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REVIEW

Hepatic steatosis and fibrosis: Non-invasive assessment

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Abstract

Chronic liver disease is a major cause of morbidity and mortality worldwide and usually develops over many years, as a result of chronic inflammation and scarring, resulting in end-stage liver disease and its complications. The progression of disease is characterised by ongoing inflammation and consequent fibrosis, although hepatic steatosis is increasingly being recognised as an important pathological feature of disease, rather than being simply an innocent bystander. However, the current gold standard method of quantifying and staging liver disease, histological analysis by liver biopsy, has several limitations and can have associated morbidity and even mortality. Therefore, there is a clear need for safe and non-invasive assessment modalities to determine hepatic steatosis, inflammation and fibrosis. This review covers key mechanisms and the importance of fibrosis and steatosis in the progression of liver disease. We address non-invasive imaging and blood biomarker assessments that can be used as an alternative to information gained on liver biopsy.

Key words: Hepatic steatosis; Fibrosis; Non-invasive assessment; Blood biomarker; Ultrasound

Core tip: Ongoing hepatic fibrosis and steatosis are well recognised features of chronic liver disease. Liver biopsy is currently the gold standard for assessing the disease although this has an associated but low morbidity and mortality risk. Therefore, alternative methods of non-

invasive assessment of liver disease are of relevance and importance. We outline the mechanisms of hepatic fibrosis and steatosis and review uses of non-invasive imaging and blood biomarkers as an alternative to liver biopsy.

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CLINICAL PROBLEM

Chronic liver disease is a major cause of morbidity and mortality worldwide. The presence of chronic inflammation and consequent fibrosis leads to the development of cirrhosis and its complications. Whilst the exact prevalence of chronic liver disease is unknown, cirrhosis of the liver was attributed for more than one million deaths worldwide in 2010, although these figures probably reflect heavy under-reporting[1]. The total worldwide prevalence of cirrhosis has been estimated at around 1% with significant regional variation owing to the presence of viral hepatitis, the metabolic syndrome and alcohol consumption[2].

Chronic liver disease has a varied aetiology, including viruses, such as hepatitis B (HBV) and hepatitis C (HCV). Worldwide, over half a billion people may be chronically infected with either of these viruses[3,4]. Metabolic causes include the increasing prevalence of non-alcoholic fatty liver disease (NAFLD). Toxic causes, such as excess alcohol consumption, aflatoxin exposure[5,6] and autoimmune disorders, such as primary biliary cirrhosis and autoimmune hepatitis, contribute to the disease burden. Over half of the deaths attributable to cirrhosis and nearly 80% of those attributable to primary liver cancers occur in those who have chronic HBV and HCV infection[7], while in many developed nations excess alcohol consumption is the commonest cause. In the developing world, aflatoxin exposure further complicates the picture, leading to high rates of hepatocellular carcinoma (HCC).

Chronic liver disease usually takes many years to progress from inflammation, associated with hepatocyte injury, to fibrosis and mostly requires long-term exposure to the causative agent. Progressive scarring or fibrosis develops during the period of time between initiation and end-stage disease. The resulting pre-cirrhotic fibrosis is a target for therapies aimed at reducing the rate of progression to cirrhosis, or even reversal of fibrosis[8].

Effective antiviral therapies and the advent of antifibrotic drugs have led to increasing demand for non-invasive, accurate and reliable biomarkers of hepatic disease severity. It is well recognised that the current "gold standard", histological analysis of liver biopsy, has limitations and engenders risk to the patient. Sampling variability and the subjective interpretation of scoring systems means that the consistency and representation of the true disease state is questionable. The procedure frequently causes discomfort and if the patient has a malignancy, there is a risk of tumour seeding[9]. Furthermore, there is frequent associated morbidity and a small, but significant, mortality rate[10] in all but a few cases. Liver biopsy is rarely performed in lower income countries, often due to a lack of expertise to interpret results[11]. On the other hand, in the United States and the developed world, magnetic resonance techniques allow a quantitative assessment of different aspects of disease from metabolic markers of inflammation, through to assessment of fibrotic load, markers of portal hypertension and prognostic indicators of the complications of cirrhosis[12]. There is a clear need for reliable and effective non-invasive markers of hepatic inflammation and fibrosis, both for the management of individual patients and for the development of new anti-fibrotic therapies. Novel imaging modalities and non-invasive biomarkers have the potential to fulfil this role and offer significant benefit in treatment monitoring and have the potential also to be of use in resource-constrained settings.

NATURAL HISTORY OF CHRONIC LIVER DISEASE

Chronic liver injury leads to initiation and perpetuation of inflammatory processes, which, by a cascade of inter-related processes and pathways, leads to deposition of fibrous tissue (Figure 1). By convention, fibrosis has been considered potentially reversible, while the end-stage of the pathological process, cirrhosis, has been considered irreversible. However, with elimination of the cause of liver injury, a number of studies have demonstrated regression of all stages of fibrosis in animal models and in humans[13-17]. Elucidation of the process of fibrogenesis enables markers of disease severity and potential targets for therapeutic intervention to be developed.

KEY MECHANISMS OF FIBROGENESIS

Fibrosis is a dynamic process of hepatic homeostasis mediated by several cellular mediators in response to an inflammatory process. In particular, hepatic stellate cells (HSC) have a central role in the pathogenesis of liver fibrosis[8,18]. These cells comprise 15% of liver cell mass[19]. HSCs are activated following liver injury from a relatively quiescent lipid and vitamin A-storing phenotype to a myofibroblastic phenotype, capable of proliferation, contraction and fibrogenesis. However,

