Non-invasive methods for diagnosis of hepatic fibrosis in chronic hepatitis B patients

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Abstract:

Non-invasive markers for assessing liver fibrosis have been developed, and they are frequently used in clinical practice. They have been validated in different studies, and some were found to be highly accurate compared with liver biopsies which have always been used as the standard reference method for evaluating the accuracy of non-invasive methods[1].The performance of a non-invasive diagnostic method is evaluated by calculation of the area under the receiver operator characteristic curve (AUROC)[2].

Methods:

Non-invasive methods to assess histology in chronic liver disease include clinical symptoms and signs, routine laboratory tests, serum markers of fibrosis and inflammation, quantitative assays of liver function, and radiologic imaging studies. However, at present, none of these tests or markers alone is accurate or reliable in predicting histology, in particular, liver fibrosis. An ideal non-invasive diagnostic test for hepatic fibrosis should be simple, readily available, inexpensive, and accurate[3].

I-Imaging:

Standard imaging techniques such as ultrasound, CT and MRI are able to detect advanced fibrosis when signs of portal hypertension are evident, but they cannot yet detect milder disease[4].

1-Transient elastography (TE): is a non-invasive technique for assessment of liver fibrosis that was first described in the medical literature in 1999. The method is easy to learn, quick, results are available immediately, and a technical assistant may perform the procedure[4].FibroScan measures the stiffness (or elasticity) of hepatic parenchyma using both ultrasound (5 MHz) and low-frequency (50 Hz) elastic waves produced by a specialized ultrasound vibrator applied to the body wall and coupled with 1D ultrasound imaging that measures the propagation speed of a wave using a pulse-echoultrasound[5].

2-Contrast enhanced ultrasonographic imaging (CEUS): uses intravenous administration of gas - filled microbubbles to enhance vascular signals and measure blood flow transit. Diminished hepatic vein transit time correlates with worsening liver disease[6].

3-Acoustic radiation force impulse (ARFI): combines conventional unltrasonographyof the liver with evaluation of local liver stiffness. As regions of evaluation can be chosen using ultrasound, ARFI allows operator avoidance of anatomical obstacles, e.g. large blood vessels[7]. Importantly, in contrast to FibroScan, liver steatosis had no statistical influence on ARFI results[8].

4-Magnetic Resonance Imaging (MRI):it is used routinely to assess cirrhosis and its complications. However, detection of less advanced stages of fibrosis is more challenging, and several novel MR imaging techniques were used for this purpose[9].

II-Serum markers of liver fibrosis:

Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. In addition to being substantially less invasive, there are practically no complications, little or no sampling errors and small observer related variability. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis[10].

Biomarkers of fibrosis are commonly divided into direct and indirect markers. Direct markers are fragments of the liver matrix components produced by hepatic stellate cells (HSCs) during the process of extracellular matrix (ECM) remodeling. Indirect markers include molecules released into the blood due to liver inflammation, molecules synthesized/regulated or excreted by the liver, and markers of processes commonly disrupted due to liver function impairment, such as insulin resistance[11].

A) Direct Biomarkers:

1-Procollagen type I carboxy terminal peptide (PICP) and procollagen type III amino-terminal peptide (PIIINP):in the healthy human liver the most abundant collagens are the fibril-forming types I and III. In its mature form, the collagen is integrated into the ECM. During fibrogenesis, type I collagen levels increase up to eightfold[12]**.** PICP levels are normal in patients with mild chronic hepatitis C and elevated in 50% of patients with moderately advanced or advanced chronic hepatitis C[13].In acute hepatitis, levels of serum PIIINP correlate with aminotransferase levels. In chronic liver disease, serum PIIINP reflects the stage of liver fibrosis[14].Unfortunately, PIIINP is not specific for the fibrosis of the liver as it is also elevated in acromegaly, lung fibrosis, chronic pancreatitis, and rheumatologic disease[12].

