

Review of diagnostic techniques of hepatic fibrosis

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Abstract

With increasing innovations aimed at the treatment of chronic liver disease (CLD), the precise staging of liver fibrosis is important to help establish efficacious management of disease activity in each patient. The development of biomarkers capable of non-invasive staging of fibrosis in the liver is challenging as fibrogenesis is a part of the normal wound healing response. There is an array of non-invasive methods, including serum biomarker assays, and imaging techniques such as transient elastography. The degree of implementation of non-invasive diagnostic tests for liver fibrosis differs all over the world, and still remains limited. Liver biopsy so far is the mainstay of diagnosing hepatic fibrosis. Precise staging of liver fibrosis is essential in management of patients. This review provides a systematic overview of various techniques, as well as both approaches based on direct and indirect biomarkers to stage fibrosis, and covers recent studies related to hepatic fibrosis.

Keywords: Fibrosis, Hepatic Stellate Cells (HSCs), Chronic Hepatitis C (CHC), Chronic Liver Disease (CLD).

Introduction

Liver fibrosis results from accumulation of extracellular matrix (ECM) as a consequence of chronic liver injury, leading to architectural changes in the liver parenchyma. Hepatic fibrosis or scarring is a wound healing process that ultimately leads to impaired hepatic function and cirrhosis. Cirrhosis can result in widespread haemodynamic disturbances.^{1,2} Constant regeneration in the setting of cirrhosis can predispose to hepatocellular carcinoma.^{3,4} Hepatic fibrosis frequently arises from injury due to viral hepatitis, especially chronic hepatitis C (CHC), and is associated with high morbidity and mortality worldwide.⁵ Other causes of hepatic fibrosis include, autoimmune hepatitis, primary biliary cirrhosis, primary sclerosing cholangitis, inherited metabolic conditions such as haemochromatosis, neonatal liver disease, and parasitic liver diseases. It has been estimated

by the Center for Disease Control (CDC) that there will be at least a four-fold rise in the prevalence of CHC infection by the year 2015.⁶ The evaluation of magnitude of liver fibrosis is vital in the management of patients with chronic hepatitis. There are both direct and indirect markers to diagnose hepatic fibrosis. Conventional serum tests include aspartate aminotransferase (AST), Alanine transaminase (ALT), alkaline phosphatase and bilirubin. Although, these tests provide useful information about hepatic function, but they do not assess fibrosis which is the main culprit of impaired hepatic function. Traditionally, diagnosis of hepatic fibrosis and necroinflammatory grade is done by liver biopsy. The most widely used scoring systems for grading activity and staging fibrosis are Knodell and Metavir systems. Various non-invasive methods are also being studied for their role in the diagnosis of hepatic fibrosis. An ideal serum marker is yet to be established. A combination of two or more markers has been evaluated in various studies in an attempt to establish diagnostic potential of non-invasive markers.

Techniques:

I- Imaging Techniques:

a) Elastography and Fibroscan: It has been reported that hepatic stiffness proportionately increases with fibrosis progression.^{7,8} Elastography has been studied as an assessment tool for the staging of liver fibrosis.^{8,9} Comparison of elastic ratio with various markers and scores showed that elastography appears to be a better technique than Forns score, hepascore, fibro index and serum markers like hyaluronic acid (HA).⁹ The F3 stage of fibrosis shows sensitivity of 85.4% and specificity of 96.4% which is higher than that shown in other stages.⁹ The role of Transient Elastography (Fibroscan) in measuring fibrosis seems promising, but there are certain limitations.¹⁰ It is not practical for patients who have ascites because the elastic waves are unable to propagate through liquids.^{7,8} It is not only a cost-intensive procedure, but also Elastography has limited significance in patients with narrow intercostal spaces and in those who are obese.^{4,7,8}

b) Acoustic radiation force impulse (ARFI) imaging sonoelastography: ARFI is an imaging technique which

