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Review Article

Pros and Cons of Existing Biomarkers for Cirrhosis of Liver

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Abstract

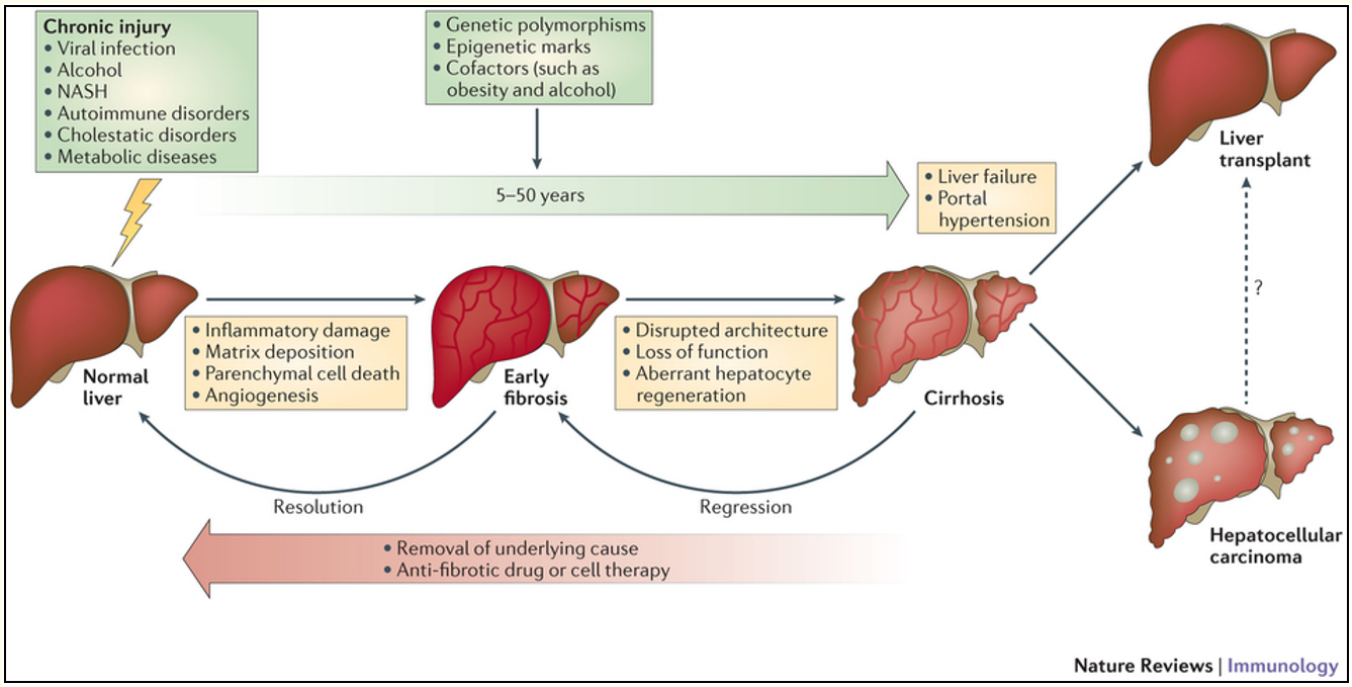
Cirrhosis of liver is natural reversible wound healing response to multi factorial chronic liver insults results in replacement of parenchyma by fibrotic tissue, formation of regenerative nodules and loss of liver functions; end stage liver disease. Diagnosing the degree of hepatocellular toxicity is crucial for tailored therapy which might be useful for the conversion of cirrhotic liver to normal liver architecture. Gold standard diagnostic tool, liver biopsy is highly invasive and complicated. Direct biomarkers involved in extra cellular matrix turnover need further validation in different geographic population. Indirect markers which are reflection of liver dysfunction may not predict early pathophysiological changes in liver parenchyma. Clinical significance of combinatorial markers has narrow applicability in regular practice due to lack of sensitivity and specificity. Hence, there is a need for biomarker which is specific for liver and can identify magnitude of clinical outcome of the disease.

Keywords: Biomarker; Cirrhosis of liver; Specificity; Sensitivity

Introduction

Cirrhosis of liver is reversible natural wound healing response results in the formation of connective tissue production and deposition and regenerative nodular formation in response to chronic liver injury. It is the final pathological result of various chronic liver diseases (CLD); fibrosis is the precursor of cirrhosis [1]. Causes of liver cirrhosis/fibrosis are multifactorial; congenital, metabolic, inflammation and toxins [2]. Studies have shown that treatment aimed at the underlying cause may improve or reverse fibrosis/cirrhosis. Resolution of liver fibrosis/cirrhosis might be due to enhanced collagenolytic activity due to reduction of expression of tissue inhibitor of metalloproteinase I (TIMP-I) (Figure 1) [3,4]. At what stage fibrosis/cirrhosis is irreversible is not well established; irreversibility attains once septal neovascularisation happens and portal pressure increases [5].

