

## Non-invasive biomarkers and FIB-4, APRI scoring system to rule out advanced liver injury

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World Journal of Advanced Research and Reviews, 2022, 13(03), 223–230

Publication history: Received on 25 January 2022; revised on 10 March 2022; accepted on 12 March 2022

Article DOI: <https://doi.org/10.30574/wjarr.2022.13.3.0198>

### Abstract

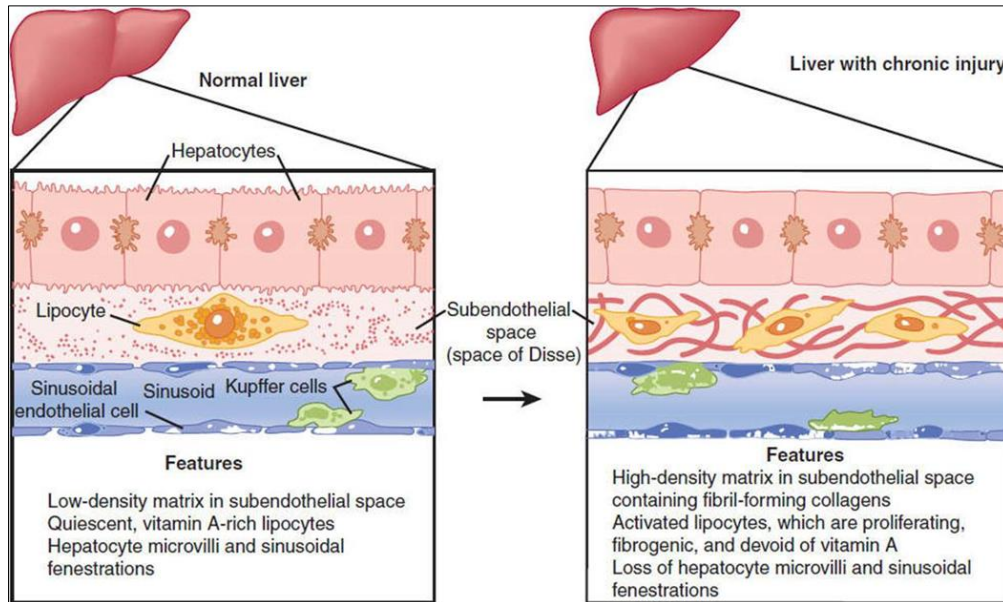
Chronic liver Injury (CLI) and its end-stages, cirrhosis, and hepatocellular carcinoma (HCC), are leading causes of morbidity and mortality worldwide with enormous socioeconomic costs. Clinical management of chronic liver injury is dependent on the extent of liver fibrosis. Liver biopsy, the gold standard, is still recommended in most patients. Liver disease diagnosis can generally be made using a carefully obtained history, physical examination, and a few laboratory tests. Initial laboratory testing should include aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and total bilirubin. Biomarkers are being developed as alternatives to liver biopsy for predicting liver fibrosis, Cirrhosis and HCC in patients with chronic liver injury. An intensive research on non-invasive alternatives. A simple, reproducible, low-cost, and non-invasive tool that can follow the evolution of the disease overtime would be beneficial for the testing physician and is desired by the patients. The aim of this review is to summarize Non-Invasive Biomarkers in addition to score systems like FIB-4 and (Aspartate Platelet Ratio Index) APRI in assessment of Fibrosis, Cirrhosis and HCC.

**Keywords:** Chronic liver Injury; HCC; Fibrosis; Cirrhosis

### 1. Introduction

During chronic liver injury, an increase of fibril-forming collagen and the replacement of the low density, basement membrane-like interstitial matrix occurs. There is also an accumulation of other matrix proteins, including elastin, hyaluronan, proteoglycans and fibronectin [1]. This type of matrix has the capacity to activate quiescent HSCs, leading to the loss of hepatocyte microvilli and the disappearance of endothelial fenestrations. This architectural change of endothelial cells also impairs the transport of solutes from the sinusoid to the hepatocytes, further contributing to hepatocyte dysfunction [2]. The Extra Cellular Matrix can also affect cell function indirectly by releasing cytokines. These include transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1), platelet derived growth factor (PDGF), hepatocyte growth factor (HGF), connective tissue growth factor (CTGF), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). During liver fibro genesis, parenchymal injury and the resulting inflammatory reaction generate a large panel of signals that stimulate the induction of specific transcription factors and morphogens in quiescent HSCs, thereby initiating the activation and the acquisition of fibro genic and proinflammatory properties [3]. Sustained activation leads to discrete changes in hepatic stellate cell (HSC) behavior, including proliferation, chemotaxis, fibro genesis, contractility, retinoid loss and WBC chemo-attractant/ cytokine release. In these phases there is a release of proinflammatory, profibrogenic and prometogenic stimuli acting in an autocrine and paracrine manner [4].