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uses sonography for assessment of liver fibrosis. Unlike Fibroscan and Elastography, ARFI can be performed on obese patients and on patients with narrow intercostal spaces. The diagnostic accuracy of ARFI when expressed as area under receiver operating curve (AUROC) showed validity of 90.2% for $F \geq 2$, being higher with progressing fibrosis.¹¹ The results showed overlap between fibrosis stages, especially F0-F1 and F2, hence the role in diagnosing early fibrosis could not be established.¹¹

II- Serum Markers

a) Enhanced liver fibrosis (ELF): The ELF score includes analyses of tissue inhibitor of matrix metalloproteinase 1 (TIMP-1), HA, and amino-terminal propeptide of type III collagen (PIIINP). In comparison to transient Elastography, ELF score showed greater association with chronic hepatitis with sensitivity of 86% and specificity of 70% in $F \geq 2$.⁴ However, transient Elastography is more discriminative in lower fibrosis stages than is the ELF score.⁴

b) APRI (AST to Platelet ratio index): APRI was used in a retrospective cohort study to evaluate fibrosis and cirrhosis. The indices used were platelets, AST and alkaline transferase. The AST-to-platelet ratio was established to augment the contrasting effects of liver fibrosis on AST and platelet count (APRI). The APRI was calculated, using the following equation:

$$\text{APRI} = (\text{AST level/Upper normal limit for AST/Platelet count } \{10^9/\text{L}\}) \times 100$$

Significant fibrosis could be predicted with sensitivity of 72.7% and specificity of 62.4% in patients of CHC.¹² The results, however, require validation in a larger cohort.¹²

c) TIMP-1 and MMP-2: Circulating levels of tissue inhibitor of metalloproteinase 1 (TIMP-1) and matrix metalloproteinase 2 (MMP-2) were explored as diagnostic markers of fibrosis in chronic liver disease (CLD). TIMP-1 could detect cirrhosis with sensitivity of 100% but only 56% and 75% specificity in two assays.¹³ The study suggested that the diagnostic potential of serum MMP-2 to detect fibrosis was low with sensitivity of 7% in two assays, and an overall diagnostic efficiency of 56% and 58%.¹³ Though TIMP-1 could detect both fibrosis and cirrhosis, it showed sensitivity for cirrhosis.¹³ However, clear descriptions of different stages of fibrosis could not be made.

d) Fibro Test-Acti Test (FT-AT): This is a non-invasive blood test that comprises six serum biochemical markers [alpha2-macroglobulin (A2M), haptoglobin, gamma glutamyl transpeptidase (GGT), and total bilirubin,

apolipoprotein A1 and alanine aminotransferase (ALT)]. FT can measure fibrosis stage and necroinflammatory grade in the liver. The results of the study showed that the use of the biochemical markers of liver fibrosis (Fibro Test) and necrosis (Acti Test) can be endorsed in patients with CHC. FT, however, requires standardisation of biochemical values.¹⁴ The serum levels of haptoglobin and apolipoprotein A can be affected by conditions unrelated to CLD. Another study assessed the utility of Fibro Test by constructing decision trees in patients with chronic hepatitis C.¹⁵ It was mentioned in the study that there are drawbacks and limitations to FT use, and that the decision tree analysis was not able to produce predictive accuracy for intermediary FT scores (particularly F3 with 43.7% false negative values).¹⁵

e) Fibrosis 4 Index (FIB 4): A FIB4 index is a biochemical test which includes platelets, ALT, AST and age. A score of -1.45 indicates moderate fibrosis with 74.3% sensitivity, while -3.25 indicates marked fibrosis with positive predictive value (PPV) of 82.1% and specificity of 98.2%. FIB4 index has been reported to be concordant with Fibro Test results.¹⁰ Though it is an economical and simple way to detect fibrosis, the results need to be authenticated. A limitation of FIB4 is that it cannot clearly outline the transitional stages of fibrosis. The study mentions cases of over-estimation, e.g. in Gilbert's syndrome, as well as under-estimation, e.g. in young patients and in unexplained thrombocytosis.¹⁴

