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Noninvasive markers of liver fibrosis in Latin America and Mexico

Misael Uribe,*,** Ylse Gutiérrez-Grobe,* Ramón Arturo Kobashi-Margáin,* Nahum Méndez-Sánchez*

* Digestive Disease Unit, Medica Sur Clinic & Foundation, Mexico City, Mexico.
** Department of Gastroenterology, Instituto Nacional de Ciencias Medicas y Nutricion Salvador Zubiran, Mexico City, Mexico.

INTRODUCTION

The basic concept of fibrosis can generally be applied to the evolution of type C chronic hepatitis. Hence, fibrosis or scarring is generally considered a healing wound response to limit tissue damage after chronic injury, in this case resulting from the effects of hepatitis C virus. Chronic type C hepatitis is classified by degrees of inflammation with or without hepatic fibrosis. The illness affects an estimated 2.7 million persons in the USA and close to one million in Mexico. It is calculated that it affects close to 170 million people worldwide. A significant proportion of these patients may develop progressive liver fibrosis that eventually becomes liver cirrhosis. Currently, cirrhosis secondary to type C hepatitis is the number one indication for liver transplantation.[1] Assessment of liver fibrosis may be an essential tool for management and an indicator for transplantation.

Even though the liver biopsy remains the gold standard, in many cases what we need to know once therapy has been indicated is the development and evolution of liver fibrosis or cirrhosis.[2]

Conventional laboratory biochemistry (commonly called liver function tests) and serological tests have poor value for assessment of liver fibrosis. Because of these limitations, histopathological examination was for many years the gold standard for evaluation, although the procedure is far from ideal. However, liver biopsy also has a number of limitations, among which are that it is high cost, is operator-dependent (with intra- and interobserver error more evident in intermediate grades of fibrosis) and although rarely associated with mortality, is more frequently associated with morbidity of variable degree or with patient discomfort. Surveys among patients requiring liver biopsies indicate that the procedure may not be accepted by a significant number of patients in whom it is needed. For example, a study carried out in France mentioned that over 50% of patients with hepatitis C are reluctant to see a hepatologist because of the fear that they will be asked to have a liver biopsy.[3] It is clear that a more accessible, easier to perform, safer, more reliable and more accepted test for evaluating liver fibrosis should be developed. Hence, research has recently investigated the possibility of developing serum biomarkers of fibrosis related to ratios of metabolic or laboratory abnormalities that can be used for assessing fibrosis. This has been supplemented by the recent appearance of Fibroscan, a method able to determine liver tissue stiffness, and the investigation of its correlation with fibrosis.

Another factor is that the small size of the liver biopsy in a high percentage of cases precludes the establishment of a reliable diagnosis. It is known that in some cases the number of portal triads in the biopsy limits diagnosis, while interpretation is variable, with intra- and interobserver disagreements. This is particularly common in chronic, unevenly distributed lesions such as cirrhosis: in these cases the unevenness of fibrosis may mean that a small sample of liver from a punch biopsy may not accurately reflect the morphological changes that have developed in the whole liver.

For many years, liver fibrosis was considered an irreversible disease. However, with the acquisition of novel therapies, the need to assess the evolution of fibrosis has arisen. It has been claimed that as many as 8% of hepatitis C cirrhosis patients may achieve complete reversion of cirrhosis,[4] but this assessment is limited by the invasive nature of a standard liver biopsy. Thus, to allow follow-up of patients a validated, noninvasive, reproducible method for evaluation of fibrosis is needed. If it is assu-

Correspondence and reprint request: Dr. Misael Uribe
Liver Research Unit, Medica Sur Clinic and Foundation,
Puente de Piedra 150, Col. Toriello Guerra, Mexico City, Mexico.
Tel.: +525-55606-6222 (4215) Fax: +525-55666-4031
E-mail: muribe@medicasur.org.mx

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med that 1.5 million people in Mexico have type C chronic hepatitis, up to 400,000 of these will need evaluation, treatment and follow-up evaluations after treatment.

There are a number of candidate noninvasive methods for such assessment. These include imaging studies, many of which have been used in the past,[5] together with currently-used imaging methods such as computed tomography (CT), magnetic resonance imaging (MRI) and ultrasound, which reliably detect gross manifestations of the presence of cirrhosis such as liver nodularity, liver-to-spleen size ratio, large caudal lobe and the appearance of varices. However once again, because of intra- and interobserver and anatomical variation, the results are not reliable and are useless to monitor fibrosis at a detailed level.[6]

ASSESSMENT OF HEPATIC FIBROSIS: BENEFITS AND PITFALLS OF LIVER BIOPSY

As stated above, standard biochemical tests (sometimes described as "liver function tests") and serological tests have poor or little value in the assessment of fibrosis. As a result, histopathological examination of a liver biopsy specimen is currently the recommended method for staging liver disease. However, as with any method, liver biopsy has limitations, among which are cost, patient morbidity and (rarely) mortality and the use of different histopathological scoring tools (interpretation).[7] It is not usually well accepted by patients because it is an invasive procedure. In addition, the liver tissue sample contained in a punch biopsy is only small (usually 2 mm x 2–3 cm) and is therefore subject to sampling variation and to intra- and interobserver error, especially in chronic liver diseases where pathological lesions are unevenly distributed throughout the parenchyma.

