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Including osteoprotegerin and collagen IV in a score-based blood test for liver fibrosis increases diagnostic accuracy

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ABSTRACT

Background: Noninvasive methods for liver fibrosis evaluation in chronic liver diseases have been recently developed, i.e. transient elastography (Fibroscan™) and blood tests (Fibrometer®, Fibrotest®, and Hepascore®). In this study, we aimed to design a new score in chronic hepatitis C (CHC) by selecting blood markers in a large panel and we compared its diagnostic performance with those of other noninvasive methods.
Methods: Sixteen blood tests were performed in 306 untreated CHC patients included in a multicenter prospective study (ANRS HC EP 23 Fibrostar) using METAVIR histological fibrosis stage as reference. The new score was constructed by non linear regression using the most accurate biomarkers.
Results: Five markers (alpha-2-macroglobulin, apolipoprotein-A1, AST, collagen IV and osteoprotegerin) were included in the new function called Coopscore©. Using the Obuchowski Index, Coopscore© shows higher diagnostic performances than for Fibrometer®, Fibrotest®, Hepascore® and Fibroscan™ in CHC. Association between Fibroscan™ and Coopscore© might avoid 68% of liver biopsies for the diagnosis of significant fibrosis.

Abbreviations: A2M, alpha-2-macroglobulin; ApoA1, apolipoprotein A1; AST, aspartate aminotransferase; AUROC, area under receiver operating characteristic curve; BMI, body mass index; C-IV, collagen IV; CHC, chronic hepatitis C; CI, confidence interval; CS, Coopscore©; GGT, gamma glutamyl transpeptidase; GST, alpha-glutathione S-transferase; HA, hyaluronic acid; hapto, haptoglobine; HCV, hepatitis C virus; LSM, liver stiffness measurement; MMP1, matrix metalloproteinase 1; NK, natural killer; NPV, negative predictive value; OD, optic density; OPG, osteoprotegerin; Plat, platelet count; PIIIP, procollagen III peptide; PPV, positive predictive value; prothr, prothrombine index; RANKL, receptor activator of nuclear factor kappa B ligand; ROC, receiver operating characteristic curve; SD, standard deviation; TB, total bilirubin; TIMP1, tissue inhibitor of metalloproteinase 1.

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Conclusion: Coopscore© provides higher accuracy than other noninvasive methods for the diagnosis of liver fibrosis in CHC. The association of Coopscore© with Fibroscan™ increases its predictive value.

1. Introduction

The evaluation of liver fibrosis is critical for the management of liver diseases. In hepatitis C virus chronically infected patients, the presence of significant fibrosis is a major criteria to initiate antiviral therapy and the presence of cirrhosis justifies monitoring for the early detection of portal hypertension or hepatocellular carcinoma. So far, liver biopsy has been considered as the reference method for liver fibrosis staging. However, it is an expansive procedure with potentially serious complications, including bleeding, hematoma and death [1]. Moreover, sampling heterogeneity and inter-observer or intra-observer variations can lead to an under or overestimation of fibrosis stage in 30% of cases [2,3]. Recently, different noninvasive methods of liver fibrosis staging have been evaluated. Liver transient elastography (Fibroscan™) is the least invasive method but of lower applicability because of more frequent failure tests and less reliable results than the blood tests, in particular in overweight patients. However, when Fibroscan™ is interpretable, it performed similar to blood tests [4–7]. A number of serum biomarkers have been investigated and combined in fibrosis scores. Three score-based tests, Fibrotest®, Fibrometer® and Hepascore® are the most currently used and have been validated in a large panel of liver diseases. These tests associate several serum markers including α2-macroglobulin (A2M), hyaluronic acid (HA), haptoglobin, apolipoprotein A1 (ApoA1), bilirubin, gamma glutamyl transpeptidase (GGT), aspartate amino transferase (AST), urea, platelets count, prothrombin index and variables like age and sex [8–10]. However, investigations are ongoing to identify new serum biomarkers that could improve the diagnostic performance of such tests. In this attempt, using of scores combining direct and indirect markers of fibrosis might be an advantage in certain situations, such as in patients with hemolysis or inflammation or who undergo antiviral therapy.

