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Topic: Advances in the diagnosis and treatment of liver fibrosis
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Advances in the diagnosis and treatment of liver fibrosis

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ABSTRACT

Liver fibrosis is the center of diagnosis and management of essentially all chronic liver diseases. While liver biopsy examination still has a role in diagnosis and drug development, it is replaced by non-invasive assessments of liver biopsy in majority of the clinical scenarios. Radiological approaches, namely transient elastography, acoustic radiation force impulse imaging, shear wave elastography, magnetic resonance elastography provide accurate diagnosis of advanced fibrosis and cirrhosis. Serum test formulae based on common laboratory parameters or more specialized parameters including those commercially available panels FibroTest®, FibroMeter® and Enhanced Liver Fibrosis are also available. Combining different modalities may further improve the accuracy. The role of all these non-invasive assessments has been further expanded from diagnostic to prognostic, e.g. risk prediction of hepatocellular carcinoma (HCC) by LSM-HCC score. Treatment of liver fibrosis can be achieved by controlling the underlying diseases, with chronic viral hepatitis as the most established disease model. Currently there are multiple clinical trials evaluating different treatment options to improve fibrosis in patients with non-alcoholic fatty liver disease. Specific anti-fibrotic treatment targets e.g. direct downregulation of hepatic stellate cell, collagen synthesis inhibitors and transforming growth factor-β antagonists have been tested in laboratory and pending further studies in clinical settings.

INTRODUCTION

Liver fibrosis is the formation of scar tissue in response to parenchymal injury secondary to chronic liver disease, e.g. chronic hepatitis B and C, non-alcoholic fatty liver disease (NAFLD) or alcoholism. It distorts the normal liver parenchyma.[1] The continuous and progressive replacement of hepatocytes by extracellular matrix and fibrous tissue leads to liver cirrhosis, which is a key risk factor for hepatocellular carcinoma (HCC).[2] Apart from its relationship with HCC, liver fibrosis is also an important treatment indication in various chronic liver diseases. Different international treatment guidelines mentioned that the severity of liver fibrosis should be considered, regardless of the level of ALT, for starting antiviral treatment for chronic hepatitis B (CHB).[3,4] There are solid evidence supporting the fact that liver fibrosis is potentially reversible.[5] Therefore, it is important to diagnose and assess the severity of liver fibrosis in order to provide appropriate

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management in order to prevent further liver damage. This article focuses on the up-to-date approaches for diagnosis, both invasive and non-invasive, and latest development in treatments of liver fibrosis, particularly in NAFLD patients for whom a handful of clinical trials are currently ongoing.

DIAGNOSIS OF LIVER FIBROSIS

There are varieties of methods for making the diagnosis of liver fibrosis, which can be classified into invasive and non-invasive approaches.

Invasive approach - liver biopsy examination

For invasive approach, it refers to liver biopsy examination, which provides liver tissue for a histopathological assessment of liver. Liver biopsy examination can be done percutaneously, transvenously (either transjugularly or transfemorally), or surgically (open or laparoscopic operations).[6] Indications for liver biopsy are for diagnostic and/or prognostic purposes, as well as for treatment planning.[7]

Liver biopsy is still regarded as the gold standard for liver fibrosis assessment in various chronic liver diseases.[8,9] Apart from general histological staging, liver biopsy can also provide information concerning morphometry, which can provide additional information on the distribution and the exact quantity of liver fibrosis.[10] A recent quantitative tool called qFibrosis utilized 87 parameters aiming for combining the results of collagen patterns, collagen architectural features and statistical analysis of features of respective collagen patterns into a single index. This requires an unstained biopsy sample for the automated analysis of liver fibrosis staging.[11] All these evidences illustrate that liver biopsy plays an important role in the diagnosis of liver fibrosis.

Apart from liver fibrosis staging, liver biopsy can provide different information important for the management of the clinicians. For example, in cases of NAFLD, the degree of necroinflammation and steatosis can be determined by liver biopsy so corresponding management can be provided for this potentially reversible situation.[12] Liver biopsy is also helpful in diagnosing adverse drug reaction and classification of liver tumors.[13] Yet, the most common reason for conducting a liver biopsy is for assessing the liver fibrosis in patients with chronic viral hepatitis and NAFLD.

Such a direct and useful method bears quite a few limitations. Sampling error is a major limitation for liver biopsy as only 1/50,000 of the whole liver parenchyma is obtained. Sampling error can be minimized by either obtaining a specimen of sufficient size (at least 2 cm in length) or from different lobes, which may not be feasible all the time.[14] Well reported complications from liver biopsy examination include pain,[15] bleeding such as wound bleeding, intraperitoneal hemorrhage, hemobilia or hemothorax,[15] transient acute hypotension or vasovagal syncope.[16] Fatal complications like uncontrolled bleeding, bacteremia and sepsis are rare but still possible.[17] In patients with HCC, liver biopsy also carries a risk of spreading the cancer cells.[18]

Non-invasive approach

Radiological assessments are either ultrasonographic-based [e.g. transient elastography, acoustic radiation force impulse (ARFI) imaging and shear wave elastography (SWE)] or magnetic resonance (MR)-based [i.e. MR elastography (MRE)].