2-Matrix metalloproteinases (MMPs): form a family of structurally related proteolytic enzymes that mediate the degradation of the ECM and the basal membranes[14,15]. The three most commonly studied human metalloproteinases are MMP-2 (gelatinase-A), MMP-3 (stromelysin), and MMP-9 (gelatinase-B). MMP-2 is secreted by activated HSCs; elevated levels of MMP-2 and its proenzyme have been observed in various liver diseases[16].

3-Tissue inhibitors of matrix metalloproteinases (TIMPs): are secreted proteins that interact with MMPs and modulate their activation and functioning. Elevation of TIMPs' levels has been observed in chronic liver disease. For example, chronic hepatitis C causes the elevation of both TIMP-1 and TIMP-2 in corollary with fibrosis progression[17].

4-Transforming growth factor-β1 (TGF-β1): is a pleiotropic cytokine involved in tissue growth, differentiation, ECM production and the immune response. Three isoforms (β1, β2 and β3) of this cytokine have been identified, but only TGF-β1 is linked to liver fibrogenesis[12,18].

5-Hyaluronic acid (HA)*:* is a glycosaminoglycan component of the ECM that is synthesized by the HSCs. In a study of NAFLD-related fibrosis of the liver, HA was found to be the best class I biomarker of fibrosis, being associated with an area under curve of 0.97[19].

6-Chondrex (YKL-40): is a mammalian homologue of the bacterial chitinases involved in remodeling or degradation of the extracellular matrix[20]. In liver diseases, serum levels of YKL-40 are closely related to the degree of histologically documented fibrosis[21].

7-Laminin: is a major non-collagenous glycoprotein synthesized by the HSCs and deposited in the basement membrane of the liver. During fibrosis, laminin accumulates around the vessels, in the perisinusoidal spaces and near the portal tracts[22].

8-Connective tissue growth factor (CTGF): is synthesized in response to profibrogenic factor TGF-β by both activated HSCs and hepatocytes. However, serum CTGF levels decrease in the end-stage cirrhosis[23].

9-Paraoxonase 1 (PON-1): is an enzyme that hydrolyzes lipid peroxides, has antioxidant properties and influences hepatic cell apoptosis. Measurement of serum PON-1 activity has been proposed as a potential test for the evaluation of liver function, however, its clinical acceptance is limited due to instability and toxicity of its substrate, paraoxon[24].

10-Microfibril-associated glycoprotein 4 *(*MFAP-4): is a ligand for integrins. In a recent study, quantitative analysis of MFAP-4 serum levels showed high diagnostic accuracy for the prediction of non diseased liver versus cirrhosis (AUROC = 0.97, P< 0.0001) as well as stage 0 versus stage 4 fibrosis (AUROC = 0.84, P< 0.0001), and stages 0 to 3 versus stage 4 fibrosis (AUROC = 0.76, P< 0.0001)[25].

Limitations of Direct serum biomarkers of fibrosis:

They reflect the rate of matrix turnover (not only deposition) and have a tendency to be more elevated when associated with high inflammatory activity. As a consequence, extensive matrix deposition might not be detected in the presence of minimal inflammation. They are not liver-specific and their serum levels may be elevated in the presence of concomitant sites of inflammation. Serum levels of markers depend on their clearance rates, which are influenced by the dysfunction of endothelial cells, impaired biliary excretion or renal function[11].

B) Indirect Biomarkers of Fibrosis:

1-AST/ALT ratio: aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are hepatic enzymes that are released into the bloodstream from damaged hepatocytes. The ratio is less than or equal to 1, while in alcoholic hepatitis, an AST/ALT ratio is often greater than 2[26].

2-The PGA index: combines the measurement of the prothrombinindex, γ glutamyltransferase levels and apolipoprotein A1. In chronic liver diseases, the PGA index has a relationship to both the inflammation and the fibrosis (P<0.01, P< 0.05 respectively). However, overall accuracy of this index is relatively low[27].

3-The AST-to-Platelet Ratio Index (APRI): is calculated as (AST/upper limit of normal range)/platelet count (10^9/L) × 100. A recent large meta-analysis suggested that APRI can identify hepatitis C-related fibrosis with only a moderate degree of accuracy[28].

