REVIEW ARTICLE

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From liver biopsy to non-invasive markers in evaluating fibrosis in chronic liver disease

Cătălina Diaconu[1], Florentina Ioniță Radu[1,3], Mariana Jinga[1,2], Petruț Nuță[1], Săndica Bucurică[1,2], Bogdan Macadon[1], Mihăiță Pătrășescu[1], Andrada Popescu[1], Vasile Balaban[1,2], Laura Voicu[1], Daniel O. Costache[1], Raluca S. Costache[1,2]

Abstract: *Chronic liver disease is a late stage of progressive hepatic fibrosis. It consists of functional and structural disruptions in most chronic liver diseases. An accurate diagnosis allows us to establish the degree of fibrosis and the stage of the disease, the prognosis of the patient and to predict a treatment response. Despite the fact that liver biopsy is considered a gold standard, non-invasive methods for diagnosing liver fibrosis have gained more and more importance. Whether we talk about serum biomarkers or imagistic methods from transient elastography to 3-D magnetic resonance elastography, the question remains: are these useful or useless? Serum biomarkers represent blood components that can reflect liver histological changes, thus they can monitor the continuous process of fibrosis. These can be subcategorized in direct (that show extracellular matrix turnover) and indirect markers (that reflect disturbances in the hepatic function). However these markers alone are not as accurate in the staging of fibrosis, only help differentiate patients without or with low grade of fibrosis from those with significant fibrosis and cannot be considered alone in the diagnosis of liver fibrosis. Imagistic methods include: ultrasound-based transient elastography, magnetic resonance elastography (MRE), 2D-shear wave elastography, acoustic radiation impulse imaging (ARFI) and cross sectional imaging, the first being the most used. Using a combination of non-invasive tools allows us to diminish the number of patients in need of liver biopsy. However, the patient must always be informed of the advantages and disadvantages of each method and its limitations.*

Keywords: *fibrosis, biopsy, elastography, biomarkers*.

INTRODUCTION

Liver fibrosis remains an important problem worldwide. At first liver fibrosis was considered irreversible, but now scientists show that it is a dynamic process [1]. Advanced chronic liver disease, also known as cirrhosis is defined by the replacement of normal liver tissue with fibrotic one, leading to the disruption of the normal architecture of the liver and formation of regenerative nodules. It is usually considered to be irreversible, eventually leading to the need of liver transplantation. Many liver diseases can lead to cirrhosis. Most encountered causes are:

[1] Carol Davila Central Emergency Military Hospital, Bucharest
[2] Carol Davila University of Medicine and Pharmacy, Bucharest
[2] Titu Maiorescu University, Faculty of Medicine, Bucharest

viral hepatitis (mostly B and C hepatic virus), alcoholic liver disease and nonalcoholic liver disease, hemochromatosis. One must bear in mind less common causes of advanced end stage liver disease such as: autoimmune hepatitis, primary sclerosing cholangitis, primary and secondary biliary cirrhosis, Wilson disease, veno-occlusive disease, alpha-1 antitrypsin deficiency or right sided heart failure [2].

In normal hepatic tissue there is a balance between the production and degradation of extracellular matrix components. In liver fibrosis the balance is skewed in favor of the latter [3]. There is a high number of cells involved in the process of fibrogenesis. However, the hepatic stellate cell is the main factor involved. Chronic liver injury leads to the activation of hepatic stellate cells that transforms into myofibroblasts. These are contractile cells that secrete extracellular matrix components, pro-inflammatory citokines and chemokines. The first such protein produced in liver fibrosis is fibronectin, leading to the formation of excess collagen type 1. This process is cyclic since the production of extracellular matrix activates other stellate cells, which produce more proteins and finally change the hepatic architecture. Portal fibroblasts are also involved in the process of fibrosis, after stellate cells, tuning into myofibroflasts. Since these are closer to the portal tract they have a more important role in cholestatic injury from liver fibrosis, encountered in primary biliary cirrhosis and primary sclerosing cholangitis [4].

Liver biopsy is considered the golden standard in evaluating the degree of fibrosis. However it is limited by sampling errors, intra- and interobserver variability in histological interpretation and by the possible complications that may be encountered. There are many histologic systems that can appreciate fibrosis, but the one mostly used is semi-quantitative METAVIR score [5].

