advanced fibrosis in NAFLD. This new stepwise algorithm, containing simple blood test, or TE as first-line test and FMVCTE as second-line procedure, could feasibly in routine clinical practice diagnose advanced fibrosis in NAFLD with very low rate of required biopsies (20%).[104] Additionally, it was suggested that MRE could be introduced as a third line, as it has shown excellent diagnostic accuracy and would further reduce the need for biopsy in NAFLD patients. The same research group most recently concluded that VCTE has comparable accuracy to LB for the prediction of liver-related events in NAFLD and that the FIB4-VCTE stepwise algorithm successfully distinguished at-risk NAFLD patients based on clinical events, similar recommendations could be applied to HCV patients in the future as well.[105]

World Health Organization guidelines suggest that APRI or FIB-4 are better options than FibroTest or FibroScan especially in resource-limited countries.[97] The American Gastroenterological Association stated that patients with chronic HCV infection should have TE rather than APRI, FIB-4 to evaluate fibrosis stages.[94] The EASL guidelines, European Association for the Study of Diabetes and European Association for the Study of Obesity for HCV infected people; recommended the use of FibroTest, APRI, and FIB-4 as well as TE, p-SWE and 2D-SWE alone and in combination with serum biomarkers.[7,106]

Overall, the main characteristic of serological methods is their ability to rule out advanced fibrosis (FIB-4<1.3, FibroMeter<0.45 or FibroTest<0.48). However, they cannot differentiate between various stages of fibrosis. Cutoff values used in untreated HCV have been shown to be inaccurate after SVR, so new lower cutoffs need to be validated in larger studies with longer follow-up. Currently, the routine use of serological biomarkers to evaluate fibrosis regression post-SVR in HCV patients is not recommended.[5]

Patients with cirrhosis before SVR with FIB-4≥3.25 con-

Table 2. Validated models for HCC risk prediction after SVR in patients with chronic hepatitis C infection

Study	Score name	Variables included	HCC predictors	Risk classes	Cumulative incidence
Fan *et al*.,[109] 2020	aMAP	Age; Sex; Albumin; Bilirubin; PLT	ALBI score	<50 Low risk; 50–60 Medium risk; >60 High risk	3 or 5 years; Low risk group; 0.0–0.8%; Medium risk group; 1.5–4.8%; High risk group; 8.1–19.9%
Shiha *et al*.,[110] 2020	GES	Age; Sex; Albumin; AFP; Pretreatment fibrosis stage (F3, F4)	Male; Age>54 years; Albumin<3.8 g/dL; AFP>20 ng/mL; Cirrhosis (F4)	GES≤6 Low risk; GES 6–7.5 Intermediate risk; GES>7.5 High risk	1-/2-/3 years; Low risk; 0.1%/1.2%/1.9%; Intermediate risk; 0.7%/3.3%/5.8%; High risk; 1.2%/7.1%/9.5%
Hiraoka *et al*.,[111] 2019	ADRES	Sex; SVR24 FIB-4; SVR24 AFP	Male; FIB-4>3.25; AFP>5.0 ng/mL	ADRES 0/1/2/3	ADRES 0/1/2/3; 0%/0.5%/8.4%/18% at 1 year; 0%/1.6%/13.4%/32.8% at 2 years
Ioannou *et al*.,[112] 2018	VHA	Sex; Age; BMI; Ethnicity; HCV genotype; Hemoglobin; PLT; Albumin; INR; AST/√ALT	Age>60; PLT<61×10^4; AST/√ALT>8.8 in noncirrhotic; AST/√ALT>11.01 in cirrhotic; Albumin<2.9 g/dL	Four subgroups; Cirrhosis/SVR; Cirrhosis/no SVR; No cirrhosis/SVR; No cirrhosis/no SVR	Cirrhosis/SVR; 4.5% at 2 years; Cirrhosis/no SVR; 13.1% at 2.6 years; No cirrhosis/SVR; 0.7% at 2.3 years; No cirrhosis/no SVR; 4.2% at 3.7 years

ADRES, after DAAs Recommendation for Surveillance Score; AFP, alpha fetoprotein; ALBI, albumin-bilirubin score; ALT, alanine aminotransferase; aMAP, age-male sex-ALBI-platelet count score; AST, aspartate aminotransferase; BMI, body mass index; DAAs, direct-acting antivirals; FIB-4, fibrosis-4 index; GES, General Evaluation Score; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; INR, international normalized ratio; PLT, platelet count; SVR, sustained virological response; SVR24, sustained virological response at 24 weeks; VHA, Veteran Health Affairs.

tinue to have a high risk of HCC (incidence >c2%) even if the FIB-4 score decreased, whereas patients with FIB-4≤3.25 before and persistently after SVR had an annual HCC risk<1%. Pretreatment non- cirrhotic patients had a low risk of HCC, except for those with pre-SVR FIB-4 scores ≥3.25 (annual risk 1.22%) and post-SVR FIB-4 scores ≥3.25 (annual risk 2.39%).[107]

