# Fibroindex, a simple noninvasive test for prediction of fibrosis in patients with chronic hepatitis C virus infection

THESIS
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*First and Foremost, Thanks are due to GOD,*
*The Beneficent and Merciful of All "*

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# *List of Abbreviations*

| | |
|---|---|
| ALT | Alanine transaminase |
| AMA | Antimitochondrial antibody |
| ANA | Antinuclear antibody |
| APRI | AST to platelet ratio index |
| AST | Aspartate transaminase |
| AUC | Area under curve |
| CBC | Complete blood count |
| CI | Confidant interval |
| CLDs | Chronic liver diseases |
| CT | Computerized tomography |
| ECM | Extracellular matrix |
| EGF | Epidermal growth factor |
| ELF | Enhanced liver fibrosis |
| ELISA | Enzyme linked immunosorbant assay |
| ET-1 | Endothelin-1 |
| FIB4 | Age , AST, ALT and platelet |
| H & E | Hematoxylin-eosin |
| HA | Hyaluronic acid |
| Hb | haemoglobin |
| HBsAg | Hepatitis B surface antigen |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |
| HSCs | Hepatic stellate cells |
| IL | Interleukin |
| INF γ | Interferon gamma |
| kPa | kilopascals |
| MCP-1 | Monocyte chemotactic protein-1 |
| MHz | Mega hertz |
| MMP | Matrix metalloproteinase |
| MRI | Magnetic resonance imaging |
| MT1-MMP | Membrane type 1 matrix metalloproteinase |
| NAFLD | Nonalcoholic fatty liver disease |
| NASH | Nonalcoholic steatohepatitis |
| NPV | Negative predictive value |
| PAI-1 | Plasminogen activator inhibitor-1 |
| PBC | Primary biliary cirrhosis |
| PC | Prothrombin concentration |
| PCR | Polymerase chain reaction |

| | |
|---|---|
| PDGF | Platelet derived growth factor |
| PGA | Prothrombin, gamma glutamyl transferase and apolipoprotein A1 |
| PICP | Procollagen I carboxy terminal peptide |
| PIIINP | Procollagen III amino terminal peptide |
| PPV | Positive predictive value |
| PT | Prothrombin time |
| PV | Portal vein |
| ROC | Receiver operating characteristics |
| ROI | Reactive oxygen intermediates |
| SD | Standard deviation |
| SHASTA | Serum hyaluronic acid, AST, and albumin |
| TGF- β | Transforming growth factor β |
| Th | T Helper lymphocyte |
| TIMP | Tissue inhibitor of metalloproteinase |
| TNFα | Tumour necrosis factor alpha |
| UPA | Uroplasminogen activator |
| | |
| WBC | White blood cell |
| γGT | Gamma glutamyl transferase |

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# Abstract

This study aimed to evaluate Fibroindex as simple noninvasive model consisting of routine laboratory tests derived from the platelet count, AST, and gammaglobulin measurements as predictor of clinically significant fibrosis in 33 patients with chronic HCV infection, and comparing its diagnostic performance to aminotransferase to platelet ratio index (APRI). The study revealed a significant positive correlation between Fibroindex and stage of fibrosis. The areas under the ROC curves for the Fibroindex were 0.962, 0.930 for predicting significant fibrosis (F2-F3) and cirrhosis (F4) respectively, better than the aminotransferase to platelet ratio index (APRI). Using the best cutoff values, significant fibrosis was diagnosed with high positive predictive value. Fibroindex had better sensitivity, specificity, positive and negative predictive values when compared with APRI. The study revealed that Fibroindex is a reliable noninvasive test in predicting HCV related fibrosis compared to liver biopsy being the gold standard for diagnosis of hepatic fibrosis.



## Key words

- Liver fibrosis
- Fibrosis biomarkers
- Fibroindex
- APRI

# Introduction

Information on the stage of fibrosis in chronic hepatitis is essential for making a prognosis and deciding on antiviral treatment ***(Castera et al., 2007).*** Liver biopsy is the gold standard for fibrosis staging in chronic HCV infection. However, liver biopsy is invasive, costly and requires an experienced hepatologist. Between 0.6% and 5% of patients who have undergone biopsy had complications ***(Regev et al., 2002).*** Moreover, recent studies have reported that inadequate sample size and sampling error frequently lead to underestimation of fibrosis stage ***(Rousselet et al., 2005).*** Therefore, there is a need for reliable, simple, and noninvasive methods for assessing liver fibrosis.