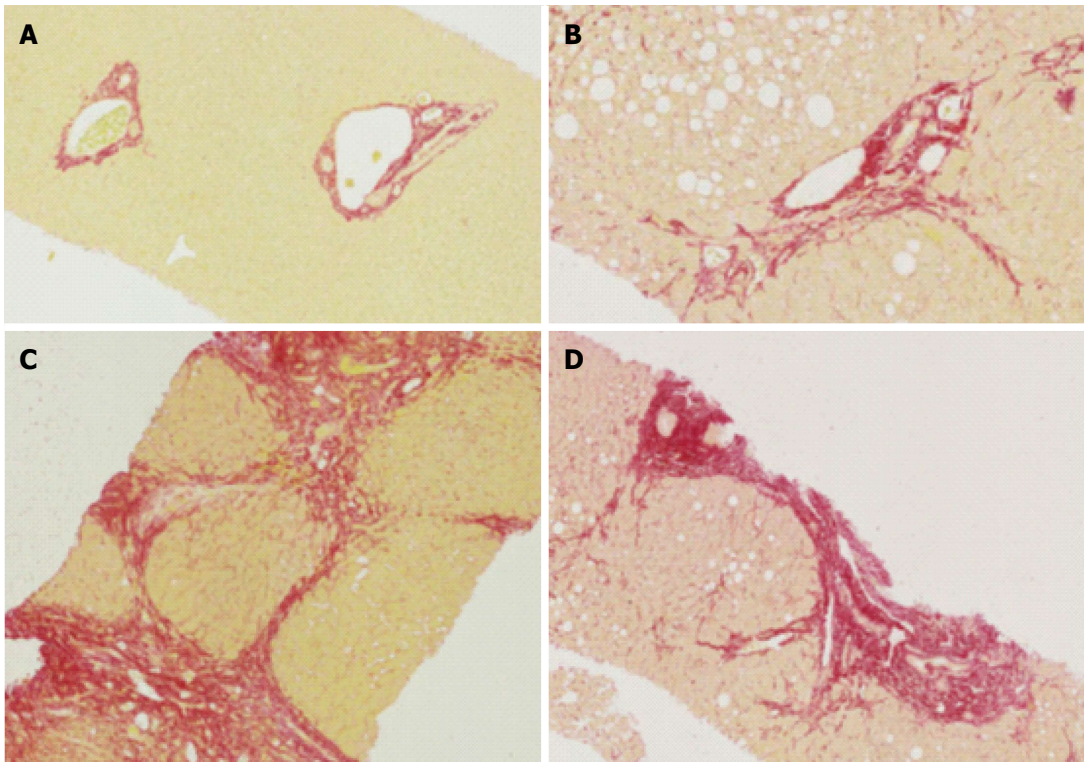

Figure 1 Histology of normal liver, fibrosis and cirrhosis. A: Representative histological images (using Sirius red staining), normal liver; B: Mild to moderate fibrosis with portal tract expansion (METAVIR F = 2, Ishak stage 3); C: Moderate "bridging" fibrosis (METAVIR F = 3, Ishak stage 4); D: Cirrhosis (METAVIR F = 4, Ishak 5 or 6).

other myofibroblastic cell populations have also been shown to be involved in fibrogenesis, including portal fibroblasts[20,21] .

HSC activation occurs in two stages: initiation and perpetuation and each involves characteristic pathways, as described by Friedman[18]. Initiating events may consist of any chronic perturbation of hepatic homeostasis, which is often, but not always associated with the presence of inflammatory cells on liver biopsy. Such perturbations of hepatic homeostasis may lead to the net over-production of unstable reactive oxygen species (ROS) derived from hepatocytes, macrophages, stellate cells and inflammatory cells. ROS are potent mediators of the initiation and perpetuation of liver injury and cause lipid peroxidation of cellular membranes. Perpetuation of HSC activation is comprised of a number of cellular responses, which lead to increased expression and responsiveness to growth factors. Perpetuation is a dynamic process, occurring along a number of pathways associated with different HSC responses. These responses include release of largely proinflammatory cytokines, proliferation and increased contractility of HSCs, chemotaxis of inflammatory cells and the net deposition of pathological ECM[18].

Resolution of fibrosis

For resolution of fibrosis to occur, removal of the injurious agent is a prerequisite. Activation of HSCs has been shown to be crucial for the development of fibrosis, while in recovery and resolution of fibrosis, the number of activated HSCs is reduced. The latter may occur as a result of reversion to the quiescent form or by apoptosis. A return to the quiescent form has been observed *in vitro*[22], but not *in vivo*. Apoptosis has been postulated as the predominant mechanism for removal of HSC activity. It has been shown that spontaneous apoptosis of HSCs occurs *in vitro*. Conversely, it has also been shown that MMP-2 levels correlate with apoptosis and may be stimulated by apoptosis[23] and that TIMP-1, by inhibition of MMP, leads to inhibition of activated HSC apoptosis[24].

HEPATIC STEATOSIS

Liver fibrosis is the key pathological feature of progressive liver disease. However, the accumulation of excessive hepatic triglyceride, hepatic steatosis, is increasingly recognised as an important factor in the pathogenesis of a number of chronic liver diseases, not simply an "innocent bystander".

Definitions

Hepatic steatosis is a pathological lesion, defined as the presence of large and small vesicles of fat, predominantly triglycerides, accumulating within hepatocytes[25]. Hepatic steatosis is frequently associated with obesity, insulin resistance and dyslipidaemia

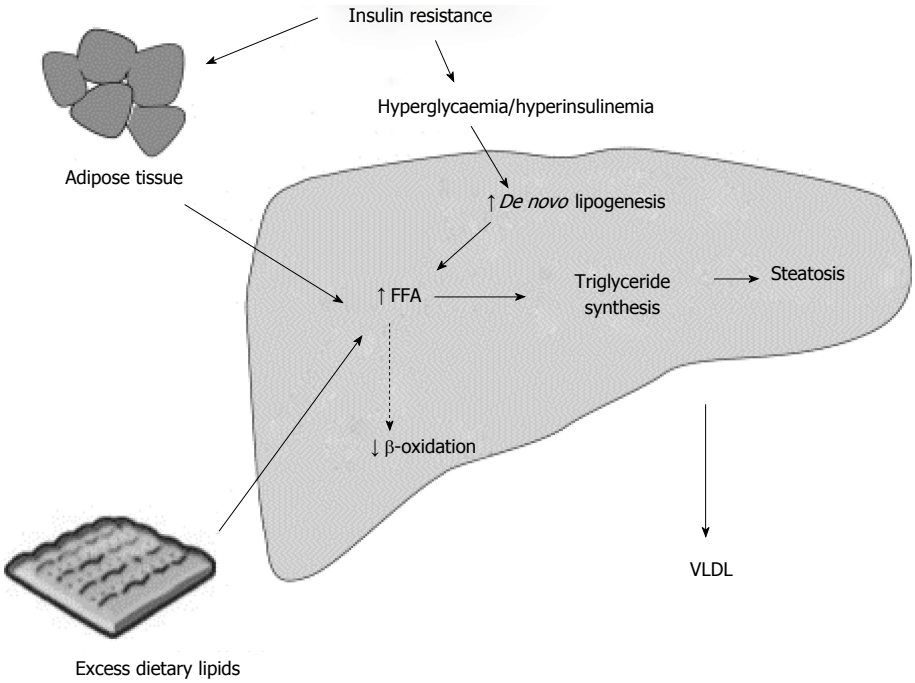

Figure 2 Summary of metabolic mechanisms leading to hepatic steatosis. Reproduced from Dowman *et al*[155] with permission from Oxford University Press.

in those who do not consume excessive quantities of alcoholic drinks, where it is termed non-alcoholic fatty liver disease[26] (NAFLD). Hepatic steatosis may also be a result of secondary causes, which include alcoholism, HCV, severe weight loss, total parenteral nutrition and drugs (such as amiodarone, diltiazem, tamoxifen, steroids and highly active antiretroviral therapy)[27]. NAFLD may thus be considered a syndrome of various aetiologies, excluding alcohol and, by convention, HCV.

Epidemiology

In a systematic review on the epidemiology of NAFLD, Vernon and colleagues reported the prevalence of NAFLD in the United States ranges from 10%-35% in published studies, depending on the investigative assessment modality used and the study population[1]. Worldwide, NAFLD prevalence ranges from 6%-35% with a median of 20%[1].