Osteoprotegerin (OPG) or Tumor Necrosis Factor receptor superfamily, member 11b, was first described in bone as a decoy receptor for RANKL (receptor activator of nuclear factor κB ligand), inhibiting its binding to RANK and subsequent osteoclast activation [11,12]. Serum OPG has been analyzed in patients with various liver diseases in an attempt to elucidate the mechanisms of bone resorption, which frequently occurs in cirrhosis. Unexpectedly, serum OPG was elevated in patients with fibrosis or cirrhosis, including those with osteoporosis [13–16]. In a recent microarray study, we identified OPG as a potential marker of hepatic stellate cell origin in liver myofibroblasts [17]. Because there are evidences that myofibroblastic hepatic stellate cells accumulate from early on in the course of liver fibrogenesis, we hypothesized that OPG could be a serum marker of liver fibrosis and we compared its diagnostic performance with that of other known variables in patients from the Fibrostar cohort, a multicentric cohort that included untreated CHC (chronic hepatitis C) patients [7]. The aim was to design a new score by selecting relevant biomarkers affected during the progression of liver injury in CHC.

2. Material and methods

2.1. Patients

HCV cohort included patients from the multicenter prospective study (ANRS HC EP 23 Fibrostar) which has been described in a previous report [7]. The study was approved by the ethics committee "CCP Sud-Est 5" (Grenoble, France). Informed consent was obtained from each patient.

Briefly, 512 untreated patients with CHC (anti-HCV antibodies positive and RNA-HCV positive) were prospectively recruited from 19 academic centers between November 2007 and July 2008. Patients with associated infection, chronic viral hepatitis B (HBs Ag positive) or HIV, with other liver diseases (drug-related hepatitis, Wilson disease, hemochromatosis, autoimmune hepatitis, alcohol consumption of >30 g/day for men and >20 g/day for women, primary biliary cirrhosis, and α1-antitrypsine deficiency), or with severe systemic diseases were excluded. Patients who received antiviral therapies during the six months preceding the inclusion were also excluded. In the present study, OPG measurements were performed a posteriori, in all the patients from the cohort for whom required serum samples were available, i.e. in 306 patients. Among them, 269 patients had an interpretable liver stiffness measurement (LSM) and were subjected to additional analyses.

2.2. Liver biopsy

Histological analysis of liver biopsies was blindly and independently performed by at least two pathologists, experts in liver histology, as previously described [7]. The stages of liver fibrosis were assessed on a five-point scale according to the METAVIR classification [18], i.e. F0 = no fibrosis, F1 = portal fibrosis without septa, F2 = few septa, F3 = numerous septa without cirrhosis, F4 = cirrhosis. In cases of discrepancy between the pathologists, reanalysis was performed to reach a consensual diagnostic. A minimal length of 15 mm and/or at least 11 portal tracts were required for considering the histological staging except when cirrhosis was evident.

2.3. Blood samples

Fasting blood samples were collected by venipuncture less than two months before or after liver biopsy. Platelets count and prothrombin time were determined on fresh blood samples in each center. Biochemical parameters were centralized in one center and performed as previously described [7]. Serum C-IV was measured using an immunoenzymatic assay with pre-coated plates and allowing measures from 15.6 to 1000 μg/l (Serum Collagen IV EIA, Argutus Medical, Dublin, Ireland). Serum OPG was measured using an ELISA assay (Human OPG/TNFRSF11B, RD systems, Minneapolis, USA). This method displays a coefficient of variation of <8% for repeatability (intra-assay variability) (tested for two concentration values, 1.7 μg/l and 0.6 μg/l) and <12% for reproducibility (inter-assay variability) (tested for two concentration values, 1.2 μg/l and 0.5 μg/l). The limit of detection, defined as the mean OD of the blank plus 2 SD, was 0.063 μg/l. Limits of linearity were estimated from 0.12 to 3.5 μg/l. Fibrotest®, Fibrometer® and Hepascore® were calculated as previously described [8–10]. All the tests were performed blindly of clinical and histological data.

2.4. Liver stiffness measurement (LSM)

Transient elastography (Fibroscan™) was performed in 269 patients from the HCV-cohort the day of blood samples collection as previously described [7]. Liver stiffness measurement (Fibroscan™) failure was defined as zero valid shots (after at least 10 attempts) and "unreliable examinations" were defined as fewer than 10 valid shots or an interquartile range (IQR)/LSM greater than 30% or a success rate less than 60%.