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Diagnosis of liver fibrosis

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Abstract. – **Hepatic fibrosis and cirrhosis represent the main consequence of chronic liver diseases of different origin. Therefore, the clinical assessment of disease severity is a must in the management of patients with chronic liver damage. Although liver biopsy may be associated with sampling error, interobserver variability and potential complications, it still remains the gold standard for establishing the severity of hepatic necroinflammation and fibrosis. In the last years, several non-invasive tests for the assessment of disease activity and stage have been proposed. However, at present all these tests are not totally accurate and reliable and need further evaluation.**

Key Words:

Liver, Fibrosis, Cirrhosis, Liver Function Test.

Definition of Liver Fibrosis

Hepatic fibrosis represents the response of the liver to different chronic insults, and is associated with significant morbidity and mortality[1]. It is now clear that the excessive accumulation of extracellular matrix in liver fibrotic diseases is mainly regulated by hepatic stellate cells (HSC)[1]. These cells comprise 15% of the total number of resident liver cells and, in the normal liver, they represent the main storage site for retinoids[1].

Following liver injury of any etiology, HSC undergo a process of activation developing a myofibroblast-like phenotype associated with increased proliferation and collagen synthesis[1-3]. Similar morphological and functional modifications can be observed when HSC are cultured on uncoated plastic dishes[4]. Using this experimental model, it has been demonstrated that the process of HSC activation results from the complex interplay of different factors, such as cytokines[5], growth factors[6-7], oxidative stress[8-10] and modification of Na^+/H^+ exchange activity[11-15].

Current evidence indicates that liver fibrosis is a dynamic and bidirectional process, and that this process involves changes in the regulation of matrix degradation in addition to incresed matrix synthesis[16]. In the extracellular space, matrix degradation occurs especially as a consequence of the action of a family of enzymes called matrix metalloproteinases (MMPs). These enzymes are secreted from liver cells (mainly HSC and Kupffer cells) into the extracellular space as proenzymes, which are then activated by specific cleavage mechanisms. The active enzymes are in turn inhibited by a family of tissue inhibitors of metalloproteinases (TIMPs)[16]. Therefore, extracellular matrix deposition and degradation are closely regulated by this combination of mechanisms.

As a result of this process, there is a progressive accumulation of extracellular matrix proteins (especially collagen types I and III) in the liver parenchyma that distorts the normal hepatic architecture by forming a fibrous scar. The main clinical consequences of advanced liver fibrosis are impaired hepatocellular function and increased intrahepatic resistance to blood flow, which in turn result in hepatic insufficiency and portal hypertension. Although the most effective treatment of liver fibrosis is to remove the causative agent, many promising new treatments can significantly reduce collagen accumulation in different experimental models of chronic liver injury[17].

Role of Liver Biopsy in Hepatic Fibrosis

One of the mayor clinical problems at present is how best to evaluate and manage patients with chronic liver diseases, and especially those with hepatitis C virus (HCV).

However, in the last years little progress has been made in improving our ability to determine the degree of liver injury as well as to predict the risk of disease progression for the individual patient.

To date, liver biopsy remains the gold standard for fibrosis assessment in patients with chronic liver diseases[18,19], but this procedure is associated with risks of complications, patient discomfort and expense. In addition, interobserver variability and sampling error may lead to erroneous staging. As regards to this, it has been shown that the interobserver and intraobserver agreement in fibrosis range from 70% to 90% and from 60% to 90%, respectively[20,21]. These findings are not surprising, since a 2 cm core of liver tissue represents only 1/50,000th of the whole organ, and since fibrosis is a heterogeneously distributed lesion. To reduce the risk for false evaluation, the use of a biopsy specimen of sufficient lenght and including a sufficient number of portal tracts is usually recommended. A recent study by Bedossa et al[22] has clearly shown that, by using the METAVIR scoring system, 65% of biopsies 15-mm in lenght are categorized correctly according to the reference value, and that this percentage increases to 75% for liver biopsy specimens 25-mm in lenght.