Extra cellular matrix (ECM) of normal liver is present between space of Disse with direct contact of low density basal lamina which contains glycoproteins, proteoglycans and glycosaminoglycans. There will be replacement of necrotic or apoptotic cells with regenerated parenchymal cells after an acute liver injury. If the hepatic injury still persists, failure of regeneration of hepatocytes and these cells will be substituted with abundant ECM and fibrillar collagen [6]. Liver fibrosis is associated with major alterations in both quantity and composition of ECM. Fibrotic liver contains 3 to 10 times more ECM compared to normal liver which is produced from collagens (I, III and IV), fibronectin, proteoglycans, elastin, laminin and hyaluronic acid [7].



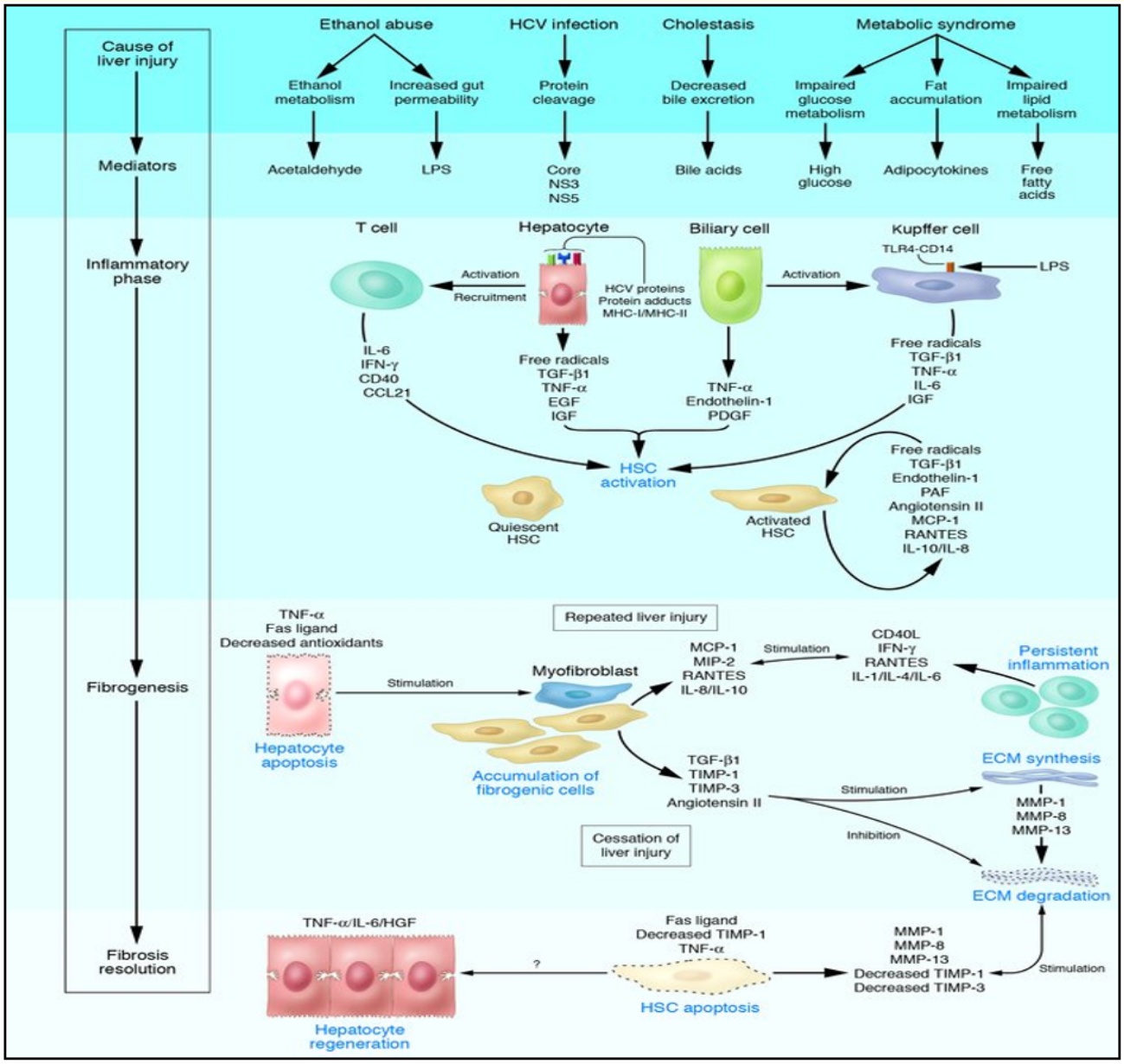
Source: http://www.medicalworld.co.in/2017/08/chronic-liver-failure.html