4-The Forns index: is based on 4 routine clinical variables: age, platelet count, cholesterol levels, and γ glutamyltransferase. This method can be used to differentiate patients with mild (F0-F1) fibrosis from those with severe (F2-F4) fibrosis, but it is less accurate in distinguishing patients with grades F2 versus F4[29].

5-The HepaScore: combines age, gender, bilirubin, γ glutamyltransferase, hyaluronic acid, and γ2-macroglobulin into a score from 0.00 to 1.00. It showed good predictive performances for significant fibrosis (AUROC = 0.81), severe

fibrosis (AUROC = 0.82), and cirrhosis (AUROC = 0.88). Importantly, HepaScore test can be automated using a single analyzer[30].

6-The FIB-4:it combines platelet count, ALT, AST and age. Use of this index correctly avoided biopsy in 71% of the validation set with an AUROC of 0.765, sensitivity of 70% and a specificity of 97% for differentiating Ishak 0-3 from 4-6[31].

7-The FIBROSpect II test:uses a combination of components in the fibrogenic cascade, such as hyaluronic acid, TIMP-1, and α-2-macroglobulin to calculate a composite score. The test is intended to differentiate mild fibrosis (Metavir stages F0 to F1) from more severe disease (Metavir stages F2 to F4), and had been shown to do well in chronic hepatitis C cohorts[29].

8-The FibroTest and FibroSure: are i+dentical tests marketed under different names in Europe and America for the assessment of fibrosis and necroinflammatory activity. The FibroTest score is computed by accessing a proprietary website and entering the patient's age, sex,haptoglobin, α_2-macroglobulin, apolipoprotein A1, γ-glutamyltransferase, and bilirubin analyses[32].The sensitivity and specificity values for FibroTest based detection of primary severe fibrosis were found to be 75% and 85%, respectively[33].

9-The FibroIndex:itrelies on platelet count, AST and serum IgG.The sensitivity and specificity of FibroIndex for detecting fibrosis in patients with HCV were 78% and 74%[34].

10-The FibroMeter: is a combination of the platelet count, prothrombin index, AST, γ2 macroglobulin, hyaluronate, blood urea nitrogen and age.An important feature of the FibroMeter is that it presents the amount of liver fibrosis as a percentage of fibrous tissue within the liver. FibroMeter has two main diagnostic targets; fibrosis stage corresponding to the Metavirstaging system and the amount of fibrosis which corresponds to morphometric determinations of the fibrotic area[35].

11-Hui's model: is another simple bio-chemical panel including body mass index, platelet count, serum albumin and bilirubin levels. It wasdemonstrated to accurately predict the absence of significant fibrosis with a high degree of accuracy[36]. Sebastiani et al. reported a moderate overall accuracy of this model for the detection of significant fibrosis (AUROC 0.71), whereas the model performances for the detection of cirrhosis had sub-optimal results[37].

12-The GP model: it includes globulin level and platelets count. It predicts significant fibrosis in patients with chronic hepatitis B. This newly designed noninvasive marker must be evaluated in different populations before common use[38].

13-The Lok index: is an evolution of the APRI combining platelet count, INR and AST/ALT ratio. This index uses two cut-off values: 0.2 to rule out cirrhosis and 0.5 to confirm cirrhosis, whereas values between these cut-offs are considered indeterminate[39].

14-The Proteomics based tests: assess patterns of protein or glycoprotein by mass spectroscopy using serum samples. Callewaert et al. developed tests based on the altered N-glycosylation of total serum protein (GlycoCirrhoTest and GlycoFibroTest), which could be both cost-effective and could rapidly determine a signature profile for n-glycans[40].

Limitations:

Although noninvasive, easy to repeat and highly applicable, serum markers have obvious limitations. Their main disadvantage is represented by their

low accuracy to detect intermediate stages of fibrosis as compared to cirrhosis[41,42].

Refernces

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