Key issues in pathophysiology

The development of hepatic steatosis occurs when the rate of synthesis or import of fatty acids by hepatocytes exceeds the rate of export or catabolism. Such an imbalance can occur in a number of ways and is summarised in Figure 2 with increased uptake of fatty acids by hepatocytes in obesity, increased hepatic fatty acid triglyceride synthesis, impaired fatty acid mitochondrial β-oxidation and reduced VLDL and triglyceride synthesis all being components to consider to a greater or lesser extent[28]. Importantly, with respect to hepatic triglyceride export, HCV core protein has been shown to inhibit this process, providing a direct route to hepatic steatosis in HCV infection[29,30].

CURRENT APPROACHES TO THE ASSESSMENT OF CHRONIC LIVER DISEASE

The evaluation of patients with chronic liver disease is largely based on assessment of the clinical history, physical examination and measurement of non-specific liver enzymes. Most pre-cirrhotic chronic liver disease is asymptomatic, where clinical signs are subtle and non-specific. Clinical chemistry, so-called "liver function tests" (aminotransferases, alkaline phosphatase, bilirubin and gamma-glutamyl transferase) and haematological indices (cell-counts and the pro-thrombin time) may be abnormal in cirrhotic and pre-cirrhotic disease, but alone, their sensitivity and specificity for the diagnosis and staging of chronic liver disease is limited[2].

Liver biopsy - the "gold standard" for disease assessment

Histological assessment of liver biopsy is the mainstay for the diagnosis and staging of chronic liver disease. Epidemiological and pathophysiological data support fibrosis being the hallmark of chronic liver disease and a predictor of outcome[31]. Most liver biopsies for the assessment of chronic liver disease are performed percutaneously, by passing the biopsy needle between the ribs into the right lobe of the liver. Guidelines for safe and effective liver biopsy have been published by the British Society of Gastroenterology[32].

Validity of histological scoring systems

Fibrosis and inflammation: The interpretation of the histology of liver tissue in chronic liver diseases is based on categorisation or scoring of inflammatory features (grade) and fibrosis and architectural disruption (stage)[33]. The first major scoring system was developed in 1981, and based on the identification of periportal necrosis, intralobular necrosis, portal inflammation and fibrosis, assessed separately and assigned a score[34], although it was later modified to include additional components[35]. The first three components of the score contribute to the necroinflammatory grade, while the fibrosis score, from 0-6, is based on architectural changes in the pattern and expansion of fibrous bands. From a practical perspective, cirrhosis is represented by both stage 5 (incomplete cirrhosis) and 6 (probable or definite cirrhosis).

The METAVIR scoring system was developed to look specifically at HCV-related liver disease. The histological activity is based on piecemeal and lobular necrosis, while the fibrosis stage is scored from 0-4, with 4 representing cirrhosis[36]. All scoring systems are designed to place a numeric value to architectural features. They consist of ordered categorical data representing qualitative and semi-quantitative descriptions, so are not quantitative measures of fibrosis. Studies of liver tissue using digital image analysis have shown that the area of fibrous tissue is not linearly related to the fibrosis score. Moreover, inflammation may cause expansion of fibrous tracts[33]. Nevertheless, histological fibrosis assessment has been validated by successive clinical studies, making the features clinically relevant[31].

Steatosis: Scoring systems quantify fat on the basis of visible hepatic lipid droplets within hepatocytes. The grade of steatosis is based on the proportion of hepatocytes containing visible lipid and is expressed semi-quantitatively on a scale of 0-3 (0, < 5%; 1, 5%-33%; 2, > 33%-66%; 3, > 66%)[37,38]. Grading is considered in the context of other histopathological lesions, such as inflammatory infiltration and ballooning of hepatocytes. However, there are considerable limitations to liver biopsy for the quantification of hepatic lipid. Quantification of lipid is based only on visible lipid droplets, so invisible (for example, membrane) lipid is not included. The assessment is only semi-quantitative, being a two-dimensional estimation of the proportion of hepatocytes including lipid, and not considering the volume of droplets. Finally, the composition of the fatty acid components is not assessed, although, using immunohistochemical techniques, the presence of lipid peroxidation products may be determined[39].

Limitations of liver biopsy

Morbidity and mortality: Percutaneous liver biopsy has a small, but quantifiable, risk of mortality, quoted as between 1 in 1000 and 1 in 10000 patients[10,40,41]. Minor complications, such as post-procedural pain or localised haematoma, occur in between 3% and 30% of cases. More severe complications, including intraperitoneal haemorrhage (requiring transfusion) or perforation of a viscus (including pneumothorax) may occur in 0.3% to 0.6% of cases[32,42]. Tumour seeding following biopsy of suspected carcinomas in cirrhosis may also occur in 2.7% of cases[43]. Good management of clotting disorders and the appropriate use of the transjugular approach for liver biopsy may improve outcome[32].

Sampling variability: Percutaneous liver biopsy typically samples less than 1/50000th of the liver, so any heterogeneity of pathological features may lead to sampling variability[44]. Autopsy studies have demonstrated that cirrhosis may be missed on a single pass liver biopsy in between 10% and 30% of cases[45,46], while a study using laparoscopic biopsy of both left and right lobes of the liver found a difference of at least one fibrosis stage between lobes in over 30% of patients[47]. The size of the liver biopsy specimen influences sampling variability. Smaller biopsy sizes (in length and breadth) were shown to lead to a lower probability of observing characteristics of more severe diseases and, consequently, led to underestimation of disease severity[48].

Subjectivity and inter-observer variation: Histological scoring systems are designed to be objective and reproducible, but interpretation is still a source of error. Inter-observer variability is low for the assessment of fibrosis, but higher for the assessment of activity or inflammation[49]. In a study of intra- and inter-observer variability, agreement was better for fibrosis than inflammation and also amongst experienced pathologists than more junior pathologists[50]. These authors concluded that the experience of the pathologist had more influence on agreement than the characteristics of the biopsy itself. Histology of liver biopsy specimens is still the mainstay for the definitive diagnosis of liver diseases and for investigation of co-existing pathology. Nevertheless, there are risks inherent in the technique and scoring systems should be interpreted in the knowledge of the limitations.

NON-INVASIVE TECHNIQUES

Non-invasive assessment techniques are suited to longitudinal studies as the risk to patients is more limited than liver biopsy. However, the measured parameters may be more susceptible to influence by confounding factors and so specificity is important to consider. Non-invasive tests of chronic liver disease may be broadly divided into serum (or blood) markers and imaging-based technologies.

Serum markers

Serum markers have been studied in detail to detect early fibrotic changes as blood tests are quick and acceptable to patients. While the results are objective, there is the possibility of the presence of confounding factors from extrahepatic disease. These have been reviewed extensively[51-53]. Serum markers may broadly be divided into "indirect" and "direct" markers, single tests and panels. Indirect markers are markers of liver function, which reflect liver fibrosis, while direct markers include serum extracellular matrix components and intermediates of fibrogenesis[54]. The strengths and limitations of the most widely used models are summarized below.