There are two categories of non-invasive methods in evaluating chronic liver disease: serologic panels of tests and radiologic tests. First ones quantify the levels of biomarkers in the serum samples and are more available. The latter consists of tests that measures liver stiffness using a “physical” approach. The two methods are complementary. Serum biomarkers are less accurate in detecting intermediate stages of fibrosis that in identifying cirrhosis, but present many advantages: high applicability, low costs, wide availability, and the possibility to be performed as outpatient [6]. Radiologic tests measure liver stiffness and are very useful in detecting cirrhosis. Combination between serum biomarkers and imagistic methods are very useful, slowly becoming a new golden standard in evaluating fibrosis in advanced liver disease [7].

Genetic risk factors for fibrosis are only now starting to gain importance. Literature shows that external factors such as alcohol and coinfections accelerate fibrogenesis. Studies now report variants in several genes that become associated with higher risk of fibrosis in chronic hepatitis C, NAFLD or AFLD [3].

LIVER BIOPSY

Considered the gold standard for the evaluation of tissue damage, including end-stage liver disease, liver biopsy is slowly falling behind non-invasive methods. Despite the fact that a high range of sensitive and accurate blood and imagistic tests can now be used to detect and even diagnose liver diseases, liver biopsy remains a valuable diagnosis tool, especially in difficult cases[8]. However the well-recognised limitations of this procedure have fuelled discussions regarding the correct diagnosis pathway in advanced chronic liver disease. The major problems of liver biopsy remain sampling and observational errors. Studies have shown that the larger the piece is, the lowest the risk of sampling errors is. The quality of the piece is in the length and the number of portal fields contained. A 20-25mm biopsy with more than 11 portal tracts is considered optimal specimen, even though some studies show that even a 15mm piece can be useful [3.9]. Even though we can obtain a large size biopsy, unfortunately it only reflects 1:50000 of the entire liver. Not only the length, but also the caliper of the biopsy needle is essential, a 16 gauge needle being the most appropriate to use [6]. Intra- or interobserver variability can also limit the usefulness of liver biopsy, leading to discrepancies in up to 20% of cases. Using histopathological scoring systems

diminished this problem [3].

Histologic scoring systems in chronic liver disease are of great importance to the progression of the disease, prognosis and helping us establish appropriate treatments. In *chronic hepatitis* the most encountered systems are: the Knodell score (histology activity index), METAVIR score, Ishak score (modified Knodell score). Other less used scoring systems are: the Scheurer system, the Batts-Ludwig system and Laennec system. *Nonalcoholic fatty liver disease* (NAFLD) can be divided in nonalcoholic fatty liver (NAFL) and nonalcoholic steatohepatitis (NASH). NAFLD can be evaluated by nonalcoholic fatty liver activity score (NAS). It sums up the degree of steatosis, lobular inflammation and ballooning of the liver cells, ranging from 0-8. Fibrosis is not included in NAS. *Primary biliary cirrhosis* uses two scoring systems: Scheuer or Ludwing, based on: the degree of fibrosis, bile duct loss, copper deposition and the degree of cholangitis and hepatitis. Even though liver biopsy in *primary sclerosing cholangitis* is uncommon, if it is performed then a histologic scoring system can be used. It allows us to define 4 stages based on the degree of inflammation and fibrosis [10].

Complication rates related to liver puncture range from 0.75% to 13.6% [3]. Minor complications after liver biopsy include transient discomfort, moderate pain and mild transient hypotension. Bleeding remains the most important complication of liver biopsy, along biliary peritonitis, perforation and pneumothorax [11]. Biopsy through transjugular route can reduce the risks in patients with coagulation disorders or advanced liver disease [9]. The number of complications is also diminished if we choose ultrasound-guided biopsies [12].

Liver biopsies should be performed as in-patient procedure since 60% of complications occur in the first 2 hours and more than 90% of complications are prone to happen during the first 24 hours[3,11].

SERUM BIOMARKERS

The "biological" approach of evaluating end stage liver disease is represented by serum biomarkers. Even though these parameters may not be liver-specific, they have been associated with fibrosis stage [6]. These so-called surrogate markers can be direct and indirect. The changes in the content of extracellular matrix are quantified by direct markers, whilst indirect markers show hepatic function disturbances. These markers can be used alone or combined, leading to different scores, which can be simple (AST to platelet ratio index (APRI), FIB-4 index, FORNS) or calculated after a formula (Fibrotest, Fibroscore, Fibromax) [3]. Simple scores might have lower diagnosis accuracy, but they have greater advantages: lower price, easy to calculate and widely available [6].