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**Figure 1** Normal Liver vs Chronic liver injury

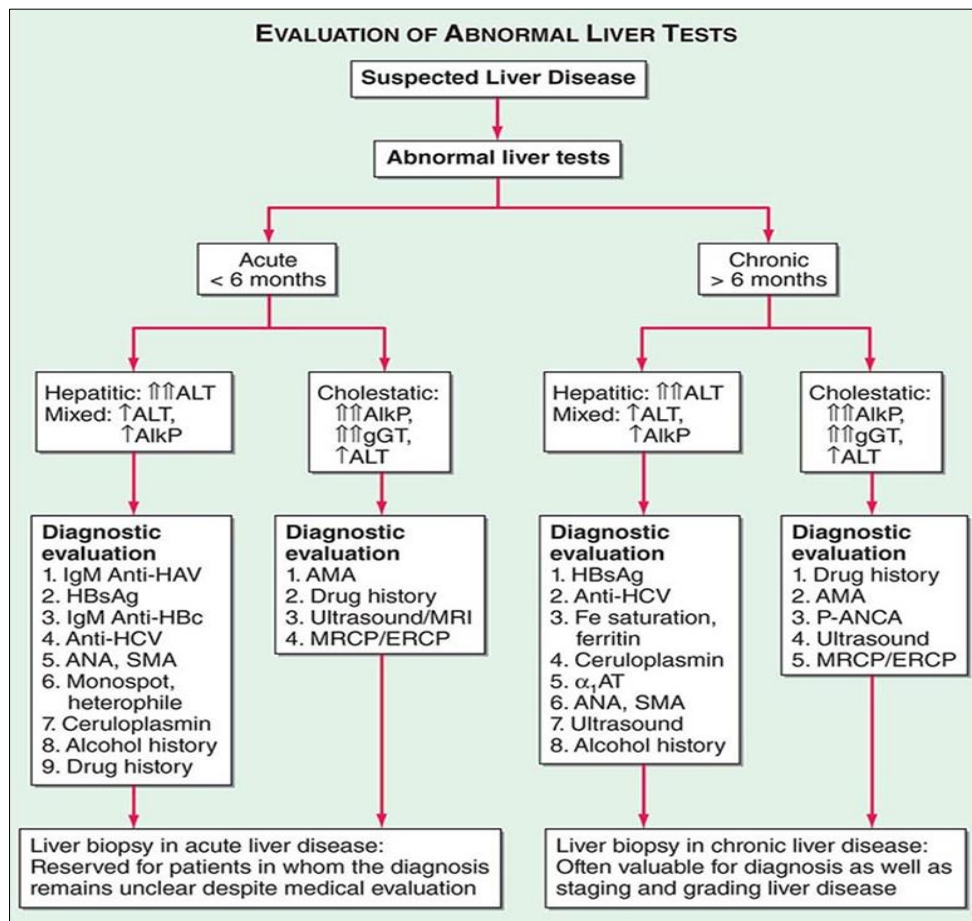
Liver fibrosis can be classified as a wound-healing response to a variety of chronic stimuli. It is characterized by an excessive deposition of extracellular matrix (ECM) proteins which includes three large families of proteins, glycoproteins, collagens, and proteoglycans [5]. Liver biopsy is considered the gold-standard method for the assessment of liver fibrosis. Histologic examination is useful in identifying the underlying cause of liver disease and assessing the necroinflammatory grade and the stage of fibrosis. Fibrosis stage is assessed by using scales such as Metavir (stages I–IV) and Ishak score (stages I–V). Specific staining of ECM proteins (e.g. With Sirius red) can be used to quantify the degree of fibrosis, using computer-guided morphometric analysis. Liver biopsy is an invasive procedure, with pain and major complications occurring in 40% and 0.5% of patients, respectively. Sampling error can occur, especially when small biopsies are analyzed. Histologic examination is prone to intra- and interobserver variation and does not predict disease progression. Therefore, there is a need for reliable, simple, and non-invasive methods for assessing liver fibrosis. Scores that include routine laboratory tests, such as platelet count, aminotransferase serum levels, prothrombin time, and serum levels of acute phase proteins have been proposed [6].