In addition, our increasing knowledge of fibrosis has led to the development of potential antifibrotic agents, so a more accurate, reproducible and noninvasive assessment of hepatic fibrosis is required to monitor progression, response or failure of treatment and clinical outcomes. However, no ideal marker of fibrosis has yet been identified. Most of the published studies compare the proposed noninvasive methods with blinded reading and grading of fibrosis in a liver biopsy by the Metavir or Ishak/Knodell methods, using receiver-operating characteristic (ROC) curves to determine their value compared with the histological specimen.[8]

TRADITIONAL NONINVASIVE IMAGING STUDIES USED TO IDENTIFY CIRRHOSIS[9]

Noninvasive assessment of hepatic fibrosis includes cross-sectional images such as CT and studies such as MRI and ultrasound. However, the clinical utility of these approaches is limited by variations in anatomy and inconsistent intra- and interobserver agreement, which results in insufficient and inaccurate assessment of the early stages of fibrosis. The most common predictors of fibrosis or cirrhosis detected by imaging methods are signs of portal hypertension, splenomegaly, large caudate lobe, large varices and the liver-to-spleen size ratio. These findings confirm advanced liver disease but are limited in their ability to give reliable data regarding less advanced fibrosis.

ELASTOGRAPHY USING ECHOSENS® FIBROSCAN

A novel technique based on transient unidirectional elastography has emerged as a promising noninvasive approach for staging hepatic fibrosis. The technique measures the mean stiffness of hepatic tissue rapidly, painlessly and noninvasively. With the use of a probe (Fibroscan; Echosens, Paris, France), which includes an ultrasonic transducer, an amplitude wave vibration of low frequency (50 MHz) is transmitted through the liver. The vibration induces an elastic shear wave that propagates through the organ. The velocity of the wave as it passes through the liver correlates directly with tissue stiffness. The harder or stiffer the tissue, the faster the shear wave propagates. Results are expressed in kilopascals (kPa): a pascal is a unit of pressure exerted per square meter and one kPa is 1,000 pascals. The Fibroscan method measures, via an intercostal window similar to the site where a liver biopsy is obtained, the stiffness of a volume of liver that is a cylinder of approximately 1 cm diameter and 5 cm in length. This is one hundred times greater in volume than a standard liver biopsy sample, and thus may be more representative of the entire hepatic parenchyma. This method is quick, inexpensive, reproducible, painless, and examines a large mass of tissue, thereby reducing sampling errors.

However, there are some limitations to the performance of a Fibroscan in patients with ascites or in those with morbid obesity, although a new probe for morbidly obese patients is already available in some places. The presence of fluid and adipose tissue attenuates the elastic wave.

Research has been initiated in this exciting area to correlate liver stiffness with the appearance and development of complications of cirrhosis, in particular its correlation with edge hepatic pressure. A recent study conducted by Samonakis, *et al*.[10] showed that the hepatic venous pressure gradient is a very good hemodynamic marker that can also be used as a marker of fibrosis progression before cirrhosis and can be performed together with a transjugular liver biopsy to give adequate samples. The authors recommend repeat histological sampling in case of discrepant results.

In addition, a study conducted by Moal, *et al*.[11] suggested that image analysis of fractal geometry can be applied to liver fibrosis. This seems to be an accurate quantitative morphometric measurement of the geometric complexity of liver fibrosis. Other characteristics such as reproducibility, rapidity, adaptability and simplicity of support should be useful in the evaluation of liver fibrosis.