Indirect markers

AST to platelet ration index (APRI) is easy to calculate based on the platelet count and AST level. APRI was first used in patients with hepatitis C virus (HCV), human immunodeficiency virus and HCV coinfection and alcoholic liver disease [13,14].

Table 1. The correlation between AST to platelet ratio index and the probability of cirrhosis.

APRI <=0.3:	Unlikely cirrhosis or significant fibrosis
APRI >0.3 and <=0.5:	Unlikely cirrhosis, significant fibrosis possible
APRI >0.5 and <=1.5:	Significant fibrosis or cirrhosis possible
APRI >1.5 and <=2:	Likely significant fibrosis, cirrhosis possible
APRI >2:	Likely cirrhosis

FibroTest and *ActiTest* were first used in patients with hepatitis B and C. *Fibrotest* uses alpha-2-macroglobulin, gamma globulin, apolipoprotein A1, alpha-2-globulin (haptoglobin), gamma-glutamyl-transpeptidase and total bilirubin. It takes into consideration patients' age and sex [1]. Even though there are advantages of FibroTest such as: high availability and applicability (>95%), there are many drawbacks: lack of specificity for liver disease, interference with other comorbidities that can change the result (Gilbert's syndrome or hemolysis) or the difficulty to differentiate intermediate stages

of fibrosis [15]. ActiTest is a modified FibroTest that involves ALT, and evaluates both fibrosis and necroinflamatory activity of the liver [1].

Hepascore involves assessment of bilirubin, GGT, alpha-2-macroglobulin, hyaluronic acid, age and sex. A metaanalysis showed that Hepascore did not bring more information than FibroTest in patients with alcoholic liver disease. However, other studies showed its accuracy in predicting fibrosis in patients with chronic HC [1,9].

AST/ALT ratio in normal patients is around 0.8. Some studies show that a value over 1 suggests cirrhosis, but its utility is not certified [1].

Other indirect markers that are not worldwide used include: FIB-4 index, NAFLD fibrosis score (cutoff higher than 0.676 is associated with positive predictive value of fibrosis and a value under -1.455 is associated with a negative predictive value of 88 percent), PGA index (alcoholic liver disease with a detection range 66-72%), Forns (similar performance with APRI), BARD score (predictive in patients with NAFLD)[1,6].

Direct markers are used in forming panels to predict fibrosis, but none are available in clinical practice. (Table 2).

Table 2. Direct markers of fibrogenesis and fibrolysis

Matrix deposition	Procollagen I peptide
	Procollagen III peptide
	Type I collagen
	Type IV collagen
	YKL-40 (chondrex)
	Laminin
	Hyaluronic acid
Matrix degradation	MMP-2
	TIMP-1, -2
Cytokines	TGF-beta
	TGF-alpha
	PDGF

Serum biomarkers aren't useful in distinguishing between intermediate stages of fibrosis, but are more accurate in detecting fibrosis. Moreover careful consideration is entitled when talking about patients with HCV-HIV coinfection since false positive results can appear. For example: APRI, FIB-4, FibroTest (due to hyperbilirubinemia induced by atazanavir), Forns Index (related to GGT increase from nevirapine) and HepaScore. Despite the fact that bioseric markers can be used for detecting cirrhosis, their utility is reduced in diagnosing significant fibrosis [6]. Combination of different scores can be effective in avoiding biopsies, but a single surrogate marker cannot replace liver biopsy [3].

RADIOLOGIC TESTS

Radiologic tests represent the "physical" approach in evaluating fibrosis in chronic liver disease. The methods include: ultrasound-based elastography, magnetic resonance elastography (MRE), acoustic radiation force impulse imaging (ARFI), 2D-shear wave elastography (2D-SWE) and cross sectional imaging [3].