Hepatic fibrosis can be estimated by imaging techniques. Ultrasonography, computed tomography, and MRI can detect changes in the hepatic parenchyma due to moderate to severe fibrosis. Due to its low cost, ultrasonography is an appealing technique. It is able to detect liver cirrhosis based on changes in liver echogenicity and nodularity as well as signs of portal hypertension. However, ultrasound is highly operator dependent, and the presence of increased liver echogenicity does not reliably differentiate hepatic steatosis from fibrosis [7]. Noninvasive methods currently in development include blood protein profiling using proteomic technology and new clinical glycomics technology, which is based on DNA sequencer/fragment analyzers able to generate profiles of serum protein N-glycans. As the technology becomes validated, the noninvasive diagnosis of liver disease may become routine clinical practice [8].

Cirrhosis results from different mechanisms of liver injury that lead to inflammation and fibrogenesis; histologically it is characterized by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent parenchymal extinction and collapse of liver structures, together causing pronounced distortion of hepatic vascular architecture. This distortion results in increased resistance to portal blood flow and hence in portal hypertension and in hepatic synthetic dysfunction. Clinically, cirrhosis has been regarded as an end-stage disease that invariably leads to death, unless liver transplantation is done, and the only preventive strategies have been screening for esophageal varices and hepatocellular carcinoma. The main causes in more developed countries are infection with hepatitis C virus, alcohol misuse, and, increasingly, non-alcoholic liver disease; infection with hepatitis B virus is the most common cause in sub-Saharan Africa and most parts of Asia [9]. The transition from chronic liver disease to cirrhosis involves inflammation, activation of hepatic stellate cells with ensuing fibrogenesis, angiogenesis, and parenchymal extinction lesions caused by vascular occlusion. This process leads to pronounced hepatic microvascular changes, characterized by sinusoidal remodeling (extracellular matrix deposition from proliferating activated stellate cells resulting in capillarisation of hepatic sinusoids), formation of intra hepatic shunts (due to angiogenesis and loss of parenchymal cells), and hepatic

endothelial dysfunction. The endothelial dysfunction is characterized by insufficient release of vasodilators, of which the most important is nitric oxide [10].

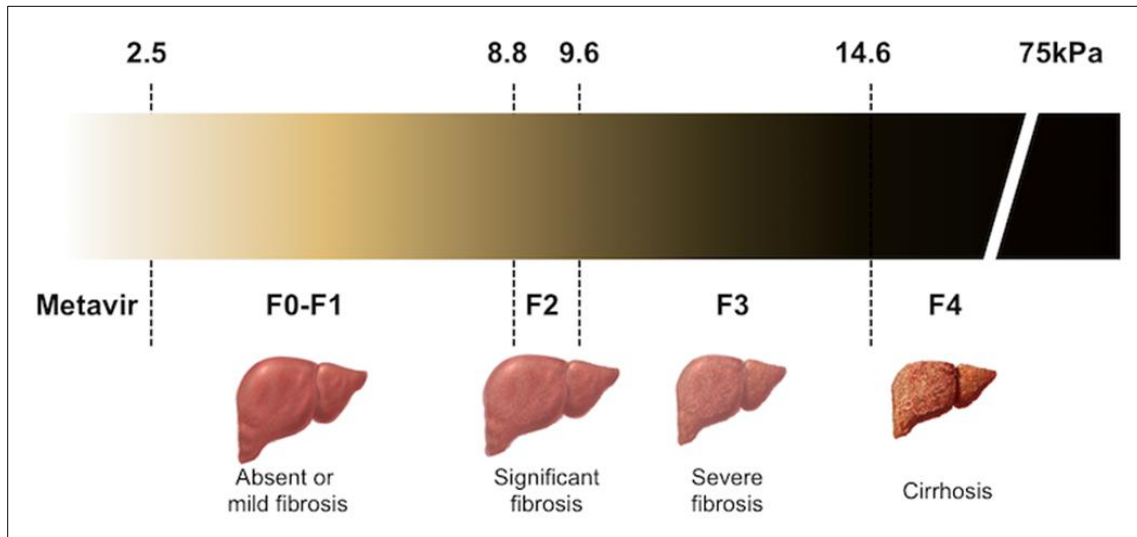
Most chronic liver disease is notoriously asymptomatic until cirrhosis with clinical decompensation occurs. Decompensating events include ascites, sepsis, variceal bleeding, encephalopathy, and non-obstructive jaundice. Imaging by ultrasonography, CT, or MRI of an irregular and nodular liver together with impaired liver synthetic function is sufficient for the diagnosis of cirrhosis. Other findings include small and shrunken liver, splenomegaly, and evidence of Porto-systemic collaterals. Differential diagnosis includes congenital hepatic fibrosis (fibrosis without regenerative nodules), nodular regenerative hyperplasia (nodules but no fibrosis), and non-cirrhotic portal hypertension. A liver biopsy is seldom needed but study of a sample can provide a definitive diagnosis and confirm the etiology in cases of uncertainty. The trans-jugular approach yields samples of equal quality to the percutaneous one, is safe, and adds additional prognostic information through measurement of hepatic-vein pressure gradient (HVPg). In early cirrhosis, however, conventional imaging can lead to false-negative diagnosis so other strategies are needed [11]. Hepatocellular carcinoma (HCC) is a primary malignancy of the liver and occurs predominantly in patients with underlying chronic liver disease and cirrhosis [12]. HCC is the most common primary liver cancer. The annual number of new cases of HCC worldwide is over one million, making it the 5th most common cancer worldwide and the 3rd leading cause of cancer-related death, preceded only by the lung and stomach cancers [13].