Ultrasonographic based

Transient elastography

Transient elastography (Fibroscan®, Echosens, Paris, France) assesses liver stiffness measurement (LSM) by transmitting shear wave followed by ultrasound wave through a probe putting on the skin overlying the liver parenchyma. The velocity of the shear wave passing through the liver parenchyma is calculated by Doppler technique. The higher the velocity, the stiffer the liver parenchyma is. As mentioned by the manufacturer, for an examination to be considered as reliable, it requires at least 10 successful attempts and the ratio of interquartile range to median of those measurements should be less than 0.3.[19] LSM reflects the degree of liver fibrosis.[20] It can even identify those with no or minimal fibrosis and differentiate them from those with severe fibrosis or cirrhosis.[21] It has been proved useful across different liver disease entity (e.g. chronic hepatitis B and C, autoimmune hepatitis).[22] However, LSM by transient elastography is found to be less reliable in obese patients.[23,24] It can be less accurate in certain situation, e.g. severe acute exacerbation of hepatitis,[25] post-treatment fibrosis stages in CHB[26] or chronic hepatitis C (CHC) patients.[27]

ARFI imaging

ARFI is another technique for estimating liver fibrosis. It is implemented in current ultrasound scanner, without acquirement of external equipment. The conventional ultrasound probe automatically produces an acoustic “push” pulse for generating shear-wave which passes through the tissue. The wave propagation speed is assessed. Again, higher the speed, higher the liver stiffness measurement is.[28,29] There are several advantages for ARFI. As it is a function of the ultrasound

scanner, no additional cost is required.[30] The ARFI not only shows the degree of fibrosis, it also provides external information for disease progression for different chronic liver disease, for example HCV.[31] Another advantage of this tool is that it can provide real-time results and easy to perform. The measurement results appear to be more accurate in overweight and obese patients, compared with transient elastography.[32] However, one prominent disadvantage for ARFI is that the range of its measurement is quite narrow (only from 0.5 to 4.4 m/s).[33] Furthermore, it is quite difficult to match the degree of fibrosis with the wave propagation speed, i.e. a cut-off, which is difficult to be defined.[34]

SWE

SWE is a 2-dimensional ultrasound technique based on the estimation of shear wave velocity from the radiation force of a focused beam of ultrasound,[35] and it can be converted results in terms of kPa by an equation.[36] No extra vibrator or detector is required as it is integrated into a conventional ultrasound system. Besides, elasticity of liver tissues can be shown in both numerical values and color (i.e. higher stiffness is reflected in red color), which can reflect the relative stiffness of the liver tissue quickly. The numerical values can be expressed in either kPa or m/s, which can be comparable with the results from transient elastography or ARFI.[37] Actually, its accuracy is higher compared to transient elastography or AFRI in assessing the degree of fibrosis, especially in those with early-stage liver fibrosis.[38] SWE with spleen stiffness index is recommended as the first line assessment for patients with liver fibrosis due to chronic hepatitis C in the latest guidelines.[39] However, only a few studies validate its clinical application.[38,40]

MRE

MRE adopts a phase contrast imaging method which depends on mechanical wave propagation to assess the degree of liver stiffness.[41] Generally, MRE is less operator-dependent and involved in less technical failure. The global picture of the liver can be viewed easily, regardless the obesity or severity of the ascites of the patients. It can also give a comprehensive assessment for the associated complications, for example portal hypertension or associated spleen stiffness.[42] Meanwhile, it is useful for diagnosis and staging of liver fibrosis, even if the fibrosis is very mild. Another advantage for MRE is that the results are readily reproducible.[42] However, MRE is more expensive and time-consuming compared to ultrasound-based approach. Respiration creates artifacts on the images. Another important limitation is that it is not applicable on patients with iron overload, or hemochromatosis, because iron might create noise for the signals received by the MR machine.[43] There are still limited studies mentioning the clinical significance of MRE results. Even though it is apparently sensitive to mild liver fibrosis, the result may sometimes be unreliable.[44]

Serum test formulae

Common laboratory parameters

Another commonly adopted non-invasive assessment is based on serum with or without clinical parameters. Examples including common parameters in clinical practice include aspartate aminotransferase (AST) to platelet ratio index (APRI),[45] Forns index,[46] Fibrosis-4 (FIB-4),[47] Fibroindex,[48] Hui index,[49] NAFLD fibrosis score (NFS)[50] and BAAT score[51] [Table 1]. These parameters are derived from routine liver biochemistry panel, so it is quite convenient. These parameters are also technically easy to obtain and with minimal inter-observer variations. Patients with advanced fibrosis can be identified by these tests.[52] However, these parameters are often validated in just one or two liver diseases. For example, two scoring systems for CHC patients, namely APRI and FIB-4, are found to be not useful in CHB patients.[53]