To help standardize assessment of liver histology among pathologists, several groups have proposed histological schemes for grading activity and staging fibrosis. Among these, the Histology Activity Index (HAI) of Knodell[23], its modification by Ishak et al.[24] and the METAVIR system[25] are the most used ones. Necroinflammation is graded on a scale of 0 to 18 in the HAI systems and on a scale of 0 to 3 in the METAVIR system. For the quantitation of fibrosis, which is more relevant in the assessment of liver disease stage, the HAI systems and the METAVIR system rely on scales of 0 to 6 or 0 to 4.

From a hepatological point of view, liver biopsy in patients with chronic liver diseases, and especially in those with HCV, may be useful to evaluate the rate of progression, to help in determinig the urgency of treatment, to exclude other forms of liver disease, to predict response to therapy and to provide baseline histology against which to compare future biopsies[26]. However, liver biopsy is an invasive procedure and adverse events are a possibility. Among these, transient pain occurs in about 30% of patients, severe complications (hemoperitoneum, pneumothorax, bile peritonitis and punctured viscera) in 3% and death in 0.03%[27-30].

Anyway, at present both the histological grade and stage apper to be the most reliable prognostic variables in monitoring patients with chronic liver diseases. At confirmation of this, it has been shown that patients with mild hepatitis and limited fibrosis progress slowly or not at all over a 10- to 20-year period, whereas those with more severe inflammation and fibrosis progress inevitably to cirrhosis[31,32]. Therefore, a baseline biopsy in patients with chronic hepatitis C may be useful to determine the urgency of initiating treatment. On the contrary, liver biopsy is not indicated in patients with persistently normal aminotransferase levels, in which liver damage has been documented not to progress[33,34], at least until their disease becomes biochemically active or until more effective and better tolerated treatment are available[26].

Non-Invasive Monitoring of Patients with Chronic Liver Diseases

Routine Laboratory Tests

Due to the "potential" limitations of liver biopsy, a non-invasive assessment of disease activity and stage in monitoring patients with chronic liver diseases would be helpful. However, how we will see later, the various non-invasive tests proposed by different authors are, at present, imperfect and lack accuracy and reliability[35].

Laboratory tests are routinely included in the evaluation of patients with chronic liver diseases. As well known, serum alanine aminotransferase (ALT) levels reflect liver injury, but a weak correlation has been found between ALT levels and the degree of inflammation and fibrosis[36]. On the contrary, the combination of different laboratory test seems able to predict the extent of fibrosis. In a recent paper[37], Forns et al. constructed a model and a score system by combining age, g-glutamyltranspeptidase (GGT), cholesterol and platelet count, and found that this model was able to discriminate patients with mild fibrosis from those with more severe fibrosis.

This model, however, may not be so accurate, since serum cholesterol may vary with HCV genotype and platelet count is poorly standardized among laboratories[38]. For this reason, two alternative biochemical tests (FibroTest and ActiTest) have been proposed[39-41], which seem to provide a more accurate estimate of fibrosis related to HCV infection and which can be computed by different laboratories with acceptable variability The tests combine five components (α_2-macroglobulin, haptoglobin, apolipoprotein A1, GGT and total bilirubin) for FibroTest and the same plus ALT for ActiTest. However, as stated above, the real clinical usefulness of these biochemical tests needs further validation and standardization.

Quantitative Tests of Liver Function

These tests are based on the turnover of a substance which is metabolized by the liver and whose clearance is influenced by hepatic blood flow and hepatic metabolic capacity. In a recent study[42], two tests of hepatic metabolism (aminopyrine breath test and galactose elimination capacity) were compared to two tests of hepatic blood flow (indocyanine green and sorbitol clearance). Although metabolic liver function was decreased also in patients with mild or moderate fibrosis, hepatic perfusion declined only in those with severe fibrosis or cirrhosis, thus suggesting that tests of hepatic metabolic function could be used in evaluating liver fibrosis. However, these tests are expensive and not easily applicable in the clinical practice, their accuracy needs to be validated, and the predictive values for the different stages of fibrosis is not known.