**Figure 2** Approach to the Patient with Liver Disease

HCC diagnosis is considerable interest to clinician’s evaluation patients with liver cirrhosis [14]. Generally, HCC appears with setting of cirrhosis with underlying chronic viral hepatitis (B or C) or alcoholism and more recently with nonalcoholic steatohepatitis [15]. In many patients, HCC is asymptomatic and then is diagnosed in an advanced stage. That is why, for cirrhotic patients, surveillance is strongly recommended to detect early HCC allowing an increase of patients suitable for curative treatment and then limit tumor related dead. Symptoms of HCC are commonly related to those of their chronic liver disease and include pain in the upper abdomen on the right side, a lump or a feeling of heaviness in the upper abdomen, swollen abdomen (bloating), anemia, weight loss, weakness or fatigue, nausea and

vomiting, yellowing skin and eyes, pale stools, and dark urine from jaundice caused by invasion of the biliary tree, fever, pain born in case of metastases [16].



**Figure 3** The METAVIRscoring system

### 1.1. FIB-4, APRI Scoring System

- **Fibrosis-4** (FIB-4) index, calculated as  $\text{age} \times \text{aspartate aminotransferase} / (\text{platelet count} \times \sqrt{\text{alanine aminotransferase}})$ , was developed as a noninvasive index to stage hepatic disease in patients with viral infection [17], and it is a simple and inexpensive measure of hepatic disorder. This index has been used across many hepatic diseases [18–19], and several studies have described the association between a high FIB4 index and poorer outcomes [20, 21], not only for hepatic disease but also for non-hepatic disease. Previous studies reported that the FIB4 index was associated with long-term mortality and readmission rate of heart failure patients [22, 23]. Some reports have shown that the FIB-4 index is not only a predictor of background liver fibrosis but also a prognostic factor after hepatectomy in patients with colorectal cancer liver metastases [24].
- **APRI** was calculated using the formula  $= \text{AST (U/L)} / (\text{upper limit of the normal range}) \times 100 / \text{platelet count (109 /L)}$ . The 40 U/L of AST was used as the upper limit of the normal range APRI reflect extent of liver injury and compensatory state of hepatic function which is simpler, cost effective [25]. Study done by Weilin Mao et al, On 193 chronic HBV infected patients. Mortality that occurred within 90 days of hospital stay was compared, which concludes that APRI is an independent predictor for mortality in patients with cirrhosis and they found positive correlation between the MELD score and APRI [26]. In a study conducted by Lieber CS1 et al, on 1308 patients, APRI has low sensitivity and specificity for the diagnosis of significant fibrosis in patients with alcoholic liver disease [27].

### 1.2. Non-Invasive Biomarkers

#### 1.2.1. Hyaluronic Acid

Hyaluronic Acid (HA) is known as a glycosaminoglycan which is a vital substance of extracellular matrix is found in the highest concentration in a fluid such as joint and eyes [28].

#### 1.2.2. Serum hepatocyte growth factor

Serum hepatocyte growth factor (HGF) has various levels in liver diseases, but it has exhibited a negative correlation with albumin concentration and prothrombin time in cirrhotic patients. In addition, individuals with hepatitis C virus had higher levels of serum HGF than those with hepatitis A or B viruses [29]

#### 1.2.3. Endostatin

Endostatin is an anti-angiogenesis factor. It is mainly produced after the degradation of collagen XVIII [30].

#### 1.2.4. Collagen type IV

Collagen type IV (CO-IV) is a substance of ECM that also with promise as a biomarker for liver fibrosis [31]. CO IV has been studied across several etiologies of chronic liver diseases [32].