FibroTest®

Some specific biochemical parameters related to fibrinolysis or fibrinogenesis are developed to improve the specificity of liver fibrosis assessment [Table 2]. One example is FibroTest® (BioPredictive, Paris, France; or known as Fibrosure® in the United States) consists of 5 components, namely GGT, total bilirubin, α-2 macroglobulin, apolipoprotein A1, and haptoglobin. Sometimes, another test, ActiTest, would also perform together with FibroTest® for assessment for liver activity, with the additional measurement of ALT. The results would be adjusted according to age and gender.[54] FibroTest® is originally used in patients with CHC.[55] Nowadays it is recommended by different associations concerning liver studies for evaluation of liver fibrosis in patients with CHB, NAFLD or alcoholic liver disease.[56-58] It is highly reliable and applicable,[59] even for patients with obesity.[60] It performs well for diagnosis of liver cirrhosis for disease entities other than CHC. However, the results are suboptimal for detecting earlier stages before cirrhosis.[61]

FibroMeter®

FibroMeter® (Echosens, Paris, France) has been validated in patients with CHB, CHC, NAFLD and alcoholic liver disease.[62] Platelets, prothrombin index, AST, α-2 macroglobulin, hyaluronate, urea and age are taken into accounts.[63] Second generation (2G) has put age into another important parameter.[62] FibroMeter® has recently reached its third generation

Table 1: Serum test formulae for liver fibrosis

Parameters or index	Formula
APRI	AST (ULN) × 100 / platelet (10^9/L)
Forns index	7.811 - 3.131 × ln(platelet count) + 0.781 × ln(GGT) + 3.467 × ln(age) - 0.014 × (cholesterol)
FIB-4	Age (years) × AST [U/l] / (platelets [10^9/L] × (ALT [U/L])$^{1/2}$)
Fibro index	1.738 - 0.064 × platelet [10^9/L]) + 0.005 × AST [IU/L] + 0.463 × gamma globulin [g/dL]
Hui index	exp(3.148 + 0.167 × BMI + 0.088 × bilirubin [μmol/L] - 0.151 × albumin [g/L] - 0.019 × platelet [10^9/L]) / (1 + exp(3.148 + 0.167 × BMI + 0.088 × bilirubin [μmol/L] - 0.151 × albumin [g/L] - 0.019 × platelet [10^9/L]))
NFS	-1.675 + 0.037 × age (years) + 0.094 × BMI (kg/m^2) + 1.13 × impaired fasting glycaemia or diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet (× 10^9/L) - 0.66 × albumin (g/dL)
BAAT score	BMI (≥ 28 = 1, < 28 = 0) + age at liver biopsy (≥ 50 years = 1, < 50 = 0) + ALT (≥ 2 × (ULN) = 1, < 2 × ULN = 0) + serum triglycerides (≥ 1.7 mmol/L = 1, < 1.7 = 0)

ALT: alanine aminotransferase; APRI: aspartate aminotransferase to platelet ratio index; AST: aspartate aminotransferase; BAAT: BMI, age, ALT, triglycerides; BMI: body mass index; FIB-4: fibrosis-4; GGT: gamma-glutamyl transferase; NFS: non-alcoholic fatty liver disease (NAFLD) fibrosis score; ULN: upper limit of normal