Figure 1: Schematic representation of course of chronic liver diseases; etiology to consequence.

In the injured liver, hepatic stellate cells (HSCs); major storage cells of vitamin A dwell in the space of Disse [8]. CLD leads to activation of HSCs and transdifferentiate into myofibroblast which have contractile, proinflammatory and fibrogenic properties. Platelet derived growth factor (PDGF) produced from Kupffer cells is the chief mitogen for activation of HSCs. Activated HSCs will be migrated and accumulated at tissue repair sites after activation which in turn secrete large amounts of ECM. Collagen synthesis by activated HSCs will be regulated at transcription and posttranscriptional levels [8]. Hepatocyte synthetic and metabolic functions will be disturbed by high density interstitial matrix which replaces normal low density matrix leads to impairment of solute transport from sinusoid to hepatocytes [7,9].

HSCs activation occurs in two phases; Initiation and Perpetuation. Early changes in HSCs which results from neighboring cells paracrine stimuli will happen in Initiation phage. Inflammatory marker cells stimulate matrix synthesis, cell proliferation and release of vitamin A by HSCs through the action of cytokine transforming growth factor β (TGF β), reactive oxygen intermediates and lipid peroxides. Seven discrete changes in cell behavior; proliferation, chemotaxis, fibrogenesis, retinoid loss, contractility, matrix degradation and inflammatory signaling and white blood cell (WBC) chemoattraction with cytokine release will occur in perpetuation phase. In fibrogenesis, fibrogenetic factors play a vital role for these discrete changes in cell behavior of HSCs (Figure 2) [7,9,10].

Diagnosing the extent of the disease is essential for tailored therapy in patients with CLD. The gold standard diagnostic tool for the assessment of severity of liver fibrosis/cirrhosis is liver biopsy. Disadvantages of liver biopsy are: highly invasive procedure and may obtain poor sample quality and tissue size which make biopsy non reproducible in relation to requirement of the sample. Direct markers are directly involved in ECM turn over whose levels are elevated with progression of the disease and have a tendency to decrease with response to treatment. But none of them are organ specific or readily available in clinical practice. Serum levels of



Source: Bataller R, Brenner DA. Liver fibrosis. J Clin Invest. 2005; 115: 209-218.

Figure 2: Cellular mechanisms of cirrhosis of liver (10)

Abbreviations: LPS: Lipopolysaccharides; IL: Interleukin; INF: Interferon; CCL21: C-C chemokine ligand 21; MCP-1: Monocyte Chemoattractant Protein–1; MIP-2: Macrophage Inflammatory Protein–2; NS3: HCV Nonstructural Protein 3; NS5: HCV Nonstructural Protein 5; TGF-β: Transforming Growth Factor β; TNF-α: Tumor Necrotic Factor α; PDGF: Platelet Derived Growth Factor; ECM: Extra Cellular Matrix; EGF: Epidermal Growth Factor; IGF: Insulin like Growth Factor; PAF: Platelet Activating Factor; MMPs: Matrix Metallo Proteinases; TIMPs: Tissue Inhibitors of Metalloproteinases; HCV: Hepatitis C Virus

cytokines do not have greater significance and could not add much diagnostic value compared to routine biomarkers. Scoring system for diagnosis/prognosis of liver fibrosis/cirrhosis include blood counts, plasma proteins, hepatic enzymes, bilirubin and prothrombin time. However, these scoring systems play a major role only after the effect. Hence in the present review an attempt has been made to discuss pros and cons of present biomarkers for liver fibrosis/cirrhosis (Table 1) [10,11].