#### 1.2.5. Kallistatin

Kallistatin, an endogenous human serine proteinase inhibitor, was originally known as a tissue kallikrein inhibitor [33]. The significantly reduced levels of serum kallistatin in patients with LC hypothesized that serum kallistatin levels could be a potential biomarker for liver cirrhosis as several studies have shown that the liver represents the major site of synthesis and secretion of kallistatin [34].

#### 1.2.6. Matrix Metalloproteinase-1

MMP-1, also known as collagenase-1, is a protein that cleaves both ECM and non-ECM substrates including collagen, gelatin, laminin, complement C1q, interleukin 1 beta, and tumor necrosis factor-alpha and thus plays a role in fibrotic and inflammatory processes [35]. It has been reported that MMP-1 overexpression attenuates fibrosis by promoting collagenase-1 degradation, alters the ECM network and thereby the cell-ECM interaction, induces hepatocyte proliferation and thus liver regeneration, and promotes HSC apoptosis and hence reduced collagen production [36].

#### 1.2.7. Alpha Fetoprotein

AFP was first described in 1953 as a fetal form of albumin and a marker for HCC. It has since become the best described and most used marker in HCC. It has been recognized for its usefulness in prognosis, its relationship to various indices of HCC human biology, its use in non-biopsy HCC diagnosis, its use in evaluation of responses to treatment and also can be used for screening of HCC which is done usually with imaging tools especially abdominal ultrasound in patients with hepatitis or cirrhosis who are known to be at risk for HCC development. However, not all HCC tumors are AFP positive or secrete elevated amounts of AFP into the serum [37].

#### 1.2.8. Circulating Cell-Free DNA

Circulating Cell-Free DNA (cfDNA) are extracellular DNA molecules released into blood from apoptotic or necrotic cells or tissues. cfDNA is elevated in various malignancies, including HCC, and has cancer-specific DNA alterations, including DNA strand integrity, mutation frequency, microsatellite abnormalities, and gene methylation, and is regarded as diagnostic, prognostic, and monitoring biomarkers for cancers [38].

#### 1.2.9. MicroRNA

MicroRNAs (miRNAs) are a family of endogenous, small (20–25 nucleotides in length), non-coding RNAs that regulate posttranscriptional gene expression by repressing messenger RNA (mRNA) translation mainly via binding at the complementary 3'-untranslated region and are well-known to play a role in human hepatocarcinogenesis. miRNAs serve as promising cancer biomarkers for diagnosis and therapy response monitoring [39, 40].

#### 1.2.10. Circular RNAs

Circular RNAs (circRNAs) are covalently closed, single-stranded, and stable transcripts that have been found to play important roles in the diagnosis of various cancers, including gastric cancer, breast cancer [41], lung cancer [42, 43], pancreatic cancer [44], and HCC [45].

#### 1.2.11. Osteopontin

Osteopontin (OPN), a secreted phosphoprotein, is associated with tumor invasion, progression, or metastasis in multiple types of cancer and has been considered to be a promising target for cancer therapy [46, 47]. HCC patients with elevated plasma levels of OPN were more likely to exhibit intrahepatic metastasis, early recurrence, and a worse prognosis [48].

#### 1.2.12. Angiopoietin-like protein 2

Angiopoietin-like protein 2 (ANGPTL2) is a secretory glycoprotein involved in vascular biology, inflammation, and tumor development [49]. ANGPTL2 is overexpressed in HCC tissues compared with non-cancerous liver tissues and able to promote HCC migration and invasion [50].

### 1.2.13. Glutamine synthetase

Glutamine synthetase (GS) is a metabolic enzyme that catalyzes the synthesis of glutamine (a major energy source of tumor cells) and has been revealed as a sensitive and specific indicator for the development of HCC [51].

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## 2. Conclusion

Non-invasive imaging techniques outperform serum scoring systems and most biomarkers are more accurate for advanced stage disease. Recent studies have started to evaluate combinations of serum biomarkers and to increase diagnostic accuracy, these tests can be used immediately to serve as essential pre-screening tools to rule-out advanced disease. Early detection and prevention will be the most effective and rational approach to substantially impact the prognosis of fibrosis patients rather than starting treatment at advanced stage. more studies are needed, it is clear that non-invasive biomarkers of fibrosis will have important clinical applications in the future and will be the key to cost-effective management of fibrosis patients and successful implementation of antifibrotic therapies. Non-invasive biomarkers are good alternatives to liver biopsy in the detection of liver fibrosis and chronic liver diseases.

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## Compliance with ethical standards

### *Acknowledgments*

Authors would like to thank professor. Hafez Ahmed, Professor of Biochemistry, Dubai Medical College for help and support.

### *Disclosure of conflict of interest*

Authors declare that they do not have any conflict of interest.

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