Serum Markers of Liver Fibrosis

As already stated, liver fibrosis is a dynamic process characterized by a balance of extracellular matrix deposition and degradation. Therefore, measurement of circulating matrix proteins related to fibrogenesis and fibrolysis could be a potential valuable tool to monitorize the fibrogenic process. Among the numerous proposed markers, serum hyaluronic acid and N-terminal propeptide of type III collage (PIIINP) have been the more extensively studied, especially in patients with chronic hepatitis C. In these patients, a significant correlation was found between serum hyaluronic acid levels and the degree of hepatic fibrosis[43,44]. In addition, serum hyaluronate correlates with histological progression in patients with alcoholic liver disease[45]. However, the accuracy of serum hyaluronic acid in diagnosing cirrhosis in extremely low[43].

The evaluation of serum PIIINP levels could be a sensitive marker of hepatic fibrogenesis, due to its correlation with hepatic transforming growth factor β1 (TGFβ1) mRNA levels[46], and because of its capacity to predict response to treatment in patients with chronic hepatitis C[47]. However, its role in predicting hepatic fibrosis has to be determined yet[44].

$TGF_{\beta 1}$ is an important cytokine which plays a key role in the fibrogenic process by stimulating activation of HSC and by modulating extracellular matrix deposition and degradation. It has been shown that progression of fibrosis is paralleled by an increase in TGFβ1 serum levels[48-50], thus suggesting that the serum kinetic of this cytokine could reflect the degree of fibrosis in patients with chronic liver diseases.

Recently, YKL-40, a new protein expressed in human liver and involved in the remodeling of the extracellular matrix, has been reported to be superior to hyaluronic acid levels in predicting hepatic fibrosis[51,52], although further studies in larger number of patients are needed to confirm the clinical utility of this marker.

Based on available data, it is unlikely that at present individual serum markers of fibrosis could replace liver biopsy in monitoring the progression of fibrosis in patients with chronic liver diseases. In fact, in order to accurately reflect hepatic fibrogenesis or fibrolysis, these markers should be organ-specific and their biological half-life should be independent of biliary and urinary excretion. Moreover, they should also be simple to perform, inexpensive, reproducible and accurate. However, up to now none of the available markers are able to fulfill the above mentioned criteria. If a combination of different markers could provide a higher accuracy remains to be determined.

Does Radiology Have a Role in Assessing Liver Fibrosis?

Imaging techniques, such as ultrasound, computed tomography (CT) and magnetic

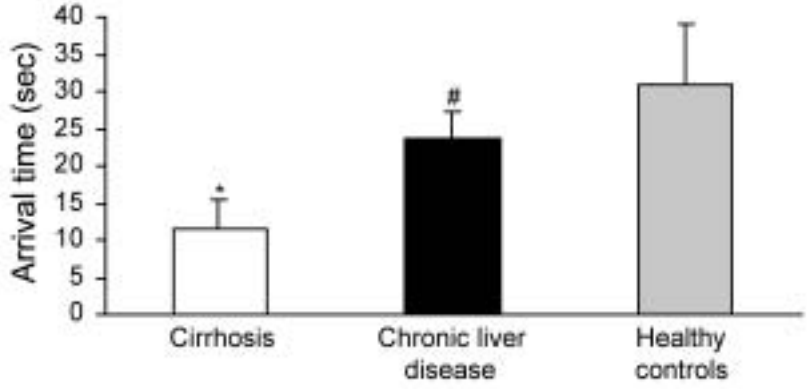

Figure 1. Arrival time (mean ± SD) of the contrast agent to the hepatic vein in healthy controls, in patients with chronic liver diseases and in patients with established cirrhosis. *$P < 0.01$ vs other groups; #$P < 0.05$ vs healthy controls.

resonance imaging (NMR), may be of help in the clinical evaluation of patients with chronic liver diseases. However, CT and NMR are quite expensive, and ultrasound is usually operator-dependent and cannot differentiate hepatic steatosis from fibrosis.