There are two main groups of noninvasive methodologies for the evaluation of hepatic fibrosis. The first group is the serum markers, which predict fibrosis stage using parameters measurable in serum, and can be categorized broadly as either direct or indirect serum markers ***(McHutchison et al., 2000).*** The second group is the liver imaging techniques (Ultrasound (U/S), computed tomography scan (CT), magnetic resonance image (MRI) and transient elastography) ***(Friedman, 2003).***

Recently, more sophisticated indices of fibrosis using indirect serum markers have been developed to enhance accuracy for detecting and staging of hepatic fibrosis in chronic HCV infection ***(Halfon et al., 2007).*** The most widely known of these indices are; Fibrotest (Haptoglobin, α2 macroglobulin, apolipoprotein A1, γGT and bilirubin) ***(Imbert-Bismut et al., 2001),*** Forns index (Age, platelet count, γGT and cholesterol) ***(Forns et al., 2002)*** and APRI (AST/platelet ratio index) ***(Wai et al., 2003).***
Particularly important among these new markers is Fibroindex consisting of platelet count, AST and gammaglobulin ***(Koda et al., 2007).*** Previous

studies reported that serum gammaglobulin is associated with liver fibrosis and portosystemic shunts and higher level in patients with score of F2 or F3 than in scores of F0 or F1 ***(Imbert-Bismut et al., 2001)***, and serum gammaglobulin was a significant parameter in differentiating cirrhosis from chronic hepatitis ***(Ikeda et al., 2000).*** The Fibroindex was derived from the platelet count, AST, and gammaglobulin measurements in a cohort of 360 patients who were divided into an estimation set (N= 240) and a validation set (N=120), in addition to longitudinal set consisted of 30 patients who had undergone a liver biopsy twice, before and after antiviral treatment, and this study reported that Fibroindex significantly increased according to fibrosis stage, and it has greater diagnostic value than either APRI or Forns index. Furthermore, in the longitudinal set, there was a significant decrease in the Fibroindex of patients whose fibrosis stage improved, and a significant increase in patients whose fibrosis stage deteriorated. There was no such correlation with Forns index or APRI, and the study reported that Fibroindex is a simple and reliable test for predicting significant fibrosis in chronic hepatitis C and could also be used as a surrogate marker during antifibrotic treatment ***(koda et al., 2007)***.

# Aim of work

This study aimed to evaluate Fibroindex as a simple noninvasive model consisting of routine laboratory tests derived from platelet count, AST and gammaglobulin measurements as predictor of clinically significant fibrosis in patients with chronic HCV infection, and comparing its diagnostic performance to aminotransferase to platelet ratio index (APRI).

# Liver fibrosis

Liver fibrosis is the excessive accumulation of extracellular matrix (ECM) proteins including collagen that occurs in most types of chronic liver diseases ***(Friedman, 2003)***. It is considered a model of wound healing response to chronic liver injury ***(Albanis et al., 2001).***

The accumulation of ECM proteins distort the hepatic architecture by forming a fibrous scar, with subsequent development of nodules of regenerating hepatocytes defines cirrhosis. Cirrhosis produces hepatocellular dysfunction and increases intrahepatic resistance to blood flow, which results in hepatic insufficiency and portal hypertension, respectively ***(Gines et al., 2004).*** In contrast with the traditional view that cirrhosis is an irreversible disease, recent evidence indicates that even advanced fibrosis is reversible ***(Arthur, 2002 ).***

## Natural history of liver fibrosis

The development of fibrosis usually requires several months to years of ongoing injury, however liver fibrosis progresses rapidly in several clinical settings including repeated episodes of severe acute alcoholic hepatitis, subfulminant hepatitis, and HCV reinfection after liver transplantation ***(Berenguer et al., 2003).***