f) Hepascore: A model of four serum markers bilirubin, glutamyl transferase, HA, macroglobulin, along with age and gender forms hepascore. This model can be useful in identifying various fibrosis stages among hepatitis C patients. In cases of significant fibrosis, hepascore provided specificity of 67% and sensitivity of 56% while in severe fibrosis, sensitivity of 82% and specificity of 86% was shown.¹⁶ However, hepascore can be altered by extra hepatic diseases. Also, this study could not define if the hepascore model is reactive to fibrosis change in same individual over time.¹⁶ There can be disparity in the results of hepascore, as the model shows vast variation in the same individual.¹⁷

g) Forns Score: This score is based on assay of age, GGT, platelets and cholesterol. Routine histopathology using Haematoxylin and eosin (H&E) was done on blocks of liver biopsy and stage 2 to 4 was taken as significant fibrosis. Using the four variables of Forns score, it was seen that insignificant fibrosis (stage 0 and 1) could be detected accurately, while significant fibrosis could not be clearly identified.¹⁸ Forns score has been reported to predict hepatic fibrosis with accuracy of 50-85%.¹⁹ The inclusion

of cholesterol, however, can lead to discrepancy in staging fibrosis because cholesterol can fluctuate with genotype.¹⁹

h) Fibrospect II (FSII): This is an assay of three serum markers HA, TIMP-1, A2M which are extracellular matrix markers.²⁰ The sensitivity of FSII was 83.5% while specificity was 60.7%. Decreased sensitivity in the middle ranges of the test could affect the differentiation in various stages of hepatic fibrosis.²¹

i) Fibrometer: This is a non-invasive test which links platelets, prothrombin index, aspartate aminotransferase,

A2M, hyaluronate, urea, and age. It indicates the amount of fibrosis as percentage of total liver mass. Fibrometer, Fibro test and Hepascore were analysed in a study that recruited chronic hepatitis Band C cases.¹⁹ It was suggested in the study that Fibrometer could not determine fibrosis stages accurately in about 24% of chronic hepatitis B and 6% of Chronic hepatitis C subjects. In another study Fibrometer showed AUROC of 0.892.²² The study mentions analytical inconsistency which may render these blood tests unreliable for detection of stages of fibrosis.²²

j) Liver Score: This index was formulated using six variables including age, ALT, GGT, apolipoprotein, A2M and HA.²³ The index was considered to be able to discriminate various fibrosis stages, with negative predictive value (NPV) of 83% and PPV of 89%.²³ The sample size, however, was small and the study mentioned that results needed to be validated.²³

k) Other Biochemical Markers: Three markers, HA, TIMP-1 and A2M, were evaluated for predictive accuracy of fibrosis.²⁴ It was concluded in the study that the three-marker panel may reliably segregate CHC patients with moderate/severe fibrosis from those with no/mild fibrosis. However, accuracy for intermediate stages of fibrosis was not established.²⁴ HA is an important component of ECM and is increased in hepatic fibrosis due to increased synthesis by HSCs.^{3,24} However, HA cannot be reliably used as a marker as it may show marked variation in results within the same individual. Another study used A2M, vitamin D binding protein and apolipoprotein A as biomarkers to predict liver fibrosis.²⁵ The level of apolipoprotein A can be altered by other pathologies like uncontrolled diabetes, which makes this marker nonspecific for liver. The sample size of the study was small (n=45), thus further studies are required to establish the role of these markers.²⁵ AHH index was formulated using the sum of scores of A2M, haptoglobin, and HA. The A2M is an acute phase protein, produced by hepatocytes

and by stellate cells during repair process, eventually leading to fibrosis. The liver parenchymal cells also produce haptoglobin probably in response to growth factors released during fibrosis. The results suggest that the AHH index could be a good model for predicting significant fibrosis in Korean patients with 89% sensitivity and 78% specificity with CLD. However, further studies are needed to evaluate the predictive value of the AHH index.³