Biomarker	Disadvantages
Serum Albumin	• Even though liver specific, concentrations will be decreased in acute and chronic renal failure. • Unable to detect early pathophysiology and compensated liver cirrhosis because of half life
Aminotransferases (AST, ALT)	• Activities of both enzymes may reach as high as 100 times the upper reference limit • Peak activities has no relationship to prognosis
Alkaline Phosphatase (ALP)	• Elevation tends to be more notable in extrahepatic obstruction than in intrahepatic obstruction • Increase may also be seen in drug therapy
Gamma Glutamyl Transferase (γGT)	• Usefulness is limited due to lack of specificity • Increased activity of the enzyme is also found in serum of subjects receiving anticonvulsant drugs • example: Phenytoin and Phenobarbital
Serum Bilirubin	• Bilirubin peaks after marker enzymes • Unable to detect early pathophysiology
Prothrombin Time (PT) International Normalized Ratio (INR)	• Cholestasis will decrease PT • Decrease in PT may be secondary to malabsorption of vitamin K
Direct biomarkers of CLD	• Still in research level and needs validation • Do not have greater significance than routine biomarkers
Serum Cytokines	• Do not have much diagnostic value • Not organ specific

Table 1: Overview of biomarkers and their disadvantages related to cirrhosis of liver [10,11].

Diagnosis of cirrhosis of liver

Accurate assessment of liver cirrhosis is essential for clinical management, to predict prognosis and for therapeutic decision.

Liver biopsy

For past 50 years, liver biopsy is the gold standard method for diagnosis and classification of liver fibrosis/cirrhosis. It provides useful information about damaging process viz., necrosis, inflammation and steatosis [12,13]. Liver biopsy is highly invasive; poor sample quality and tissue size make this process non reproducible. This diagnostic procedure depends on the experience of pathologist; inter observer variations. Liver biopsy causes pain in 84%, bleeding in 0.5%, and damage to biliary system with approximately 0.01% mortality rate [14]. Because of these limitations there is an urgent need for the development of non-invasive diagnostic biomarker for liver fibrosis/cirrhosis.

Liver fibrosis/cirrhosis biomarkers are categorized into direct markers and indirect markers. Direct markers reflect ECM turnover where as indirect marker reflect alterations of hepatic function [16].

Direct markers of cirrhosis of liver

Direct markers; molecules produced by HSCs and other hepatic cells that are involved in deposition and removal of ECM. Serum levels of these markers are raised which is directly proportional to progress of the disease. In response to treatment, they have a tendency to decrease [17]. Serum estimations of these markers might be useful for effective treatment, but none of them are organ specific. Based on their molecular structure, direct biomarkers are categorized (Table 2) [18].

Collagens

During firbogenesis, enzymatic cleavage of procollagen at carboxy and amino terminal ends by procollagen C- peptidase and procollagen N-peptidase releases peptides into blood stream [19]. Concentrations of these peptides in serum reflect diseases progression. Type I and IV collagen are crucial components of ECM; compared to normal liver, 8 fold increases will be seen in cirrhotic liver. Serum estimation of type I and IV has positive correlation with grading of liver fibrosis/cirrhosis.

Collagens • PICP • PIIINP • Type IV collagen **Glycoproteins and polysaccharides** • Hyaluronic acid • Laminin • YKL -40	**Collagenases and their inhibitors** • MMPs • TIMPs **Cytokines and proteomic markers** • TGF – β1 • PDGF • Microfibril associated protein – 4
Abbreviations: PICP: Procollagen I Carboxy Peptide; PIIINP: Procollagen III Amino Peptide; MMP: Matrix Metallo Proteinase; TIMP: Tissue Inhibitors of Metallo Proteinase; TGF – β1: Transforming Growth Factor β1; PDGF: Platelet Derived Growth Factor	

Table 2: Classification of direct biomarkers for cirrhosis of liver according to structure [18].

Because of low sensitivity and specificity (78% and 81%) of these markers, they have limitation in clinical use. PICP and PIIINP serum levels do not have any correlation with histological grading of liver fibrosis/cirrhosis which makes them not reliable for the establishment of liver fibrosis/cirrhosis grading [20-22].

Glycoproteins and polysaccharides

The main component of ECM, hyaluronic acid (HA) is a glycosaminoglycan which is synthesized from HSCs. During diseases progression, increased production and decreased hepatic elimination or both leads to increased concentrations in serum which reflects degree of necroinflammation [23]. Non-collagenous glycoprotein, Laminin is synthesized by HSCs and deposited in basal membrane of liver around the vessels, in perisinusoidal space and portal triad. Laminin concentrations in circulation are elevated in liver fibrosis/cirrhosis irrespective of etiology; correlates with severity of fibrosis/cirrhosis and liver inflammation [24]. YKL-40 (chondrex, human cartilage glycoprotein-39), a glycoprotein will be expressed from liver. It can be used as a marker to assess liver fibrosis/cirrhosis and helps distinguish between mild stage and extensive stage of liver fibrosis/cirrhosis [25].