SUGGESTED SERUM MARKERS OF FIBROSIS

A large number of serum biomarkers have been proposed for the assessment of liver fibrosis. These can be divided into direct and indirect markers.[12] Indirect markers reflect alterations in hepatic function but do not directly reflect extracellular matrix metabolism. Examples include platelet count, coagulation studies and liver aminotransferase ratios. Several studies have also evaluated the accuracy of combinations and ratios of these measures. The most widely used are listed below.[13]

1. The aspartic aminotransferase/alanine aminotransferase (AST/ALT) ratio.
2. The AST-to-platelet ratio index or APRI = AST level (IU/L x 100/platelet count (1 x 10^9/L).
3. The PGA index, which combines the measurement of the prothrombin index, gamma glutamine transferase level and apolipoprotein A1 and has been validated particularly in alcoholic liver disease.[14]
4. Fibroindex, which is derived from the platelet count, AST and gamma globulin measurements and has been proposed as a marker of fibrosis in chronic hepatitis C virus infection.[15]
5. The FIB4, which combines platelet count, ALT, AST and age. This test has been shown in at least two studies involving patients with chronic hepatitis C virus infection to have good predictive accuracy for advanced fibrosis.[16,17]
6. Fibrometer, which involves a combination of the platelet count, prothrombin index, AST, Alpha-2 macroglobulin, hyaluronate, blood urea, nitrogen and age.
7. Fibrotest and Fibrosure, which are identical tests given different names in Europe and America. The test involves assessment of alpha-2 macroglobulin, alpha-2 globulin, gamma globulin, apolipoprotein A1, gamma glutamine transferase and total bilirubin.
8. Actitest, which is a modification of the Fibrotest that incorporates ALT and reflects both liver fibrosis and necroinflammatory activity. This test appears to improve identification of more advanced fibrosis associated with histological inflammation.
9. Proteomics and glycomics: this test reflects the pattern of protein or glycoprotein expression that can be assessed by mass spectroscopy. It can be used in combination with Fibrotest in some cases. Direct markers of fibrosis can be divided into two categories:

    a) Markers associated with matrix deposition (laminin, procollagen type 3, type 1 and type 4 collagens, hyaluronic acid and o-chondrex).
    b) Markers associated with matrix degradation (matrix metalloproteinases (MMP)-2, 3 and 4, and tissue metalloproteinases 1 and 2.

10. Cytokines and chemokines associated with hepatic fibrosis (tumor necrosis factor alpha, transforming growth factor beta, platelet-derived growth factor (PDGF). The serum assay approach remains somewhat promising because this test may represent an integrated readout of liver activity. However, these markers are surrogates and can not yet replace the liver biopsy.

In summary, because fibrogenesis is a balance between factors produced by stellate cells that tend to induce synthesis of extracellular matrix and those that promote the degradation of the matrix, including a reduction in MMPs or a reduction in plasmin synthesis and low activation of serum pro-MMPs, detection of these substances or signs of advanced liver disease as evidenced by indirect markers such as platelet counts, serum enzymes or combinations of these and more recently the measurement of liver stiffness, which correlates with liver biopsy, remain as potential markers of liver fibrosis. It is well known that the formation of collagen fibers including collagen 1, 3, 5 and 11 is important in the liver

capsule together with their presence in the large basils and the portal triads. In fibrosis, collagen production increases three to ten times. In addition, the extracellular matrix and collagen 1, 3, 4 and 6 increase under conditions of scarring. When these invade the subendothelial space, this increases fibronectin, laminin, tenasin, proteoglycan, decorin, fibromodulin, glipican, glycosamine, chondroitin and dermatain sulfate, which produce the characteristic extracellular matrix. It is important to realize that the activation of the stellate cell is the initial event that triggers these other events.

Clinically speaking, there are two well-established methods for evaluation of fibrosis in liver biopsies, the Ishak/Knodell and Metavir systems, although these do not always correlate exactly. For instance, in these two methods the correlation between the grading of the area of fibrosis and the grading for its severity from 1 to 6 or 1 to 4 is not exactly linear. It is worthwhile to remember that the Metavir system classifies necroinflammation from 0 to 3 and fibrosis from 1 to 4. In this case, the fibrosis is established as F0 or F1 by the presence of inflammation but no fibrosis or minimal portal fibrosis, as F2 with portal fibrosis in some septa and as F3 with portal fibrosis in many septa or septa that already include a nodular form of normal liver tissue that is characteristic of F4 liver cirrhosis. These are the main principles that are expressed in the Metavir system. It is worthwhile to consider that while the utility and usefulness of liver biopsy is without question, emerging methods such as Fibroscan promise to become the method of choice for the follow-up of patients, particularly when the large population who will eventually require treatment is considered. This applies not only to those infected with hepatitis C virus but also to those with hepatitis B virus or liver transplant patients, to allow clinicians to follow them with the least uncomfortable methods and to identify the presence of early stages of fibrosis. In terms of acceptance, it is important to know that there are a number of studies that show that in France for instance, half of the patients with hepatitis B or chronic hepatitis C do not accept referral to a hepatologist, mainly because of their fear of being asked to have a liver biopsy. This was published by Poynard in Gastroenterologie Clinique et Biologique in 2003,[3] who raised the question whether it is acceptable to delay diagnosis and possibly impair the treatment for this reason.