The natural history of liver fibrosis is influenced by both genetic and environmental factors. Epidemiological studies have identified polymorphisms in a number of candidate genes that may influence the progression of liver fibrosis in humans. These genetic factors may explain the broad spectrum of responses to the same etiological agent found in patients with chronic liver diseases ***(Bataller et al., 2003).***

## Pathogenesis of liver fibrosis

The normal liver parenchyma is composed of an epithelial component (Hepatocytes), an endothelial lining distinguished by fenestrations or pores (Sinusoids), tissue macrophages (Kupffer cells), and liver specific pericytes known as hepatic stellate cells (HSCs). The sinusoid is the liver microvascular unit, with the subendothelial space of Disse separating the hepatocytes from the sinusoidal endothelium. This space contains a basal membrane-like matrix essential for maintaining the function of all resident liver cells and for ensuring optimal metabolic exchange between the blood stream and hepatocytes. Portal tracts are key structures in the architecture of liver tissue and include branches of the portal vein, the hepatic artery, bile ducts, lymphatic ducts and stromal cells (Portal myofibroblasts and fibroblasts) ***(Schuppan et al., 2001).***

Hepatic fibrosis is a result of wound healing response of the liver to repeated injury. After an acute liver injury (e.g. viral hepatitis) parenchymal cells regenerate and replace the necrotic or apoptotic cells. This process is associated with an inflammatory response and a limited deposition of ECM. If the hepatic injury persists, inflammatory lymphocytes infiltrate the hepatic parenchyma. Some hepatocytes undergo apoptosis, and Kupffer cells activate, releasing fibrogenic mediators. HSCs proliferate and undergo a dramatic phenotypical activation, secreting large amounts of ECM proteins. Sinusoidal endothelial cells lose their fenestrations, and the tonic contraction of HSCs causes increased resistance to blood flow in the hepatic sinusoid then eventually the liver regeneration fails, and hepatocytes are substituted with abundant ECM, including fibrillar collagen ***(Friedman, 2003)*** (Figure 1).

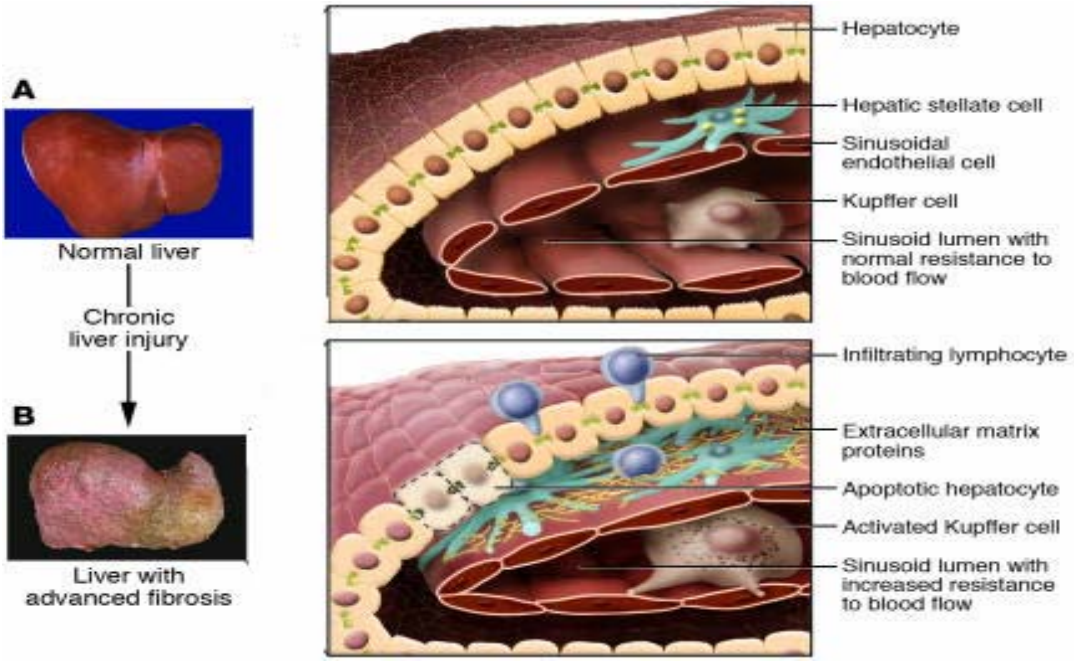


**Figure (1):** (A) Normal liver, (B) Injured liver with fibrosis ***(Bataller and Brenner, 2005)*.**

## Extracellular matrix in normal and fibrotic liver

Extracellular matrix (ECM) refers to a group of structural and supporting molecules. These include; collagens, noncollagen glycoproteins, matrix bound growth factors, proteoglycans and glycosaminoglycans ***(Schuppan et al., 2001)***.