Table 2: Different non-invasive approach

Non-invasive tests	Features	Advantages	Disadvantages
Radiological			
Transient elastography	Ultrasound-based liver stiffness measurement by shear wave velocity by a specific probe	Useful across different liver disease entity Special probes designed for different body built Measure liver fat at the same time with CAP Can identify no or minimal fibrosis	Less reliable in obese patients Less reliable in severe acute exacerbation of hepatitis Less reliable in post-treatment fibrosis stages in CHB or CHC patients
Acoustic radiation force impulse imaging	Ultrasound-based wave propagation speed measurement by conventional probe	No additional apparatus except ultrasound machine Can reflect disease progression Real-time results Less technical difficulties Accurate in overweight or obese patients	Narrow range of measurement Difficult to define a cut-off More experienced operators need
Shear wave elastography	Ultrasound measurement of shear wave velocity	No additional apparatus except ultrasound machine Elasticity can be reflected by numbers or colors Sensitive for early-stage fibrosis Results can be expressed into kPa or m/s	Limited studies on its clinical application
Magnetic resonance elastography	Phase contrast imaging depending on mechanical wave propagation	Less operator-dependent and less technical failure Limited effect by obesity or ascites Can assess complications Sensitive for early-stage fibrosis Reproducible results	High cost Limited availability in some countries/regions More time-consuming Not applicable on patients with iron overload or hemochromatosis Limited studies on its clinical application
Serum test formulae			
Common laboratory parameters	Refer to Table 1	Results from routine liver function test, convenient to perform No inter-observer variations	Cannot be used for all chronic liver diseases
FibroTest	Consists of GGT, total bilirubin, α-2 macroglobulin, apolipoprotein A1, and haptoglobin	Useful in different chronic liver disease Reliable Applicable Accurate in overweight or obese patients	Suboptimal for early stage fibrosis
FibroMeter	First 2 generations: consists of platelets, prothrombin index, AST, α-2 macroglobulin, hyaluronate, urea and age 3rd generation (3G): hyaluronate does not take into account	With high fibrosis classification accuracy Good predictive value for severe fibrosis in different liver disease entities	High cost
Enhanced liver fibrosis	Consists of 3 direct blood markers: procollagen III amino terminal peptide, hyaluronic acid and tissue inhibitor of metalloproteinase I	Good prognostic factor for clinical outcomes in patients with chronic liver diseases Similar results by using fresh blood or cryopreserved blood Sensitive for advanced fibrosis or cirrhosis	Not sensitive for early stages of fibrosis Age, low CD4+ T-cell count and other factors can affect ELF results

AST: aspartate aminotransferase; CAP: controlled attenuation parameter; CHB: chronic hepatitis B; CHC: chronic hepatitis C; ELF: enhanced liver fibrosis

(3G), which does not take hyaluronate into account. Therefore, the cost has been reduced but with similar effectiveness.[64] FibroMeter®, both 2G and 3G, has been shown with high fibrosis classification accuracy.[65] Besides, it appears to have a good predictive value towards the occurrence of severe fibrosis in those with NAFLD[66] and chronic hepatitis B or C.[67] Even though the hyaluronate-free FibroMeter® 3G is in use nowadays, the cost is still high compared to common parameters (e.g. FIB-4 or NFS).[68]

Enhanced liver fibrosis

Enhanced liver fibrosis (ELF) score is an algorithm consists of 3 direct markers in blood, namely procollagen III amino terminal peptide (PIIINP), hyaluronic acid and tissue inhibitor of metalloproteinase I (TIMP-I).[69] ELF can be a good prognostic factor for the clinical outcomes of patients with chronic liver disease. The increase in one point in ELF can lead to doubling of the risk of clinical outcomes in patients, especially liver-related clinical outcomes.[70] ELF results are even similar when using fresh blood or cryopreserved blood. Therefore, it has a high predictive value for identifying patients with risk to develop progressive chronic liver disease at an early stage.[71] It is sensitive in identifying advanced fibrosis or cirrhosis, but not for lower fibrosis stage.[72,73] Meanwhile, it is noted that different factors can influence the result of ELF score, with the most significant factor being age.[74] Other factors include low CD4+ T-cell count, co-existing extra-hepatic fibrosis, *etc*.[75] Therefore, the results of ELF should be interpreted with particular clinical context.

Novel serum markers

There are some other novel serum fibrosis markers that raise the attention of the clinicians. Glycosylated Wisteria floribunda agglutinin-positive Mac-2 binding protein (WFA$^+$-M2BP) is a marker which is related to fibrosis-related glyco-alteration. It can be measured by a glycan-based immunoassay, FastLec-Hepa. A cut-off index would be calculated based on the measured value.[76] It is found to be useful for detecting early stages of fibrosis in chronic hepatitis B patients in a recent study.[77] Another novel marker, YKL-40 (CHI3L1), is an emerging inflammation biomarker which was shown to be related to the early stage of liver fibrosis.[78] In NAFLD patients, macrophages in liver were showed to express YKL-40. This makes YKL-40 be possible as a biomarker as liver fibrosis.[79] However, further studies need to be conducted to show the effectiveness and impact of both biomarkers on making the diagnosis or management of patients with liver fibrosis due to any chronic liver diseases.

Combination of different approaches

It is common for using both radiological and biochemical methods to increase the accuracy in determining the degree of fibrosis. Both types of methods can play a supplementary role to each other. For example, the performance of ELF improves with the assistance of transient elastography.[80] With the use of ELF-LSM algorithm, a significant proportion of patients can avoid liver biopsy.[69] Another example is Hui Index and transient elastography. Since LSM result is confounded in patients with elevated ALT, Hui index, a score independent of ALT level, is a good choice for supplementation of transient elastography. Studies have shown that the combinations can help predict hepatic event-free survival in chronic hepatitis B patients.[81] Another combination for assessment of liver fibrosis in CHB patients is Forns index (another ALT-free index)-LSM algorithm.[82] FibroMeter® and transient elastography combined together can help improve diagnostic accuracy and avoid liver biopsy in CHC patients.[83] For the diagnosis of cirrhosis in CHC patients, using the algorithm FibroTest® and transient elastography improves the performance. However, this combination does not show extra benefit for diagnosis of advanced fibrosis compared to the sole use of FibroTest®.[84]