APRI

APRI (AST to Platelet Ratio Index) was proposed as an alternative to biopsy in patients with chronic HCV infection[55] and it is calculated as (AST/upper limit of normal range)/platelet count (10^9/L) × 100. A recent meta-analysis by Lin and colleagues showed APRI had AUROC scores for the diagnosis of significant fibrosis, severe fibrosis, and cirrhosis of 0.77, 0.80, and 0.83 respectively, demonstrating a potential use for identifying HCV-related fibrosis[56]. However, APRI fails to identify a significant proportion of people in the earlier stages of fibrosis and therefore is limited in its ability to identify only significant and untreated chronic HCV-related fibrosis[55,57].

Enhanced liver fibrosis score

The enhanced liver fibrosis score (ELF®) score (Siemens, Munich, Germany) combines three direct markers of fibrosis including hyaluronic acid (a component of the extracellular matrix), TIMP-1 (an inhibitor of matrix metalloproteinases, which break down collagen) and PIINP (a marker of collagen synthesis at disease site). Therefore, the premise of this scoring system is that a higher score will indicate a higher rate of fibrogenesis. ELF® has been shown to have good performance for the detection of significant fibrosis in chronic HCV (93% sensitivity and 83% specificity)[58] but also in NAFLD (sensitivity 89% and specificity 96%) and ALD (100% sensitivity and 16.7% specificity)[59], although results for the latter two have been less rigorously evaluated. Results also need to be adjusted appropriately as scores can be influenced by gender, age, and sex[60].

FibroTest®

FibroTest® (BioPredictive, Paris, France) uses five different serum markers in its model and has been validated in meta-analysis in multiple aetiologies including NAFLD (AUROC 0.84; 95%CI: 0.76-0.92), alcohol-related liver disease (AUROC 0.86; 95%CI: 0.80-0.92) and both chronic HBV infection (AUROC 0.80; 95%CI: 0.77-0.84) and HCV infection (AUROC 0.85; 95%CI: 0.82-0.87)[61]. However, results are limited by false-positive results, attributed to increases in bilirubin or decreases in haptoglobin; in particular HCV patients on ribavirin. Results can also be affected by acute inflammation, Gilbert's syndrome and cholestasis[52].

FIB-4 index: The FIB-4 index combines several markers of liver function into the following formula: age (years) × AST [U/L]/(platelets [10^9/L] × (ALT [U/L]). The FIB-4 index was specifically developed as an alternative to biopsy in patients with chronic HCV infection, although it has shown use in other causes of liver disease. In a study of 529 HCV-infected patients, the FIB-4 index enabled the correct identification of patients with severe fibrosis (F3-F4) and cirrhosis with an Area under Receiver Operated Curve (AUROC) of 0.85 (95%CI: 0.82-0.89) and 0.91 (95%CI: 0.86-0.93), respectively[62]. However, a lack of universal agreement amongst studies for positive and negative cut-off values has proved problematic.

Forns index: The Forns index is another formula that assesses liver function by combining age, cholesterol, gamma-glutamyltranspeptidase and platelet count. Forns and colleagues showed using a best cut-off score of < 4.2, the presence of significant fibrosis (F2-F4) could be excluded with high accuracy (negative predictive value of 96%) in 125 (36%) of 351 patients with chronic HCV infection[63]. The Forns index has also been shown to be more accurate than other serum markers including FIB-4 and aspartate APRI (AUROC 0.795, 0.764 and 0.774 respectively) in the prediction of significant fibrosis in patients with chronic HCV[64].

IMAGING-BASED MODALITIES AS ALTERNATIVES TO BIOPSY

Various imaging modalities have been proposed as alternatives to liver biopsy. Given that there is strong evidence that liver stiffness measurements (LSM) increases with the degree of fibrosis[65,66], the most successful imaging modalities have used techniques to measure liver stiffness and correlate this with the degree of fibrosis, most notably ultrasound-based transient elastography (TE) and acoustic radiation force impulse imaging (ARFI®, Siemens, Munich, Germany). Their potential use in the measurement of hepatic steatosis and differentiation of hepatic steatosis grade has also been investigated. The use of other modalities, including MR techniques, to assess morphological and biochemical changes in chronic liver disease (CLD) will also be discussed.

TE

TE was first developed as Fibroscan® (Echosens, Paris, France), where a vibrator generates low frequency shear waves through the liver which are then transmitted to an ultrasound receiver. The velocity of the waves is

dependent on the tissue elasticity and therefore, the rate of propagation through the liver can be used as a measure of liver stiffness and converted into a numerical value (kPa). Meta-analyses have shown TE has a very high sensitivity and specificity for detecting cirrhosis, but its accuracy was much reduced in the earlier stages of fibrosis[67,68]. This is partly due to the lack of validation for stiffness cut-off values in earlier stages of disease, but also possibly due to the multiple processes that contribute to liver stiffness other than fibrosis[67,69]. Fibroscan® machines now have been calibrated to measure hepatic steatosis levels by using a novel Controlled Attenuation Parameter (CAP®; Echosens, Paris, France), with results showing a sensitivity and specificity between 78% and 100% and an excellent correlation between different steatosis grades, determined by the percentage of hepatocytes with fatty infiltration (Spearman Rank $\rho = 0.81$, $P < 10^{-16}$)[70]. Its use has been validated in patients with chronic hepatitis C infection (AUROC 0.80; 95%CI: (0.75-0.84) for S $\geqslant$ 1, 0.86; 95%CI: 0.81-0.92 for S $\geqslant$ 2 and 0.88; 95%CI: 0.73-1.00 for S = 3) respectively and has shown correlation in patients withNAFLD (0.49, $P = 0.00069$) although this accuracy reduces with increasing steatosis grade in patients with NAFLD[71,72].

Acoustic radiation force impulse imaging®

Acoustic radiation force impulse imaging (ARFI)® (Siemens, Munich, Germany) is another form of ultrasound elastography that measures soft tissue displacement following the exposure of high-energy acoustic pulses. The measurement of displacement can then be quantified and interpreted as a measurement of liver stiffness. Meta-analysis by Friedrich-Rust and colleagues used AUROC to show ARFI® is effective with scores of 0.87 for discriminating significant fibrosis, 0.91 for severe fibrosis and 0.93 for cirrhosis[73]. Additionally, ARFI® has also shown comparable results with TE for detection of significant fibrosis and cirrhosis and was significantly more likely to obtain reliable measurements[74]. However, its uses for detecting earlier stages of fibrosis though remain limited.

ElastPQ®

ElastPQ® (Philips, Best, Netherlands) is a newer ultrasound method of non-invasive assessment of liver stiffness and uses two-dimensional shear wave elastography to generate an absolute measurement of liver stiffness. ElastPQ® showed comparable accuracy to ARFI® when differentiating 176 patients with and without chronic liver disease (83.7% *vs* 83.1%), although measurements of liver stiffness for ElastPQ® were significantly lower, compared to ARFI® and thus required different cut-off values[75]. Additionally, in a study of 291 patients with chronic HBV, who underwent biopsy or partial hepatectomy, ElastPQ® values showed good correlation between the stage of liver fibrosis and grade of necroinflammatory activity while being unaffected by levels of steatosis[76]. However, whilst ElastPQ® remains an exciting prospect, further comparisons with other non-invasive imaging modalities and liver biopsy are needed.