III- Proteomics: Proteomics may help discover novel markers with diagnostic potentials. The study of proteomics detects mass of proteins and defines the amino acid sequence. The segregation of proteins is done either by 2-dimensional polyacrylamide gel electrophoresis (PAGE), liquid chromatography or difference gel electrophoresis (DIGE).²⁶ These segregated proteins are then processed through mass spectrometry technique, which detects the peptide mass and amino acid sequence. Hepatic fibrosis in advanced stages leads to changes in structural proteins. This alteration in protein structure forms the base of proteomics use in hepatitis C virus (HCV) patients. Though proteomics forms a very comprehensive method, procedural errors limit its use. There is low reproducibility between studies by different research groups.²⁶

IV- Biopsy Techniques: Most serum markers are not liver-specific. The serum markers of fibrosis can be affected by disturbed metabolism, as they are the end products of synthesis or breakdown of extracellular matrix, and may represent impaired hepatic clearance. Hence, coexisting pathologies and altered blood parameters, not related to CLD, must be taken into account.⁷ There can also be analytic variability due to laboratory skills. Although dynamic changes can be interpreted by these markers, but they do not seem to appreciate existent fibrosis.⁷ Due to insufficient precision and lack of validation, extensive use of non-invasive markers is not recommended.²⁷ Liver biopsy has been the cornerstone for diagnosis of liver pathologies and represents the gold standard for the assessment of hepatic fibrosis.

a) Conventional Liver Biopsy: Metavir, Knodell and modified Knodell system of scoring are widely used to assess the degree of fibrosis and necroinflammatory activity. Most of the studies have used Metavir scoring system as a reference of hepatic fibrosis.²⁸ In Metavir system of scoring, histopathology grading of fibrosis is done on a 5-point scale from 0 to 4. The activity, which is the amount of inflammation, is graded on a 4-point scale from A0 to A3.²⁸ The blind percutaneous liver biopsy (PLB) is commonly being performed as an outpatient

procedure and involves 0.25% to 3.8% risk of major complication.²⁹ The complications associated with PLB are rare, despite rich blood supply of liver. Most of the liver biopsies are now done under ultrasound guidance, which, as compared to blind PLB, is considered to be cost-effective.³⁰ The ultrasound-guided liver biopsy minimises the possible risks associated with blind PLB.³⁰ Biopsy provides a source of additional information regarding any unrecognised pathology of liver. Special stains can be used to diagnose the various stages of fibrosis.

b) Immunohistochemistry: Hematopoietic stem cells (HSCs) represent the key source of fibrogenesis. Liver injury results in activation of otherwise quiescent stellate cells, leading to their transdifferentiation into fibrogenic, proliferative and contractile myofibroblasts.³¹ The HSC activity is in proportion to extent of fibrosis and necroinflammatory activity. The expression of contractile filaments, Alpha Smooth Muscle Actin (α SMA), were identified in stellate cells. This forms the basis of using α SMA as an immunohistochemical marker which detects HSC activation. Hence, it has been shown to be a useful marker for early diagnosis of hepatic fibrosis (standardised coefficient of correlation=0.76; $p < 0.001$).³² Further study of immunohistochemical markers may open new avenues for early diagnosis of hepatic fibrosis by appreciating early HSC activation. This may promise better patient care

Conclusion

The degree of implementation of non-invasive diagnostic tests for liver fibrosis differs all over the world and still remains limited. Various serum markers and non-invasive techniques have been evaluated. However, clear demarcation between the stages of fibrosis cannot be made. Liver biopsy so far is the mainstay of diagnosing hepatic fibrosis. Conventional biopsy using H&E stain may not be able to accurately detect fibrosis. There is a need to establish role of special stains which can reflect liver collagen and ECM. Immunohistochemistry is one such procedure which can help in the diagnosis of fibrosis and can even help predict fibrosis before actual evidence of fibrous deposition. This may help in better management of patients with liver fibrosis.

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