Non-invasive tests - from diagnostic to prognostic

Portal hypertension and related complications

The role of all these non-invasive tests is moving from diagnostic to prognostic. They are useful to predict liver-related complications and hence the prognosis of patients with chronic liver diseases. For example, a LSM with 13.6 kPa can be a predictive value the presence of portal hypertension.[85] Combing LSM with APRI or Fibroindex increases the sensitivities for portal hypertension predication.[85] Liver stiffness with ARFI greater than 2.34 m/s indicates a poor liver reserve function.[86] Assessment of spleen loss modulus by MRE is a good method for recognizing patients with severe portal hypertension or esophageal varices with high bleeding tendency.[87] Combing LSM and spleen stiffness measurement (SSM) may exclude the presence of large esophageal varices with high sensitivity[88] and can be adopted in the risk stratification and variceal screening strategy.[89]

Survival

Survival for chronic liver disease can be predicted using non-invasive test. LSM[90,91] or FibroTest® has a high prognostic value for patients with chronic viral hepatitis.[92,93] The usage of LSM and Hui index for predicting hepatic-event free survival in CHB patients is shown to be accurate.[81] FibroMeter® is shown to be useful for assessment of liver prognosis in CHC patients with milder disease.[94] ELF score can be used

to assess transplant-free survival of the patients with primary sclerosing cholangitis,[95] HCV/HIV co-infected women[96] and the prognosis if patients with different chronic liver diseases.[70]

HCC

There is good evidence show the strong predictive and even diagnostic role of the non-invasive tests for HCC. ARFI is used for differentiating benign and malignant liver tumors by the assessment of virtual touch tissue imaging (VTI) and virtual touch tissue quantification (VTQ), as VTI appears to be stiffer and VTQ is higher in malignant lesion than its benign counterpart.[97] For MRE, the measurement of loss modulus in liver tumor can help differentiating the benign lesions from the malignant ones, with the former having a lower value.[98] Non-invasive test is also an important part of some HCC risk score. For example, LSM-HCC score, which is optimized from CU-HCC score with LSM, further increases the negative predictive value to close to 100% for HCC prediction in 3 to 5 years in CHB patients.[99] Both FibroTest and LSM results can help predict the occurrence of HCC in patients with viral hepatitis.[100] Patients with ELF higher than 10.4 is known to have higher risk of liver-related events, in which HCC is at the top of the list.[101] Non-invasive tests can also play some part in prognosis of HCC. For example, in HCC patients receiving partial hepatectomy or transarterial chemoembolization, LSM and APRI is an independent prognostic factor.[90,91,102]

TREATMENT OF LIVER FIBROSIS

Treatment for underlying diseases

With very potent antiviral agents, patients with chronic viral hepatitis often have liver fibrosis and even cirrhosis regressed after sustained viral suppression or viral clearance.[103,104]

CHB

There is ample evidence to support the fact that effective antiviral treatment reverses liver fibrosis in majority of CHB patients.[104,105] Cumulative entecavir therapy for 3 to 7 years regressed liver fibrosis in 88% of 57 CHB patients, including all 10 patients with advanced fibrosis or cirrhosis.[105] This observation was further confirmed by a larger cohort of 348 patients who tenofovir disoproxil fumarate, in which 176 (51%) had regression of fibrosis at week 240.[104] More importantly, most (71%) patients with cirrhosis at baseline had regression of cirrhosis. Data from the same trial revealed that body mass index at baseline was the single negative predictor of liver fibrosis regression.[106]

Importance of metabolic factors on liver fibrosis regression was also supported by data from Chinese and Korean cohorts established that metabolic syndrome is a risk factor of advanced liver fibrosis and cirrhosis independent of viral factors in CHB.[107,108] New-onset metabolic syndrome and some of its components (namely central obesity and low high-density lipoprotein cholesterol) were found associated with liver fibrosis progression, independent of change in viral load and ALT level.[109] Therefore controlling metabolic factors in CHB patients already have good viral suppression with antiviral treatment would be important, not only to enhance liver fibrosis regression and hepatic events, but also to minimize cardiovascular death.[110]

Indirect evidence of antiviral treatment reversing liver fibrosis also came from two studies using serial LSM results to assess the change in liver fibrosis in large cohorts of asymptomatic CHB patients revealed low incidence rate of liver fibrosis progression, defined as an increase in LSM by 30% or more.[111,112] It was because patients who had active disease, as evidenced by raised ALT and high HBV DNA, had been started on antiviral treatment.