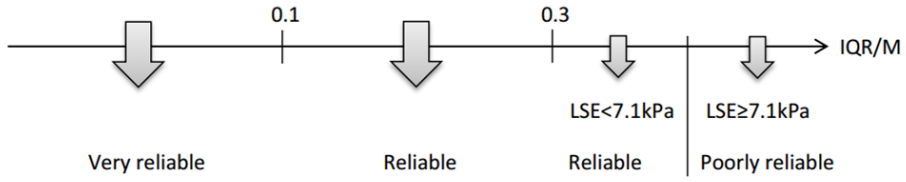
Trasient elastography was first evaluated as valid marker for fibrosis in a clinical study in 2002, therefore it remains rather new in the diagnostic pathway. It assesses the liver fibrosis by calculating the velocity of a low-frequency shear wave (50Hz) produced by a mechanical probe placed directly on the skin. The wave penetrates the liver tissue depending on the stiffness of the liver, correlating to the extent of liver fibrosis. The technique goes as following:

- the transductor is placed in contact with the skin with the tip covered in coupling gel;
- the position is in the 9^{th} to 11^{th} intercostal space (where liver biopsy is performed),
- we select an area of liver at a maximum of 6 cm deep, free of vessels;
- we gather 10 consecutive successful measurements. If one value is not valid, the entire set must be repeated [16].

There are several markers that evaluate if the result is valid: 10 consecutive valid results, a success rate (valid shots/total number of shots) above 60%, interquartile range lower than 30% of the median liver stiffness measurements, serum aminotransferase levels less than 5 times the normal value. Liver stiffness is expressed in kilo Pascal (kPa), ranging

from 1.5 to 75kPa. A three category system is used: very reliable (IQR/M≤0.10), reliable (0.10<IQR/M≤0.30 or IQR/M>0.30 and liver stiffness evaluation (LSE) <7.1kPa) and poorly reliable (IQR/M>0.30 and LSE≥7.1kPa) [16,17].

Figure 1. The accuracy of transient elastography is based on a system made from: interquartile range/median liver stiffness measurements (IQR/M) and LSE (liver stifness evaluation) [3].



The largest series report evaluating TE show that in 3.1% of cases the measurement was impossible and in 15.8% of cases the results were unreliable (mostly due to patient obesity). Therefore a new probe was developed: XL, 2.5MHZ transducer. This allows us to measure liver stiffness from 35 to 75mm depth [3,6]. In their study Myers et al concluded that in 276 patients with chronic liver disease (42% viral hepatitis, 46% NAFLD) and a BMI>28 kg/m^2, using an XL probe was more successful than using M probe. The number of unreliable results was lower when using XL probe (25%) than with M probe (50%). Stiffness values calculated with XL probe are lower by a median of 1.4kPa than when obtained with M probe [18,19].

Other limitations in using transient elastography are: narrow intercostal spaces, ascites, cardiac failure, high necroinflammatory activity (quantified by a high AST/ALT ratio), steatosis and cholestasis [20]. Excessive alcohol ingestion or food intake might modify the results of TE, therefore a fasting period prior to the investigation in recommended, 3-6 hours [6,21].

A special S probe was introduced for evaluating liver stiffness in children or patients with small intercostal space [22].

A meta-analysis of 50 studies comparing transient elastography and liver biopsy showed that we can choose an imagistic method in distinguishing cirrhosis from lesser degrees of fibrosis, but it is less reliable in detecting lower grades of fibrosis thus it has moderate negative predictive values. Moreover the study shows that transient elastography is better in diagnosing nonalchoholic steatohepatitis and alcohol-related disease than in chronic viral hepatitis. Transient elastography is useful in detecting cirrhosis with an optimal cut-off value of 13.01 kPa[3]. However one must take into consideration that the target population might have different characteristics and it should be pre-tested [6]. TE should not be used alone in evaluation on liver fibrosis in some patients, but if it predicts significant fibrosis there is no need for biopsy [3,4]

The performance of TE in hepatitis B and C is similar if we compare the two. When evaluating hepatitis e antigen (HBeAg-) negative patients with normal ALT values, non-invasive methods, especially TE could be used to estimate levels of HBV-DNA and identify patients in need of liver biopsy [6].