Standard ultrasound

Ultrasound is widely used clinically to detect hepatic steatosis, but it can also detect the vascular changes of chronic liver disease with contrast enhancement[77]. Ultrasound is also able to detects hepatic steatosis, based on the premise that steatosis causes increased echogenicity of the hepatic parenchyma, leading to a brighter image when compared to the cortex of the ipsilateral kidney[78]. Other conditions, such as fibrosis may also lead to increased echogenicity, resulting in a potential for confusion. A review of the non-invasive measurement of fat content found the sensitivity of ultrasound for the detection of steatosis to range from 60% to 94%, while the specificity ranged from 84% to 95%[79]. However, histologically-assessed mild steatosis resulted in a sensitivity of just 55%[80]. In addition, obesity reduces the accuracy of ultrasound due to technical considerations and increased attenuation of signal caused by subcutaneous fat. Ultrasound performs more poorly for the *quantification* of hepatic lipid, although subjective grading systems, categorizing steatosis into mild, moderate and severe groups have been proposed[81]. Dynamic microbubble contrast-enhanced studies are thought to exploit the intra- and extrahepatic vascular changes that generate shortening of hepatic vein transit times with increasing disease severity[82,83] (Figure 3).

Computed tomography

CT enables the assessment of hepatic steatosis on the basis of radiographic density[84], although the ability to quantify hepatic lipid is not clear[85]. Ionising radiation associated with CT-scanning confers a small excess risk to subjects, making it less suited for repeated measurements.

Magnetic resonance imaging

T_1 and T_2 mapping are MR techniques that can be used for *in vivo* tissue characterisation either individually or in combination. T_1 relaxation times correlate with increased levels of extracellular fluid associated with inflammation and fibrosis, whereas T_2 mapping primarily reflects the amount of iron deposition[86]. For example, T_1 mapping of the liver has used a modified Look-Locker inversion recovery sequence with motion recovery[87] and T_2 on multi-gradient-echo acquisition[88]. Since elevated iron levels interfere with T_1 measurements, correction algorithms are applied to provide more accurate readings[86]. T_1 mapping has shown good correlation with the histological degree of fibrosis in a cohort of 79 patients with chronic liver disease of multiple aetiologies with a ROC of 0.94 for any degree of fibrosis[88].

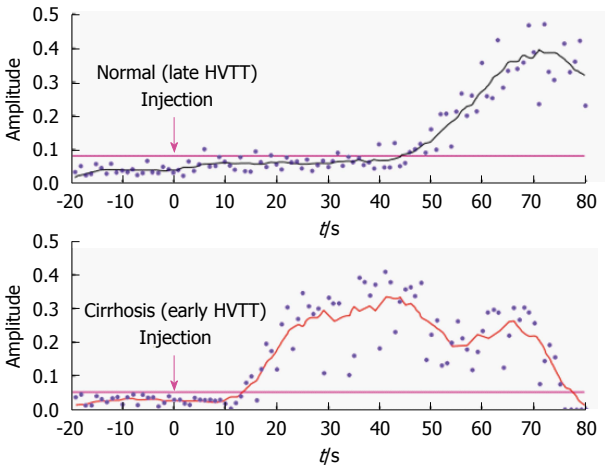

Figure 3 Hepatic vascular transit times in normal patients and patients with cirrhosis. Time intensity curves from the hepatic vein show earlier arrival of contrast in the cirrhotic liver[83].

Additionally, Pavlides and colleagues have used T_1 mapping to show a correlation with the degree of liver disease and the risk for developing clinical events in 112 patients. Optimal T_1 cut-off points were created by comparing multiparametric MR data with histological staging of fibrosis from previous studies. This was used to create a "liver inflammation and fibrosis" (LIF) staging score. Pavlides subsequently found that patients with severe liver disease (LIF > 3) were at higher risk for developing clinical events, compared to patients with LIF < 1 (P = 0.02) and LIF 1-1.99 (P = 0.03)[86]. T_1 mapping has also been shown to differentiate Child-Pugh A patients from Child-Pugh B/C patients effectively (P < 0.00001)[89]. T_1 mapping is a promising diagnostic tool that has shown to be effective in differentiating different stages of fibrosis and also has shown potential for predicting clinical events. However, further research is needed to validate effective scoring systems and the influences of other compounding factors for it to become a valid alternative to liver biopsy in clinical practice.

Magnetic resonance elastography

While ultrasound-based transient elastography techniques such as Fibroscan® measure liver stiffness in a defined region (about 5 cm^3, right lobe of the liver), Magnetic Resonance Elastography (MRE) can be used to predict fibrosis stage effectively in patients with chronic liver disease, while producing a wider and more representative map of liver stiffness in both 2D and 3D planes. This is performed using similar principles to TE, whereby propagating shear waves are generated and imaged using phase contrast MRI, which includes oscillating motion sensitising gradients (MSGs). The subsequent cyclic spin displacement of protons, which in the presence of synchronised MSGs, are encoded as phase shifts within the MRI signal[90].

MRE has been validated in multiple studies and meta-analyses[91-94]. One meta-analysis by Singh and colleagues using 12 retrospective studies, comprising 697 patients with CLD of varying aetiology, showed MRE had high diagnostic capability for detecting significant fibrosis (F ⩾ 2), advanced fibrosis (F ⩾ 3), as well as cirrhosis with ROC values of 0.88 (0.84-0.91), 0.93 (0.90-0.95), and 0.92 (0.90-0.94) respectively, as determined by liver biopsy[91]. Another meta-analysis by Su and colleagues, comprising 13 studies and 989 patients, also demonstrated good sensitivity and specificity of MRE for the staging of fibrosis. The pooled sensitivity and specificity for F ⩾ 1, F ⩾ 2, F ⩾ 3 and F = 4 were 0.87 (95%CI: 0.84-0.89) and 0.92 (95%CI: 0.87-0.96), 0.87 (95%CI: 0.84-0.90) and 0.92 (95%CI: 0.89-0.95), 0.88 (95%CI: 0.85-0.91) and 0.91 (95%CI: 0.88-0.93), 0.91 (95%CI: 0.87-0.94) and 0.92 (95%CI: 0.89-0.94), respectively. The pooled ROC values for F ⩾ 1, F ⩾ 2, F ⩾ 3 and F = 4 were 0.9502, 0.9663, 0.9644, and 0.9768, respectively[92]. MRE can also be used to stratify risk of cirrhosis progression in patients with chronic HCV infection[95] and may have greater potential than TE for assessment of NAFLD in patients with high risk of NASH or cirrhosis, due to the multiparametric nature of MRI which allows for a comprehensive assessment of the liver[96].