Another important issue raised by Pinzani[18] and Bedossa[19] in France and in Italy is the poor representation that a small biopsy can give of a widespread lesion such as liver fibrosis. Furthermore, the proportion of concordance and discordance between the interpretations of different pathologists is high. For instance, there is a study in which three pathologists were asked in blinded assessment to reach consensus about the F0, F1, F2, F3 and F4 value of biopsies using the Metavir biopsy score. As seen in the figure, in samples graded F0, more than 20% of the diagnoses from the three pathologists fell into a different category, they more or less agreed in only 70 or 60% of samples graded F1, F2 and F3 but had only 20% discordance in F4. Thus, the intermediate areas of fibrosis graded F1, F2 and F3 are the critical points where we can expect poor agreement between different expert histopathologists.

There is little disagreement that patients with chronic liver disease need to be monitored for evolution, because the main risk occurs when the patient develops liver cirrhosis. In addition, there are many hepatitis C patients on treatment, and these patients and their doctors need to have information about the development of their fibrosis.

In summary, the key issues for liver biomarkers, whether they are serum markers or markers based on physical characteristics of liver (stiffness) like the Fibroscan, are the need to demonstrate accuracy, reproducibility, to be liver specific, to have predictive stability, be able to measure progression and regression of fibrosis and to be able to correlate their outcomes with a meaningful clinical endpoint. Fortunately, tests that can make these conclusions are starting to appear, both from studies of large numbers of patients and as systemic analyses,[20] because from the patients' point of view tests should be acceptable, cheap and be able to be clinically used under conditions that are not necessarily those needed for research. If we compare the clinical issue of biopsy versus biomarkers, the accuracy of biopsy is from 0.75 to 0.85 and for biomarkers is from 0.80 to 0.95, while for reproducibility, the interobserver K for biopsy is very variable and for biomarkers is excellent. Thus, the reliability of biopsy is positive and that for biomarkers is equivocal. Therefore, biopsy may be a useful baseline study but is not necessarily the best option for patient follow-up.[21] The correlation of biopsy with clinical outcome is positive or equivocal, and that for biomarkers equivocal, while the acceptability is low for biopsy and high for biomarkers.

EVALUATION OF FIBROSCAN

As previously stated, Fibroscan is a method that requires very simple equipment. The patient does

not need to be fasting and the examination time varies from five to eight minutes. The result is recorded as the average value of a minimum of 10 successful acquisitions. The sample size relative to liver size is 1 to 50,000 with biopsy and 1 to 500 with Fibroscan.

The experience of a number of groups shows that the cutoff value for absent or mild fibrosis (F0-F1) is up to 7.0 kPa, for significant fibrosis (F2) is up to 9.5 kPa, for severe fibrosis (F3) is up to 12.5 kPa and for cirrhosis (F4) is from 12.5 to 75 kPa. The arrow shows the levels where patients begin to have potential complications related to portal hypertension.

The correlation between liver stiffness and cirrhotic complications, presence of portal hypertension and complications of chronic liver disease has been investigated by Fourcher et al., who have stated that the presence of stiffness below 27.5 kPa is associated with no varices, that Child B or C varices appear at 37.5 kPa, ascites appears at 49.1 kPa, hepatocellular carcinoma at 53.7 kPa and variceal bleeding at 62.7 kPa.[22]

European studies show good correlation between F2, F3 and F4 and the area under the ROC curve with Fibroscan in patients with chronic hepatitis C virus infection.[23] According to Carrion et al., Fibroscan studies show a linear correlation between hepatic portal pressure and tissue stiffness.[24] According to the studies of Poynard,[25] which show a correlation between the grade of fibrosis by Fibrotest and the fibrosis detected by biopsy, these correlations are also suggested by Fibrotest, the most frequently used serological test for fibrosis.

There are other variables, but the most important factor is that we are developing a pathway for approaching diagnosis by combining biopsy and biomarkers. Liver biopsy should be performed at the outset in all patients; however, after this some patients can be followed by serum biomarkers, some by clinical biomarkers including an annual Fibroscan or by a combination of Fibroscan and serum tests. The data from patients with hepatitis C virus in the USA compared with that from patients with hepatitis C virus in Europe shows a good correlation between the specificity and sensitivity of the methods.

In summary, biomarkers are already used in clinical practice, but validation and commercialization is necessary for large-scale uptake in the USA and Mexico. A combination of Fibroscan and serum tests may be optimal, and biomarkers are complementary to biopsy. Many more large and prospective studies plus longitudinal studies must be undertaken to confirm these initial results. All patients are candidates for biomarker measurements.

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