CHC

Data from last century illustrated the conventional interferon regresses liver fibrosis in CHC patients with sustained virologic response (SVR).[113] Similar findings have been reported in sustained responders to pegylated interferon.[114,115] Regression of liver fibrosis, which occurred in 82% of patients, was sustained at 5 years after SVR; more impressively recovery of normal or nearly normal liver architecture is possible.[103]

Now it is the era of direct-acting antiviral (DAA) agents in treating CHC patients, which leads to an SVR close to 100%.[116] Studies evaluating liver fibrosis regression in DAA-treated CHC patients often adopted non-invasive assessments like transient elastography. A small study of 54 DAA-treated patients with baseline cirrhosis revealed more pronounced reduction in LSM happened between baseline to end-of-treatment visit, but less obvious in the post-treatment period. Hence the authors concluded that decreased LSM was likely accounted by the reduced necroinflammation and probably to a less extent to regression of cirrhosis.[117] Another study of larger sample size already made use of serum makers on top of LSM revealed that FIB-4 and APRI improved to the same extent of LSM after SVR.[118] Yet whether this indicated a true regression of fibrosis or merely resolution of chronic liver inflammation remained to be determined.[118]

NAFLD

Similar to chronic viral hepatitis, controlling underlying

metabolic risk factors is central in the management to improved liver fibrosis in NAFLD patients. A weight reduction of 10% or more by aggressive lifestyle modification appears to resolve fibrosis in most if not all cases (at least with mild-moderate fibrosis).[119,120] Thiazolidinediones [peroxisome proliferator-activated receptor (PPAR)-γ agonists] such as pioglitazone and rosiglitazone are insulin sensitizers and were found to be effective to reduce fibrosis in two meta-analyses;[121,122] but the finding was not confirmed when more recent and bigger studies were included in the analysis.[123] The largest study of pentoxiphylline and also a recent study of obeticholic acid both showed a significant reduction of fibrosis,[124] the magnitude was not pathologically significant (far less than one fibrosis stage by the non-alcoholic steatohepatitis (NASH) Clinical Research Network system.[125]

In terms of pharmacological agents, there has been much interest in anti-fibrotic therapy in NAFLD as fibrosis is one of the strongest prognostic markers for NAFLD. Lysyl-oxidase like 2 (LOXL2) is involved in a relatively late step in hepatic fibrogenesis, the crosslinking of extracellular matrix proteins such as collagen and elastin.[126] Simtuzumab, a humanized monoclonal anti-LOXL2 antibody was once evaluated in Phase 2 trials in nonalcoholic steatohepatitis (NASH) patients with significant fibrosis and cirrhosis.[127] Nonetheless, the pharmaceutical company developed this agent announced it discontinued testing of simtuzumab, as it failed to show efficacy in Phase 2 trials of NASH as well as primary sclerosing cholangitis.[128] More recent data also support that the hepatic expression of the apoptosis signal-regulating kinase 1 (ASK1) marker, phosphorylated-P38 (p-P38), correlates with fibrosis stage in patients with NAFLD.[129] Therefore, selonsertib, an oral molecule that inhibits ASK1, together with simtuzumab, was found to be effective to regress liver fibrosis in NASH patients with stage 2 or 3 fibrosis. Selonsertib alone is currently evaluated in NASH patients with advanced fibrosis and cirrhosis (Clinicaltrials.gov Identifier NCT03053050 and NCT03053063) [Table 3].

Cenicriviroc is a C-C chemokine receptor type 2 and type 5 (CCR2/CCR5) antagonist, which interrupts the inflammatory cascade in NASH that leads to fibrogenesis. In animal models, the drug has been shown to have anti-inflammatory and anti-fibrotic activity.[130,131] In an ongoing two-year Phase 2b trial with cenicriviroc, it significantly improved liver fibrosis for at least one stage at 48 weeks when compared to placebo (20% *vs.* 10%; $P = 0.023$).[132] Galectins are cell surface glycoproteins that can mediate cell migration, matrix interaction and inflammatory signals. GR-MD-02 and GM-CT-01, two galectin inhibitors, bind to terminal galactose residues in glycoprotein and reduce fibrosis in animal NASH.[133] GR-MD-02 has favorable

Table 3: Active clinical trials in the clinical trials.gov on anti-fibrotic treatments