Recent studies have shown that three-dimensional spin-echo echoplanar imaging (3D-SE-EPI) could have better diagnostic accuracy than conventional two-dimensional gradient-recalled echo (2D-GRE). In a study of 179 patients with either chronic HBV or HCV infection, Shi and colleagues showed AUCs for the characterisation of F ⩾ 1, F ⩾ 2, F ⩾ 3, and F = 4 were 0.957 (95%CI: 0.913-0.983), 0.971 (0.932-0.991), 0.991 (0.961-0.999), and 0.979 (0.942-0.995) for 3D-SE-EPI compared with the AUCs for 2D-GRE at each fibrosis stage which were 0.948 (0.901-0.977), 0.959 (0.915-0.981), 0.979 (0.943-0.995), and 0.976 (0.938-0.994) respectively[97]. A higher diagnostic accuracy for 3D-SE-EPI compared with 2D-GRE has also been shown for patients with NAFLD advanced fibrosis[98].

Proton magnetic resonance spectroscopy

Since a Magnetic Resonance Spectroscopy (^{1}H MRS) spectrum of the liver is dominated by lipid and water resonances, ^{1}H MRS has been used for the assessment of hepatic fat. The percentage liver fat has been estimated from the number of protons in the lipid and water resonances by calibration with hepatic lipid extracts[99]. Such measures have been used to assess racial differences in the prevalence of hepatic steatosis and the technique has also been applied in a population of over 2000 participants[100,101]. Simpler lipid-to-water resonance ratios have been used to compare hepatic steatosis between obese and lean individuals and have demonstrated a change in intrahepatic lipid in response to dietary intervention[102-104]. ^{1}H MRS also has also been applied to the assessment of steatosis in living-

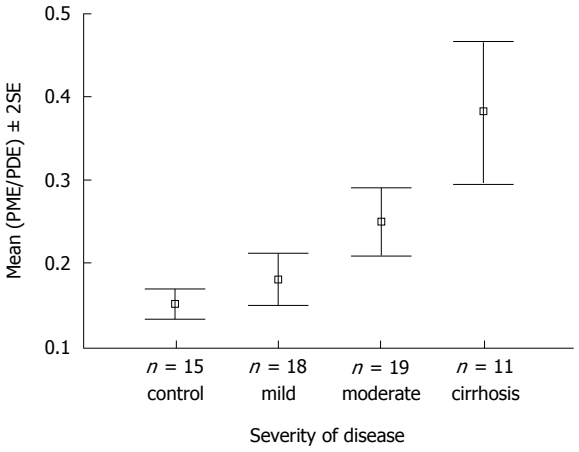

Figure 4 ^{31}P magnetic resonance spectroscopy of patients with increasing severity of liver disease *vs* controls. PME/PDE ratios obtained from *in vivo* hepatic ^{31}P MRS correlating with severity of liver disease in patients with hepatitis C[116]. MRS: Magnetic resonance spectroscopy; PME: Phosphomonoester; PDE: Phosphodiester.

donor liver transplantation[105] and for quantification of steatosis in HIV mono-infected individuals who are at greater risk of liver fibrosis and cirrhosis[106].

Proton density fat fraction

MRI techniques can also be used to calculate the proton density fat fraction (PDFF), a marker of hepatic steatosis, using MRS as a reference. PDFF is defined as the ratio of density of mobile protons from triglycerides and the total density of protons from mobile triglycerides and mobile water which then reflects the concentration of fat within that tissue in the absence of confounding factors[107]. PDFF has shown at least equivalence in accuracy for quantifying hepatic steatosis with both ^{1}H MRS and with histological grade, across several studies with various aetiologies of chronic liver disease[108-112]. Additionally, in a retrospective study of data from 506 adults, PDFF estimation accuracy was not affected by age, sex and BMI with the authors concluding these confounders have a clinically negligible effect[113]. Further validation of these techniques would be of benefit as ^{1}H MRS techniques are not widely available.

Phosphorus MRS

Phosphorus (^{31}P) MRS currently remains a research tool and allows observation of metabolites associated with energy metabolism as well as membrane phospholipid turnover. The latter include phosphomonoesters (PME), thought broadly to represent membrane precursors including phosphoethanolamine, phosphocholine and phosphodiesters (PDE), which are thought to represent membrane degradation products including glycerophosphocholine and glycerophosphoethanolamine. Studies in chronic liver disease both *in vitro*[114,115] and *in vivo*[116,117] have demonstrated a correlation between the PME/PDE and disease state or severity (Figure 4). A study in patients with acute hepatitis A demonstrated an acute rise in PME/PDE, which decreased with resolution of disease[118]. Moreover, in patients with hepatitis C, the PME/PDE decreased significantly in those responding to antiviral treatment but did not change in non-responders[119].

Combination techniques

Combinations of non-invasive techniques can be used to improve accuracy. Serum biomarker models can be combined to create more accurate algorithms, such as the Fibropaca algorithm, Leroy algorithm and SAFE biopsy, with some studies suggesting their use could reduce the number of liver biopsies by 79%[120]. However, the use of serum biomarkers in combination with imaging techniques has proved highly useful. One study of 183 patients with chronic HCV by Castera and colleagues demonstrated that a combination of serum FibroTest® and ultrasound-based Fibroscan® (TE) (the Bordeaux algorithm) showed an increased AUROC score for F2 (0.88 *vs* 0.83) and F3 (0.95 *vs* 0.90), compared to Fibroscan® alone, thus avoiding the need for biopsy in a large proportion of patients[121]. The Anger's algorithm combines the serum biomarker model, Fibrometer® (Echosens, Paris, France) and ultrasound-based Fibroscan®. TE has also demonstrated good diagnostic accuracy and required significantly fewer biopsies than the Bordeaux algorithm for significant fibrosis (20.2% *vs* 28.6%, $P = 0.02$) and cirrhosis (9.3% *vs* 25.3%, $P = 0.001$)[122]. Most recently, the role of combination techniques as a cost-effective screening tool has been validated by Harman and colleagues in a community setting in Nottingham, United Kingdom (practice population 10479). High-risk patients were identified using risk factors for chronic liver disease and subsequently investigated them using a serial biomarker algorithm and liver stiffness measurement (Figure 5). Of the 504 identified as being high risk, 62 patients (12.3%) had normal biomarkers and were not further investigated. 378 patients then agreed to undergo TE which found 98 patients (26.8% of valid scans) had clinically significant fibrosis (defined as LSM < 8kPa). Most interestingly, 71/98 patients (72.4%) of these patients had normal liver enzymes and would have been otherwise missed by conventional algorithm models. This techniques also managed to identify 140% more patients with definite cirrhosis ($n = 11$)[123].

IMAGING MODALITIES TO DETECT FEATURES OF CLD

Imaging modalities are can be used to detect a number of signs or physical parameters, which are of relevance to chronic liver disease.