In the last years, microbubble contrast agents have been developed for ultrasonography[53]. These agents are confined to the intravascular space and enhance doppler signals by about 20 dB. As well known, hepatic cirrhosis is associated with several hemodynamic changes: arterialization of the liver; formation of intrahepatic shunts between the branches of the hepatic artery, the portal vein and the hepatic veins; hyperdynamic circulatory state with increased cardiac output and reduced systemic vascular resistance.

On the basis of these observations, it has been postulated that the hemodynamic changes in patients with cirrhosis would re-

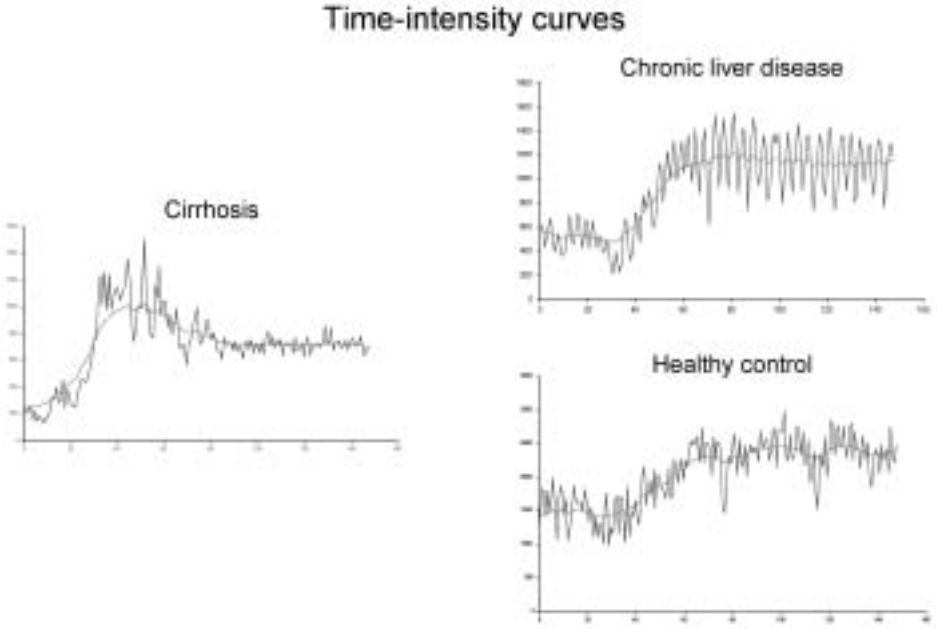

Figure 2. Examples of hepatic vein time-intensity curves of a patient with established cirrhosis, a patient with non-cirrhotic chronic liver disease and a healthy control. As evident, the arrival time of the contrast agent is earlier in cirrhotic patients with respect to the other groups.

Table I. Etiology of hepatic cirrhosis.

Etiology	*N. of patients*
HCV infection	11
HCV infection + alcohol abuse	5
Alcohol abuse	5
HBV infection	3

Table II. Etiology of hepatic cirrhosis.

Etiology	*N. of patients*
HCV infection	17
Alcohol abuse	4
Non-alcoholic steatohepatitis (NASH)	3
Primary biliary cirrhosis (stage 1)	2
Granulomatous hepatitis	1
Autoimmune cholangitis	1

sult in a earlier appearance of the hepatic venous signal from a microbubble bolus injected into a peripheral vein[54]. By using Levovist, which consists of galactose microparticles and a small admixture of palmitic acid, Albrecht et al. have demostrated that the arrival time of the contrast agent to the hepatic vein (usually the middle one) was significantly earlier in patients with established cirrhosis with respect to patients with chronic liver diseases and to normal volunteers[54], thus suggesting that this tecnique could be used as a simple non-invasive test to discriminate cirrhotic from non cirrhotic patients and, possibly, to evaluate liver damage progression in patients with chronic liver diseases.