TE might be used in monitoring liver fibrosis: either after or during treatment or to evaluate the natural progression of the disease. A most discussed topic in the world of hepatology is the role of TE in predicting portal hypertension. A cut-off value of 27.5kPa can predict the presence esophageal varices and the need of endoscopy to evaluate the extent of the disease and the need to start primary prophylaxis with non-selective β-Blockers, whilst a cut-off value of 62.7kPa can suggest a high risk of esophageal bleeding. Studies show that a cut-off value >25kPa is associated with > 45-fold risk to develop hepatocellular carcinoma in patients known with viral hepatitis [3,23]

Shear wave elastography (SWE) is divided in: point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI) and 2D-shear wave elastography (2D-SWE). ARFI excites

liver tissue by using short-duration acoustic pulses (~262µsec) that propagate shear waves and lead to localized µ-scale displacement in the tissue. The target zone (10x15mm) is set 10-20mm under the liver capsule. Ten consecutive valid measurements are required and the accuracy is evaluated with the interquartile range. The failure rate is lower when using ARFI to evaluate fibrosis than TE (2.9% vs 6.4%, p<0.001), especially in patients who suffer from obesity or associate ascites. Food intake, necroinflammatory activity and serum levels of aminotransferases might influence the results. ARFI is better in evaluating fibrosis in chronic hepatitis C than in chronic hepatitis B, NAFLD, HIV-HCV coinfection and other liver diseases. *2D-SWE* uses a combination of radiation force from focused ultrasonic beams and a very high frame rate ultrasound imaging sequence. Failure rate is lower than TE, especially in patients with ascites, but not in patients with obesity where XL probe is more accurate. However quality criteria in SWE are not yet well defined [6, 24].

Table 3. The correlation between the number of cases and SIRS criteria

Cirrhosis caused by:	Trasient elastography evaluation cut-off value (kPa)
Hepatitis C	13.01
Hepatitis B	11.7
Alcoholic fatty liver disease if drinking	22.7
Alcoholic fatty liver disease if abstinent	12.5
Biliary liver disease	17.9
Non-alcoholic fatty liver disease	10.3

Magnetic resonance elastography (MRE) involes placing a probe on the patient's back that emits low-frequency vibrations that pass through the liver and can be measured. With an optimal cut-off of 4.71 it can suggest cirrhosis with a sensitivity of 91% and a specificity of 81%[1]. Some studies show a better diagnostic accuracy and technical success than TE[25]. Moreover, MRE has the advantage of scanning the entire liver tissue, thus it cannot miss areas of fibrosis, hepatocellular carcinoma, focal liver lesions, and it is independent to an acoustic window and is operator independent [1]. However due to the costs and excess of time MRE requires, it is not used for routine clinical practice use [6].

Other imagistic methods such as computerized tomography are not currently in use for evaluating fibrosis in chronic liver disease, due to low sensitivity and specificity and high costs [3].

We now know that non-invasive tests have striking advantages against liver biopsy when talking about patient safety. Therefore by combining the serologic biomarkers with imagistic methods (preferably) or even different bio-markers we can limit using liver biopsy only in case of doubt or discordance between serological biomarkers and imagistic method. The combination of non-invasive markers allows rapid staging of the liver without the need for liver biopsy for hepatitis C. The current gold standard in hepatitis C is to associate TE and serum biomarkers [6].

In hepatitis B, TE is superior to serologic biomarkers and it is best used to detect the stage of fibrosis in patients with active viremia (DNA-HBV>2000 IU/ml) and normal ALT. TE is not indicated in hepatitis with very high levels of ALT >10 times the normal value. Liver biopsy should be used when in doubt [6].

Non-invasive methods in evaluating fibrosis in NAFLD have a lower accuracy compared to liver biopsy and in time patients might require histological confirmation. However, follow-up can be performed with serum biomarkers or TE once every 3 years to evaluate the progression of the disease [6].

Despite the fact that non-invasive tests cannot replace hepatic venous pressure gradient (HVPG) in evaluating portal hypertension or endoscopy that reveals clinical implication of portal hypertension (oesophageal varices), these might be useful in selecting patients that require these procedures. HVPG over 10mmHg is predictive of varices and decompensation, whilst values under 10 mmHg has a 90% negative predictive value for the development of clinical decompensation in 4 years. However measuring HVPG is both expensive and invasive therefore repeating measurements is impractical [4,6].

CONCLUSION

Non-invasive markers do not abolish the need for liver biopsy. They should be used to select the patients in need of liver biopsy more carefully.

Smart combinations of non-invasive markers can greatly reduce the number of liver biopsies performed and the risks associated, but when the diagnosis is unclear one must use liver biopsy.

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