Clinicaltrials.gov	Drug	Phase	Disease	Target sample size	Status
NCT01965418	Fufang Biejia Ruangan	4	Chronic hepatitis B	100	Recruiting
NCT02241616	Entecavir + Fuzheng Huayu + TCM Granule	4	Chronic hepatitis B	350	Recruiting
NCT00956098	Oltipraz	2	Chronic hepatitis B or C	81	Completed
NCT02138253 (POLT-HCV-SVR)	IDN-6556	2	Chronic hepatitis C	60	Ongoing, finished recruitment
NCT02744105	Dietary Supplement: Spirulina	N/A	Chronic hepatitis C (in beta-thalassemia)	60	Ongoing, finished recruitment
NCT02217475	Cenicriviroc	2	NASH fibrosis	200	Ongoing, finished recruitment
NCT03059446	Cenicriviroc	2	NASH fibrosis	200	Recruiting by invitation
NCT03028740 (AURORA)	Cenicriviroc	3	NASH fibrosis	2000	Recruiting
NCT02530138	Synbiotic	2/3	NASH fibrosis	42	Recruiting
NCT02686762	Emricasan	2	NASH fibrosis	330	Recruiting
NCT02704403 (RESOLVE-IT)	Elafibranor	3	NASH fibrosis	2000	Recruiting
NCT02548351 (REGENERATE)	Obeticholic Acid	3	NASH fibrosis	2000	Recruiting
NCT03053050 (STELLAR 3)	Selonsertib	3	NASH advanced fibrosis	800	Recruiting
NCT03053063 (STELLAR 4)	Selonsertib	3	NASH cirrhosis	800	Recruiting
NCT01899859	GR-MD-02	1	NASH cirrhosis	31	Completed
NCT02462967	GR-MD-02	2	NASH cirrhosis	156	Ongoing, finished recruitment
NCT02806011	Livercellgram	2	Alcoholic cirrhosis	50	Recruiting by invitation
NCT01452308	Simtuzumab	2a	Any	20	Completed

NASH: non-alcoholic steatohepatitis

safety profile in a phase I study in NASH patients with advanced fibrosis and is now under investigation in patients with NASH cirrhosis (ClinicalTrials.gov Identifier NCT01899859 and NCT02462967; Table 3). The pharmaceutical company is going to present the data from this Phase 2 clinical trial by early December 2017.[134]

Other liver diseases

Ursodeoxycholic acid (UDCA) was found to reduced serum ALT, GGT and PIIIP in an early study.[135] Candesartan, an angiotensin receptor blocking agent, together with UDCA, when compared to UDCA alone for 6 months, induced more significant improvement of fibrosis in histological and quantitative measurements in patient with compensated alcoholic liver disease.[136] UDCA combined with budesonide, but not UCDA alone, led to fibrosis regression in patients with primary biliary cholangitis (PBC, previously known as primary biliary cirrhosis). Obeticholic acid (OCA) is a semi-synthetic 6-ethyl analogue of the endogenous bile acid chenodeoxycholic acid (CDCA) that is 100 times more potent than CDCA as a Farnesoid X receptor (FXR) activator. OCA has been shown to have anticholestatic, anti-inflammatory and antifibrotic effects.[137] OCA is found to be effective to improve liver biochemistries in a Phase 3 trial.[138]

Specific anti-fibrotic treatment targets

Direct downregulation of hepatic stellate cell

Hepatic stellate cells (HSC) are the main collagen-producing cells in the liver and their activation promotes liver fibrosis. Targeting HSC is a popular strategy for treating liver fibrosis.[139] Liver fibrosis can be reversed via a few mechanisms, which include inhibition of HSC activation; promotion of HSC phenotypic conversion; immune clearance of HSC; promotion of HSC apoptosis; induction HSC senescence.[140] Several drugs have been tested to down-regulating HSC activation, which include a few antioxidants (e.g. namely vitamin E, phosphatidylcholine, silymarin, resveratrol), gamma interferon, peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists (e.g. pioglitazone), endothelin receptor antagonists, histone deacetylase (HDAC) inhibitors *etc*.[139] Yet none of these agents has been approved as anti-fibrotic agents.

Several novel targets have been identified for the treatment of liver fibrosis through suppression of HSC activation. Interleukin (IL)-30 attenuates hepatic fibrosis by inducing natural killer group 2D (NKG2D)/ribonucleic acid export 1 crosstalk between activated HSCs and natural killer T cells and is therefore an ideal therapy for liver fibrosis. Hydrogen peroxide-inducible clone-5 (Hic-5), a transforming growth factor (TGF)-β1-inducible focal adhesion protein, facilitates cell proliferation, ECM expansion and vascular restoration and restructuring.[141] Hic-5 expression also plays a critical role in attenuating fibrosis by enhancing TGF-β1-induced small mother against decapentaplegic (Smad)2 phosphorylation via the downregulation of Smad7 in both human and mouse activated HSCs.[142]

Although several drugs show potent anti-fibrotic activities in experimental models of hepatic fibrosis, there is presently no effective pharmaceutical intervention specifically approved for the treatment of liver fibrosis. Targeted delivery systems that bind specifically to receptors solely expressed on activated HSCs or trans-differentiated MFBs are essential to increase treatment efficacy as well as to reduce adverse effects. The applicability and efficacy of sequestering molecules, selective protein carriers, lipid-based drug vehicles, viral vectors, transcriptional targeting approaches, therapeutic liver- and HSC-specific nanoparticles, and miRNA-based strategies are potential and promising treatment strategies.[143]