Our group also have recently investigated this aspect[55]. Sixty-five consecutive subjects have been evaluated: 24 patients with histological or clinical diagnosis of cirrhosis (10 Child A, 7 Child B and 7 Child C), 28 patients with chonic liver diseases and 13 healthy controls. The etiology of liver damage in patients with cirrhosis and in those with chronic liver diseases is shown in Tables I and II.

As evident from Figures 1 and 2, the arrival time of the contrast agent (Levovist) to the hepatic vein was significantly lower in patients with cirrhosis with respect to non-cirrhotic patients with chronic liver diseases and

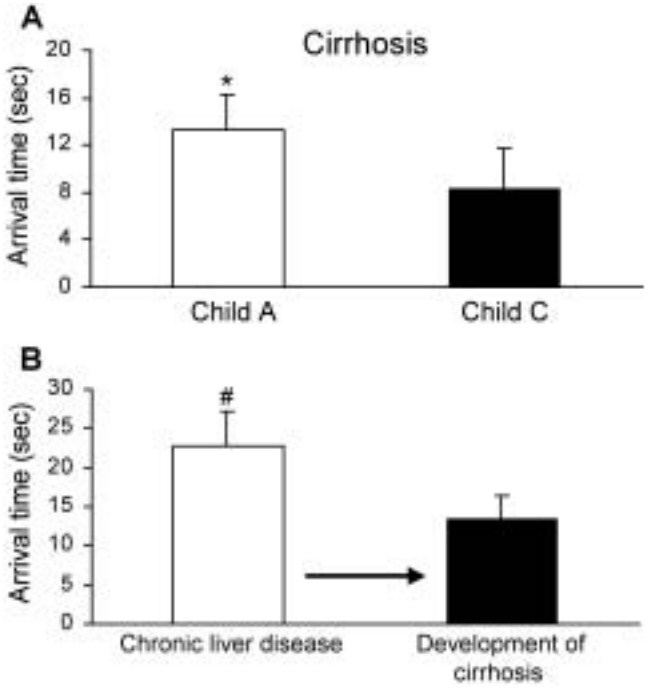

Figure 3. **A,** Arrival time (mean ± SD) of the contrast agent to the hepatic vein in patients with established cirrhosis divided according to the Child score. *$P < 0.05$ vs Child C. **B,** Arrival time (mean ± SD) of the contrast agent to the hepatic vein in patients with chronic liver disease who developed cirrhosis. *$P < 0.01$ vs the other group.

to healthy controls. In addition, in the cirrhotic group, the arrival time of the contrast agent was significantly lower in Child C patients with respect to Child A patients (Figure 3, panel A).

During the study, five patients with chronic liver diseases developed portal hypertension and, for this reason, they underwent to a second doppler evaluation. As shown in Figure 3, panel B, the arrival time of the contrast agent was significantly lower than that observed at baseline.

Therefore, all these results suggest that this novel technique could represent a simple non-invasive test for the diagnosis of cirrhosis in clinical practice and in the follow-up of patients with chronic liver diseases which, as previously stated, require several biopsies for treatment planning. Of course, studies which correlate ultrasonographic findings to hepatic histology (and especially fibrosis) are needed to confirm the potential usefulness of this tecnique.

Concluding Remarks

In the last years, significant progress has been made in the development of non-invasive tests for monitoring patients with chronic liver diseases. These tests should be simple, cheap, and widely available; moreover, they should be able to accurately reflect the entire spectrum of hepatic inflammation and to reliably distinguish mild from moderate and severe fibrosis, in order to make treatment decisions and disease monitoring easier to clinicians. At present, none of the currently available tests can replace liver histology in the evaluation and follow-up of patients with chronic liver diaseses, due to the complexity of factors which may influence the progression of liver damage. However, all the efforts performed in these last years make us to believe that an accurate and non-invasive monitoring of patients with chronic liver diseases will become a reality in the next future.

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