HA has sensitivity and specificity of 88%-95% and 86%-100% respectively in liver fibrosis/cirrhosis especially in nonalcoholic fatty liver diseases, but positive and negative predictive value of HA has been reported as 61% and 98%-100% respectively [26]. Laminin cut off concentration at 1.45 U/ml has sensitivity and specificity of 87% and 74% respectively with positive predictive value of 77% and negative predictive value of 85%. Estimations of serum HA and laminin has good prognostic value for liver fibrosis/cirrhosis complications [27]. Between HA and YKL-40, HA is a better predictive marker for liver fibrosis/cirrhosis [28].

Collagenases and their inhibitors

ECM of liver degradation will takes place by matrix metallo proteinases (MMPs) activity. In humans, three MMPs are expressed viz., MMP-1 (Collagenases), MMP–2 (Gelatinase A) and MMP–9 (Gelatinase B) (29). Intra cellular synthesis of these enzymes will be secreted as zymogens. Activation of MMPs will be by membrane type matrix metalloproteinase (MT1–MMP); tissue inhibitors of metalloproteinases (TIMPs) will inhibit the activities of MMPs [17]. Concentrations of MMP-1 will be inversely correlated with histological severity of liver fibrosis/cirrhosis [30]. HSCs secreted MMP–2 has high diagnostic accuracy approximately 92% for diagnosis of liver fibrosis/cirrhosis; 2 to 3 fold increase of MMP–2 will be seen in fibrotic liver compared to normal liver [31]. Kupffer cells secrets MMP–2 which has negative correlation with histological grading of the disease [32].

MMPs degradation of ECM will be inhibited by TIMPs; TIMP–1 will interact with all the 3MMPs, TIMP-2 is specific for MMP–2. Serum levels of TIMPs will be elevated during progression of liver disease. Portal inflammation has correlation with MMP-1/TIMP-1 ratio which is useful for the diagnosis of hepatic cell injury [33].

Cytokines and proteomic markers

HSCs proliferation will be increased by TGF–α which has positive correlation with progression of the disease [34,35]. ECM production of HSCs will be stimulated by TGF-β1 which in turn inhibits hepatocyte growth and proliferation [36]. Enhancement of TGF-β1 indicates diseases progression. False positive results will be seen for TGF-β1 due to platelet derived platelet TGF–β [37]. Platelet Derived Growth Factor BB (PDGF-BB) stimulates proliferation of

HSCs and migration. There will be positive correlation between levels of PDGF–BB and severity of hepatic disease [38]. Disulfide linked dimer, Microfibrillar associated Protein – 4 (MFAP4) which forms higher oligomeric structure of ECM [39].

MPAF4 has fibrinogen like domain at C– terminal and an integrin binding motif at N-terminal end [40]. According to Christian., *et al,* MPAF4 has sensitivity and specificity of 91.6% and 95.6% respectively; ideal proteomic marker [41]. Major intermediate filaments of hepatocytes, cytokeratin-18 fragments (CK18), produced by caspase induced apoptosis by cleavage of CK18 at different positions [42]. According to Yilmaz., et al. and Yang., et al; concentrations of M30 antigen (a neoepitope in CK18) and M65 (cytosolic pool of CK18) can distinguish advanced fibrosis/cirrhosis with early stages [43,44].

Indirect markers of cirrhosis of liver

Indirect markers include measurement of activity of enzymes viz., aminotransferases, alkaline phosphatase (ALP) and gamma glutamyl transferase (γGT) and estimations of bilirubin and albumin in blood [45].

Aminotransferases

(Aspartate amino transferase EC 2.6.1.1 and Alanine amino transferase EC 2.6.1.2)

Increased transaminase activity in circulation will be seen mainly in liver diseases. Serum activities of AST and ALT are elevated when disease processes affect hepatocyte integrity. Activities of these enzymes will reach more than 100 times to upper reference limit in liver diseases. There will not be any relationship between prognosis and peak activities; fall with worsening of patient's condition [46]. AST/ALT ratio >1 has sensitivity of 81.3% and specificity of 55.3% to predict cirrhosis [47].