Kawaguchi *et al*. demonstrated that the enhanced liver fibrosis (ELF) test could be a useful marker for predicting HCC in patients with chronic HCV after achieving SVR with DAA therapy. The study showed that a high enhanced liver fibrosis test score 24 weeks after treatment was strongly associated with the development of HCC.[108] Cumulative HCC incidence increases when two or more independent predictors are combined in a multivariable analysis. The studies with external validation are listed in Table 2.[109-112]

Future directions in noninvasive liver fibrosis assessment

MicroRNAs (miRNA) include 18–25-nucleotides and are noncoding RNAs that have roles in various cell processes by targeting messenger RNA (mRNA) and thereby regulate gene expression.[113] Each cell type expresses different sets of miRNAs during stages of tissue development. For example, various liver cells express 227 miRNAs and miR-122 accounting for 70% of those known and is liver-specific.[114] MiRNA can be detected in serum, plasma, urine, and organ tissues. Importantly, expression of miRNAs is a dynamic process, so that in one stage of the disease one type of miRNA can be upregulated, followed by its downregulation in the next stage.

In recent years, much research has focused on incorporation of miRNA analysis into fibrosis staging. For example, a panel of tests including miRNA-129, miRNA-223, platelet count and AST level was a reliable test for prediction of advanced stages of fibrosis ≥F3 (AUROC=0.91) and F4 (AUROC=0.96), more accurate than APRI, or FIB-4. However, it was inferior to those tests in predicting milder stages of fibrosis.[115] Other studies explored serum levels of various miRNAs to assess their potential role in fibrosis staging, but no highly specific miRNA type was found to differentiate between each of fibrosis stage with high accuracy.[116–118]

Conclusions

A variety of different NITs for liver fibrosis assessment have emerged and are widely available in clinical practice. In low prevalence HCV populations, NITs should be used to rule out advanced fibrosis, rather than diagnosing it. Since patients with cACLD require monitoring for HCC and/or varices even after the SVR, staging of liver fibrosis before therapy is still required. The routine use of NITs to assess fibrosis regression post-SVR in HCV patients is presently not recommended by guidelines. Although, use of physical methods following SVR may improve the categorization of residual risk of liver-related events in patients with cACLD prior to HCV antiviral therapy. Annually LSM can be done while confirmation evidence is awaited. Serum miRNA detection in HCV infection is an interesting and rapidly growing field of research but lacks diagnostic precision for various stages of liver fibrosis.

It is our opinion that NIT for liver fibrosis assessment provides numerous benefits to HCV patients due to its main qualities rapid, cost-effective, and repeatable for longitudinal evaluation (Table 3). A liver specialist should be consulted when choosing specific NITs and creating diagnostic pathway for advanced fibrosis assessment in specific subgroups and specific purposes.[5]

It is more crucial from a clinical standpoint to rule in or rule out advanced fibrosis than to provide a precise stage. Future studies with larger sample sizes and longer follow-up will establish standardized cutoff values for prediction liver-related complication after SVR, since this is of great importance in current clinical practice.

Table 3. Advantages and disadvantages of currently available noninvasive methods for evaluation of hepatic fibrosis in patients with chronic hepatitis C infection

Feature	Serological methods	Noninvasive physical methods		
		TE	p-SWE/2D SWE	MRE
Cost	+	++	++	+++
Availability	***	**	*	-
Validation	***	**	-/+	-/+
Applicability	***	*	**	***
Diagnostic accuracy for excluding liver cirrhosis	*	***	**	***
Diagnostic accuracy for excluding advanced fibrosis	*	**	**	***
False positivity	+	+	+	+
Discrimination between intermediate stages of fibrosis	–	–	–	–
Detecting fibrosis regression after SVR	–	–/+	–/+	–/+

+, yes; –, no; –/+, partially; *, good; **, high; ***, excellent. MRE, magnetic resonance elastography; p-SWE, point shear wave elastography; SVR, sustained virologic response; 2D SWE, two-dimensional shear wave elastography; TE, transient elastography.

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Conflict of interest

GW has been an editor-in-chief of *Journal of Clinical and Translational Hepatology* since 2013. MS and RS have been editorial board members of *Journal of Clinical and Translational Hepatology* since 2013. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptualization (KB, MSB, MS), methodology (GI) formal analysis (RS), investigation (KB, MSB), data curation (KB, MSB, RS, SV), writing - original draft preparation (KB, MSB), writing - review and editing (MS, RS, SV, AT, GW), visualization (RS), supervision (KB, MS), project administration (MS), funding acquisition (MS). All authors have read and agreed to the published version of the manuscript.

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