Morphological changes

The later stages of chronic liver disease are cha-

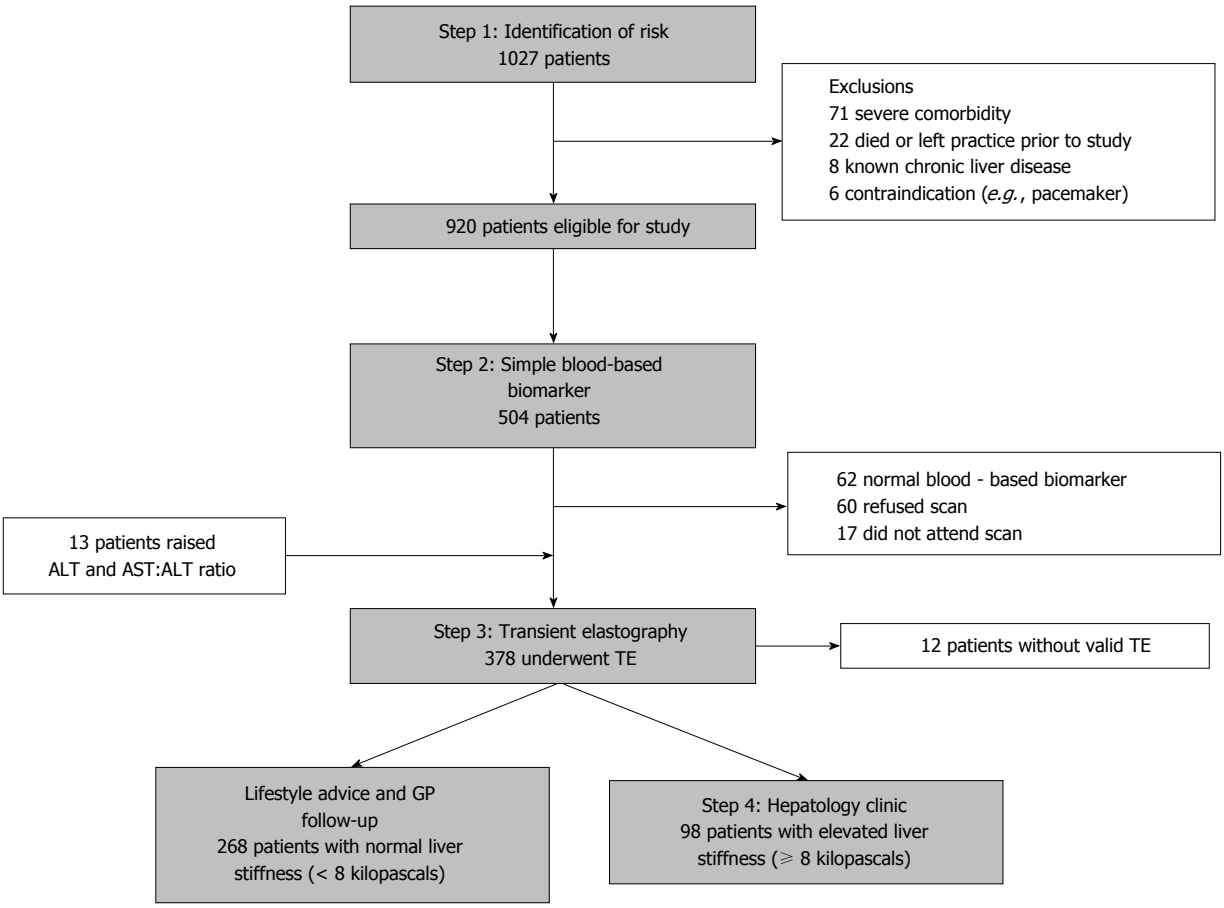

Figure 5 Diagnostic algorithm and patient flow chart of the non-invasive biomarker and transient elastography pathway[123]. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GP: General practitioner; TE: Transient elastography.

racterised by a number of intra- and extrahepatic structural changes. A number of these structural changes are manifestations or consequences of other pathological processes. The cirrhotic liver is typically small with an uneven border or perimeter. These features are caused by the contraction of thick bands of fibrous tissue interspersed by regenerative nodules. Ultrasound-visible features, such as liver surface nodularity, caudate lobe hypertrophy and the presence of detectable hepatic venous blood flow have been shown to identify those with severe fibrosis or cirrhosis from a cohort of patients with chronic liver disease, although it is doubtful that the severe fibrosis group alone would be identifiable by such a technique[124]. Routine MRI, computed tomography (CT) and ultrasound indices can detect gross morphological changes associated with cirrhosis, but none is sensitive or specific. Moreover, pre-cirrhotic disease is not adequately discriminated[125-127]. However, dynamic superparamagnetic iron oxide-enhanced and gadolinium-enhanced MRI demonstrates reticular-nodular patterns, thought to represent septal hepatic fibrosis, the presence of which, taken with an overall (subjective) qualitative assessment, allows the discrimination of moderate and severe from mild fibrosis[128] (Figure 6). Digital image processing of unenhanced CT scans has enabled assessment of the distribution of high-attenuation patterning, again presumed to represent fibrosis and distinction between moderate and severe fibrosis[129]. However, these techniques do not provide a quantitative measure of disease severity, and a number of the techniques remain unvalidated by other centres. Water molecules are tightly bound in the fibrotic extracellular matrix, providing the rationale behind the application of diffusion-weighted MR imaging to chronic liver disease. An apparent diffusion coefficient (ADC) is derived, representing proton, and hence water, mobility. A reduced ADC is observed in cirrhosis and with increasing fibrosis stage, and has been interpreted as being due to restriction of water diffusion in fibrotic tissue[130,131], or possibly by reduced capillary perfusion[132]. However, the precise relationship between the ADC and fibrosis is currently unclear.

Portal hypertension

Portal hypertension is the cause of much morbidity and mortality associated with chronic liver disease, through development of varices of porto-systemic anastomoses and through activation of vasodilatory pathways and development of ascites and the hepatorenal

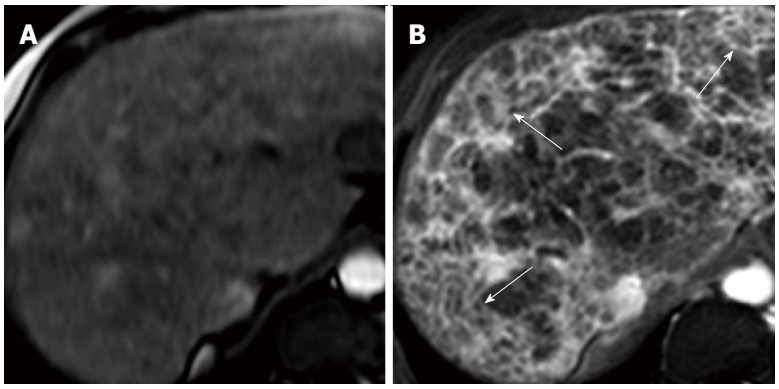

Figure 6 Transverse MR images of cirrhotic liver *in vivo*[128]. A: SPIO-enhanced two-dimensional spoiled gradient echo (SPGR) image with echotime of 2.65 ms; B: Double-enhanced SPGR image at the same level, showing hyperintense reticulations and hypointense nodules (arrows), thought to represent fibrous septal bands surrounding regenerative nodules.