Collagen synthesis inhibitors

Continuous accumulation of extracellular matrix (ECM) extremely rich in collagen I and III in response to liver injury leads to scar deposition and liver fibrosis.[144] Activated HSCs are indeed a major source of collagen in the liver and can abundantly secrete ECM proteins, tissue inhibitors of metalloproteinases, and matrix metalloproteinases (MMPs) that elicit liver architecture remodeling.[145] Apart from modulating HSC, there are some therapeutic agents directly targeting collagen synthesis.

Halofuginone is an analog of febrifugine - an alkaloid originally isolated from the plant Dichroa febrifuga.[146] Animal model with established liver fibrosis halofuginone elicited reductions in the levels of collagen, collagen αI gene expression, and α-smooth-muscle-positive cells, and even complete resolution of liver fibrosis.[147] Regeneration of the liver, which was blocked in rats with established fibrosis, occurred at an almost normal rate in halofuginone-treated rats.[148] Nonetheless, there has not been a clinical study specifically that use halofuginone to treat liver fibrosis in human.

TGF-β antagonists

TGF-β1 is the key pro-fibrogenic cytokine involved in liver fibrosis, as it regulates the production and deposition of ECM.[149,150] There are several approaches to interfere with TGF-β signaling. TGF-β expression can be down-regulated by applying anti-sense oligonucleotide mRNA. A targeted blocking of a specific

isoform of TGF-β by means of monoclonal antibodies is also feasible. Activation of TGF-β receptors can be inhibited by the use of specific inhibitors, thereby halting downstream signaling. Local activation of TGF-β induced by αvβ6 integrin and by tropomyosin-related kinase (TSP)-1 can be prevented.[151] The amino acid sequence Leu-Ser-Lys-Leu (LSKL) naturally occurs in the region of the amino terminus of the LAP and that it can hamper the activation of latent TGF-β by TSP-1 through competitive inhibition.[152] LSKL peptides significantly decrease DMN-induced liver atrophy and fibrosis in an animal model.[153] Yet LSKL has not been developed clinically. More recently nanoconjugate siRNA against TGF-β1 equipped with an N-acetylglucosamin targeting moiety intending to reach HSCs via desmin was reported to colocalize with HSCs and to reduce liver fibrosis.[154]

Connective tissue growth factor inhibitor

CTGF is a mediator of ECM accumulation and coordinates a late common pathway to fibrosis.[155] Blocking connective tissue growth factor (CTGF) activity reduces liver fibrosis and preserves liver function.[156] FG-3019 is a recombinant human anti-CTGF monoclonal immunoglobulin G antibody. FG-3019 reduces collagen deposition in nonclinical models of liver. FG-3019 was tested in CHB patients in a Phase 2 randomized trial; unfortunately the study terminated due to an unexpected prominent effect of entecavir alone in this patient population.[157]

CONCLUSION

With the wide applicability of non-invasive assessments of liver fibrosis, the management of 2 billion patients with chronic liver diseases worldwide has been revolutionized. While liver biopsy examination still has an important role in the diagnostic process, non-invasive assessments including transient elastography and serum biomarkers have high accurate to diagnose advanced fibrosis and cirrhosis. Transient elastography and serum biomarkers can be used alone or in combination, either simultaneously or in a stepwise approach. Meanwhile, ARFI and SWE are effective for staging liver fibrosis, especially when ultrasound is the first imaging tool for assessment of diffuse liver disease. Treating underlying chronic liver diseases is still the cornerstone of liver fibrosis regression. Potent antiviral treatments for chronic viral hepatitis lead to regression of liver fibrosis and even cirrhosis in majority of patients. Numerous ongoing clinical trials in NAFLD patients will bring us treatment to treat NASH fibrosis and cirrhosis soon. Plentiful therapeutic agents specifically targeting the fibrogenesis pathways, in particulars HSC and TGF-β1 work well in animal models. We look forward to assess these agents in human and hopefully they can modify the natural history of chronic liver diseases, and more importantly, to improve patient outcome in the near future.

DECLARATIONS

Authors' contributions

Drafting of the manuscript: J.Y.K. Cheng, G.L.H. Wong
Critical revision of the manuscript for important intellectual content: J.Y.K. Cheng, G.L.H. Wong

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None.

Conflicts of interest

Grace L.H. Wong has served as an advisory committee member for Gilead Sciences. She has also served as a speaker for Abbott, Abbvie, Bristol-Myers Squibb, Echosens, Furui, Gilead Sciences, Janssen and Roche.

Patient consent

Not applicable.

Ethics approval

Not applicable.

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