Alkaline phosphatase (ALP) (EC 3.1.3.1)

Zinc metalloproteinase enzyme, ALP, at alkaline pH, catalyses the hydrolysis of phosphate esters. Canalicular membranes of hepatocytes express ALP which will be enhanced by biliary tree obstruction [48]. Enhanced activity of ALP is more in extrahepatic obstruction when compared to intrahepatic. Increased activity of ALP can also be seen as consequence to drug therapy [49].

Gamma glutamyl transferase (γGT) (EC 2.3.2.2)

Elevated activities of γGT are found in serum of alcoholic hepatitis patients. Moderate elevations occur in infectious hepatitis. As a consequence of drugs viz., Phenytoin and Phenobarbital, activity of γGT will be enhanced; lack of specificity [48,49].

Albumin

Albumin levels in circulation can be maintained until hepatocellular damage is more than 50%. Severity of the liver disease can be assessed by concentrations of albumin in circulation; reduced levels can also be seen in acute kidney disease [49].

Bilirubin

Multifactorial liver damage can be assessed by sequential estimations of serum bilirubin. High levels of bilirubin will be seen only after hepatic enzymes increased activity in case of acute hepatitis [48,49].

Prothrombin time (PT)

Cholestasis can be differentiated by severe hepatocellular diseases with the help of serial PT estimations. Prolonged PT will be seen in case of severe hepatocellular damage. Due to malabsorption of vitamin K in cholestasis, PT will be decreased [17,49].

Combinatorial use of biomarkers

Combination of multiple biomarkers as a panel might increase specificity and sensitivity for diagnosis/prognosis of the disease (Table 3) [50,51]. Murawaki., *et al.* has reported the useful of HA and MMP–2 for differential diagnosis of fibrosis/cirrhosis stages. These markers could not replace liver biopsy; overlap among stages and grades [52]. Diagnostic performance of HA, TIMP–1 and PIIINP was compared with liver biopsy; sensitivity greater than 90% with specificity greater than 90% was revealed in the study conducted by European Liver Fibrosis study [53]. According to Patel., *et al*. and Hind; HA, TIMP–1 and α2–macroglobulin can differentiate moderate/severe liver fibrosis with no or mild liver fibrosis caused by HCV [54,55]. There may be chance for false positive results for scores viz., APRI, FIB-4 and Forns index in case of acute hepatitis. Fibro test might give false positive results with respect to haemolytic and hyper bilirubinemia [56]. World Health Organization (WHO) in the year 2015 stated that APRI and Fibro test are preferred non-invasive tests for hepatitis B infectious patients [57]. Low performance has been documented for APRI when compared to FIB-4 and Fibro test for CLD caused by viral infection [58]. Before comparing FIB-4 cut offs with Fibro test and APRI; needs further validation [59].

Test	Parameters	Sensitivity %	Specificity %
APRI	AST/platelet count	57	93
AST/ALT	AST/ALT	51	71
Bonacini Index	ALT/AST, INR, platelet count	46	98
ELF Index	Age, HA, PIIINP and TIMP-1	90	69
FIB – 4	Platelet count, AST, ALT and age	65	97
Fibro Index	Platelet count, AST and γ globulin	35	97
Fibrometer Test	Platelet count, INR, AST, α2 macroglobulin, HA, urea and age	80	84
FibroSpect II	HA, TIMP-II and α2 macroglobulin	76	73
Forns Test	Age, platelet count, γGT and cholesterol	30	95
Globulin - albumin Ratio	Globulin and albumin	43	98
GUCI	Platelet count, AST and INR	80	78
Hepascore	age, gender, bilirubin, γGT, HA and α2 macro-globulin	84	71
Lok Index	Platelet count, AST, ALT and INR	68	72

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; INR: International Normalized Ratio; HA: Hyaluronic acid; PIIINP: Procollagen III amino peptide; TIMP-1: Tissue inhibitor of metalloproteinase I; TIMP-II: Tissue inhibitor of metalloproteinase II; γGT: Gamma glutamyl transferase

Table 3: Main scoring system for CLD with sensitivity and specificity [55].

Conclusion

Diagnosing the degree of CLD is essential for tailored therapy and successful management of the disease. Liver biopsy, gold standard diagnostic tool comes with complications. Direct markers can give indication about prognosis of disease with response to treatment; not organ specific. Scoring systems have narrow applicability in clinical practice due to limitations in specificity and sensitivity. Considering these limitations, there is an urgent need to introduce a biomarker which should be organ specific, accurate and precise, freely available in peripheral tissue, easily measurable having diagnostic significance much earlier than the scoring systems or disease onset and eliminate need for invasive liver biopsy.

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Declaration of conflict of interest

The authors declare no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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