syndrome. Increased portal pressure is caused by increased intrahepatic resistance to flow which results from both vascular factors and fibrosis[133]. The structural results of portal hypertension, such as splenomegaly, ascites and the presence of venous collaterals are also readily assessed by conventional imaging techniques, but these features tend to be associated with decompensated cirrhosis and not pre-cirrhotic disease stages. A number of ultrasound-based studies have aimed to assess portal pressure indirectly as a surrogate for disease severity[134]. However, ultrasound Doppler indices of portal flow were found not to correlate reliably with increasing severity of disease[135,136]. A number of studies have pointed to a relationship between portal hypertension and liver stiffness measurement (LSM) measured by Fibroscan®. Foucher and colleagues demonstrated a correlation between liver stiffness measurement and splenomegaly, the presence of oesophageal varices and a history of bleeding varices[137]. A relationship between LSM, measured by Fibroscan® and the presence of varices has also been described, although evidence for the relationship between LSM and size of varices is mixed[138,139]. Vizzutti and colleagues went on to demonstrate correlation between LSM and the hepatic venous pressure gradient (HVPG), particularly at lower HVPG values (< 10-12 mmHg). This represents a complex relationship, which was less apparent at higher HVPG values[139]. It has been stated that the "progressive rise in portal pressure...[is]...due mainly to an increase in intrahepatic vascular resistance from the accumulation of fibrillar extracellular matrix"[140]. However, increased arterial and portal inflow may contribute directly to the liver stiffness, while haemodynamic changes characteristic of advanced portal hypertension, including extrahepatic haemodynamic changes, may not be detected by changes in liver stiffness.

Intrahepatic vascular changes

Vascular remodeling is increasingly seen as a pathological feature of chronic liver disease. In the development of fibrosis, obliteration of the small hepatic and portal veins may lead to a congestive hepatopathy, which is exacerbated by a co-existent hyperdynamic circulation[141]. This results in inflammation and oxidative stress, both triggers for fibrogenesis. Intrahepatic vascular remodeling within the fibrotic liver is performed by the contractile HSCs, mediated by changes in levels of nitric oxide (NO), consequent to derangement of endothelial NO synthase. This contributes to high resistance and constricted sinusoidal vessels[142]. Such mechanisms may also contribute to the development of intrahepatic vascular shunts. Imaging techniques assess changes in physical properties consequent to vascular alteration. While vascular changes occur with increasing fibrosis, imaging techniques do not assess fibrosis directly, so may be considered surrogate markers in this context.

Inflammation and cell turnover

Hepatic inflammation is associated with cellular inflammatory infiltrate, tissue oedema and hepatocyte swelling. Each of these is likely to affect the physical properties of liver tissue and, as such, can be measured by imaging modalities. These properties include: nuclear relaxation (T_2), assessed by MR techniques; water perfusion and diffusion, as assessed by DWI; liver stiffness; changes in attenuation, assessed by CT and echogenicity, assessed by B-mode ultrasound.

Liver stiffness

The association between liver stiffness on Fibroscan® and disease activity, or necroinflammatory score on histology has been shown by a step-wise increase of liver stiffness measurements (LSM) with necroinflammatory activity in a cohort of patients with disease of varied aetiology[143]. The relationship between LSM and biochemical activity in patients with chronic viral hepatitis has also been studied using Fibroscan®. The LSM was lower, stage-for-stage, in those with biochemical

remission (assessed by ALT) than those with a higher ALT[144]. Studies have specifically addressed the effect of hepatic inflammation on LSM. In one, 18 patients without a past history of liver disease, but with acute viral hepatitis, were studied. The LSM on Fibroscan® at the peak aminotransferase level exceeded 12kPa (the cut-off for prediction of cirrhosis) and furthermore, in all but one subject, the LSM returned to within normal range (below 7kPa). In addition, the LSM correlated with the aminotransferases at onset and with the AST at follow-up[145]. In another Fibroscan® paper, 20 patients with acute hepatitis of varying aetiology were studied. In those followed up longitudinally, the aminotransferases returned to a level commensurate with the fibrosis stage at biopsy[146]. While it is known that acute hepatitis is associated with an inflammatory infiltrate, tissue oedema and hepatocyte swelling, (all of which are likely to affect LSM), there was no histological confirmation of these features in these studies, as liver biopsy was not clinically indicated[147].

The development of ultrasound-based TE has enabled the rapid acquisition of objective liver stiffness measurements *in vivo*[65]. Multiple regression analysis in early studies demonstrated a relationship between elasticity measurements and fibrosis stage, but not to the histologically-measured disease activity, necroinflammatory score or the degree of steatosis[65,66]. A number of studies have confirmed the correlation between liver stiffness and hepatic fibrosis in chronic HCV infection[66,148], and other chronic hepatic conditions[149-151] with meta-analyses and systematic reviews also being recently published[68,152]. However, studies have also indicated the presence of co-existing factors that may contribute to liver stiffness, including inflammation, portal hypertension and possibly steatosis[137,147,150,151]. It has also been stressed that "biological tissue is a composite material and it is difficult to separate the influence of each component of the tissue on the total in modulus estimates"[153]. Substantial differences in cut-off values for cirrhosis have been observed on Fibroscan® between those with chronic hepatitis and those with alcohol-related chronic liver disease and NAFLD, perhaps representing modification of liver stiffness by coexisting fat[150,151,154]. Thus, there is circumstantial evidence that steatosis affects liver stiffness although the magnitude of the effect is likely to be smaller than that of other contributing factors, such as fibrosis, inflammation and portal hypertension.

Summary

The development of chronic liver disease is a major cause of morbidity and mortality worldwide and usually occurs over many years from progressive fibrosis and associated hepatocellular injury, including steatosis. Non-invasive assessments using imaging modalities and serum markers have now been shown to be effective at detecting significant fibrosis and cirrhosis to at least some degree. However, recent evidence suggests that various combinations of these techniques may be helpful both as an alternative to liver biopsy, which has significant associated morbidity and mortality, and as a cost-effective tool to identify sub-clinical disease.

SUMMARY OF KEY POINTS

Chronic liver disease develops over many years following ongoing injury and is characterised by progressive scarring caused by fibrosis. Hepatic steatosis is increasingly being recognised as an important factor in a number of chronic liver diseases and occurs when the rate of synthesis or import of fatty acids by hepatocytes exceeds the rate of export or catabolism. Serum markers measure both direct and indirect markers of liver fibrosis although these have had limited success as individual markers of fibrosis. Imaging modalities to assess liver disease can be used to quantify morphological changes, portal hypertension, vascular remodelling and liver stiffness associated with increasing fibrosis. The most widely used and successful of these are transient elastography and acoustic radiation force impulse imaging although MRI techniques are very promising. Combination techniques involving transient elastography and various serum markers can provide good diagnostic accuracy and a reduced need for liver biopsy in patients with significant fibrosis. This has also proved successful as a screening tool in for patients in a community setting.

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