In the normal liver, collagens (Types I, III, V, and XI) are largely confined to the capsule, the area around large vessels, and the portal triad, with only scattered fibrils containing types I and III in the subendothelial space, with subendothelial deposits of glycoproteins and proteoglycans.

In advanced fibrosis, significant changes occur in the ECM quantitatively and qualitatively. These include, increase in fibril-forming collagens (Types I and III), non-fibril forming collagens (Types IV and VI), glycoproteins (Cellular fibronectin and laminin) and glycosaminoglycans (Perlecan, decorin, aggrecan, lumican, and fibromodulin). These

processes represent a change in the type of ECM in subendothelial space from the normal low density basement membrane-like matrix to the interstitial type ***(Bataller and Brenner, 2005).*** These quantitative and qualitative changes in the composition of ECM have the following implications:

ECM is a dynamic regulator of cell function. The replacement of the low density matrix with the interstitial type leading to loss of hepatocyte microvilli and disappearance of endothelial fenestrations, which impairs transport of solutes from the sinusoid to the hepatocyte ***(Gressner, 1998).***

ECM directly perturbs hepatocyte function, activates HSCs and directly influences the function of surrounding cells through interaction with cell surface receptors, including integrins and nonintegrin matrix receptors ***(McGuire et al., 1992).***

ECM can also indirectly affect cell function via release of soluble cytokines ***(Safadi and Friedman, 2002).***

## Cellular sources of extracellular matrix

Activated hepatic stellate cells (HSCs) are the primary source of ECM in normal and fibrotic liver. In addition, related mesenchymal cell types from a variety of sources may have measurable contributions to total matrix accumulation, including sinusoidal endothelial cells, portal fibroblasts, and myofibroblasts of bone marrow origin ***(Forbes et al., 2004).***

**Hepatic stellate cells (HSCs)**

Formerly known as lipocytes, Ito cells are resident perisinusoidal cells in the subendothelial space between hepatocytes and sinusoidal endothelial cells. This cell type was first described by Von Kupffer in 1876.

They are the primary site for storing retinoid (Vitamin A) within the body and the primary source of ECM in normal and fibrotic liver. Stellate cells actually comprise a somewhat heterogeneous group of cells which are

functionally and anatomically similar but differ in their expression of cytoskeletal filaments, their retinoid content, and in their potential for activation ***(Geerts, 2001)***. Animal and human studies have defined a process of changes within stellate cells that collectively are termed "activation ". During activation, stellate cells undergo a transition from a quiescent vitamin A-rich cell into prolifrative, fibrogenic, and contractile myofibroblasts. Stellate cell activation is a key event in liver injury, and proliferation of stellate cells occurs in regions of greatest injury, and is typically preceded by an influx of inflammatory cells and associated with subsequent ECM accumulation ***(Friedman, 2004)***.

**Sinusoidal endothelial cells**

They are an important contributor to early fibrosis. Increased expression of cellular isoforms of fibronectin by these cells is an important early event following acute liver injury because their appearance creates a microenvironment that activates stellate cells ***(Geerts, 2001).***

**Portal fibroblasts**

These cells derived from small portal vessels and have fibrogenic potential; they proliferate around biliary tracts in cholestasis-induced liver fibrosis to initiate collagen deposition ***(Magness et al., 2004)***.

**Myofibroblasts of bone marrow origin**

Culture of hematopoietic stem cells with various growth factors has been shown to generate HSCs and myofibroblasts of bone marrow origin that infiltrate human liver, undergoing tissue remodeling. These data suggest that cells originating in bone marrow can be a source of fibrogenic cells in the injured liver ***(Forbes et al., 2004).***