

Hepa-index, a New Biomarker Indicator of Hepatic Fibrosis in Patients with Chronic Liver Disease

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ABSTRACT

AIM: Noninvasive methods for assessment and follow-up of hepatic fibrosis are important for the management of patients with chronic liver disease. Our aim was to assess a new panel of surrogate biomarkers for prediction of severe hepatic fibrosis in patients with chronic liver disease of different aetiology.

METHODS: 118 patients [62 males (52.5%) and 56 females] (47.5%) were prospectively enrolled with a mean age of 55.6 years \pm 14.9. The aetiology of chronic liver disease was hepatitis B virus infection ($n=12$), hepatitis C virus infection ($n=20$), autoimmune hepatitis ($n=36$), alcoholic steatohepatitis ($n=10$), non-alcoholic steatohepatitis: ($n=12$), hepatocellular carcinoma ($n=16$). 12 patients had no evidence of liver disease. Biomarkers of hepatic fibrosis and liver function tests (α_2 -macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, ALT, total cholesterol, AST, albumin, CA19-9, CA125, CA 15-3, INR, platelet count, hyaluronic acid, nitric oxide) were analyzed in serum. As reference for staging of fibrosis we used FibroTest and FibroScan. Biomarkers were correlated to hepatic fibrosis by univariate and multivariate analyses as well as

logistic regression.

RESULTS: Univariate and multivariate analysis indicated that platelet count, α_2 -macroglobulin, total bilirubin, GGT and total cholesterol were the most relevant biomarkers related to the stage of hepatic fibrosis. A new panel for prediction of severe hepatic fibrosis was created using these relevant parameters. Applying this panel; severe hepatic fibrosis was predicted with a sensitivity of 97.4% and a specificity of 85.9% in comparison with FibroTest. Also a sensitivity of 78.8% and specificity of 90.9% was obtained by the panel in comparison to FibroScan.

CONCLUSION: The new noninvasive panel allows accurate prediction of severe liver fibrosis in different types of chronic liver disease.

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Key words: Noninvasive; Biomarkers; Fibrosis; Liver; FibroTest; FibroScan

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INTRODUCTION

Liver fibrosis (LF) is a significant health problem with a worldwide mortality attributable to its consequences (cirrhosis and primary liver cancer) of around 1.5 million deaths per year^[1]. LF occurs in response to almost all causes of chronic liver injury^[2] and is characterized by the excessive deposition of extracellular matrix (ECM) involving molecular and histological rearrangement of various types of collagens, proteoglycans, structural glycoproteins and hyaluronic acid.

Assessing LF is important for both predicting disease progression and monitoring efficacy of therapeutic measures^[3]. Invasive diagnosis using liver biopsy with histological examination is most commonly used as reference standard for the assessment of fibrosis but is hampered by several disadvantages: large sampling error,

consistent inter-observer disagreement, high emotional cost of patient and enormous health care commitment in case of rare but possible severe complications. Besides, liver fibrosis is a dynamic process that cannot adequately be mirrored by the snapshot of a biopsy^[4]. Thus, noninvasive methods of measuring the degree of hepatic fibrosis have been developed, such as surrogate serum fibrosis markers^[5-8], liver stiffness measurement using FibroScan (Echosense, Paris, France)^[9-12], various imaging methods^[13] and glycomics^[14,15]. Biochemical markers of LF, because they can be tested noninvasively, reproducibly, and reliably, may constitute a true alternative to liver biopsies^[16]. Several noninvasive direct and indirect serum markers, capable to predict the presence of significant fibrosis or cirrhosis in patients with chronic liver disease have been reported^[17]. In addition to widely used tests such as FibroTest or FibroMeter, other either indirect (aspartate aminotransferase, prothrombin time, platelets) or direct (type III procollagen-N-peptide, hyaluronic acid, metalloproteinases) markers, usually used in combination, have been evaluated^[18,19]. Simple scores such as AST-to-platelet ratio index (APRI), Enhanced Liver Fibrosis (ELF) or FIB-4 have also been widely studied and have revealed interesting, albeit non-comprehensive, data on liver fibrosis, especially in terms of significant, extensive fibrosis or cirrhosis^[5,20-23]. Recent studies showed the usefulness of some new markers as predictors for severe hepatic fibrosis (tumor markers CA19-9, CA125 and CA15-3)^[20], extensive fibrosis [serum hyaluronic acid (HA)]^[24-26], or progression of chronic liver disease [serum nitric oxide (NO)]^[27]. The combination of non-invasive tests to assess LF has recently also been used in chronic hepatitis C patients with the purpose of establishing new fibrosis stage classification based on the combined assessment of FibroMeter and FibroScan and has high diagnostic accuracy^[28], but new markers that are more accurate and, above all, able to predict the outcome of liver fibrosis are still needed^[18]. Aim of this study is to develop a new noninvasive index for predicting severe hepatic fibrosis.

PATIENTS AND METHODS

Patients

118 patients [62 males (52.5%) and 56 females(47.5%)] were enrolled, mean age 55.59±14.9 years; with different types of chronic liver diseases. 12 patients with chronic hepatitis B infection (HBV) (10.2%), 20 patients with chronic hepatitis C infection (HCV) (16.9%), 36 patients with autoimmune hepatitis (AIH) (30.5%), 10 patients with alcoholic steatohepatitis (ASH) (8.5%), 12 patients with non alcoholic steatohepatitis (NASH) (10.2%) and 16 patients with hepatocellular carcinoma (HCC) (13.6%) were prospectively recruited from the outpatient clinics of the Department of Gastroenterology, Hepatology and Infectious Diseases (Otto-von-Guericke University, Magdeburg, Germany) between March and June 2010 and were classified as test group. Another 12 patients (10.2%) with no evidence of liver disease were additionally recruited and classified as control group. Patients with other causes of liver disease e.g., Primary biliary cirrhosis (PBC), Primary sclerosing cholangitis (PSC), Wilson's disease, and haemochromatosis were excluded. The study protocol was approved by the ethical committee of Otto-von-Guericke University, Magdeburg, Germany and informed consent to participate in the study was obtained from all subjects included.

Laboratory tests

After full history taking and clinical assessment, all patients were subjected to laboratory tests including: liver function tests; [alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma

Table 1 Biochemical characteristics.

Variables	Mean	SD	Reference range
α ₂ -MG (g/L)	2.6519	1.05208	1.30-3.00 g/L
Haptoglobin (g/L)	1.1668	0.77716	0.30-2.00 g/L
Apo-A 1 (g/L)	1.6108	0.43921	1.10-1.60 g/L
Bilirubin total (μmol/L)	13.05	13.644	<21.0 μmol/L
GGT (IU/L)	90.585	131.5504	Male: 10.2-71.4 IU/L, Female: 6-42 IU/L
ALT (IU/L)	36.249	24.4120	Male: 10.2-49.8 IU/L, Female: 10.2-34.8 IU/L
Cholesterol (mmol/L)	5.0097	1.25477	<5.2 mmol/L
AST (IU/L)	40.29	19.442	Male: 17-83 IU/L, Female: 17- 58 IU/L
Albumin (g/L)	43.995	5.0903	35.0-52.0 g/L
CA125 (U/mL)	53.781	136.9339	<35 U/mL
CA19-9 (U/mL)	25.617	37.5370	<27 U/mL
CA15-3 (U/mL)	21.474	11.5471	<25 U/mL
INR	0.9871	0.16925	<1.25
Platelet count (Gpt/L)	222.95	98.619	150-375 Gpt/L
NO (μmol/L)	50.242	22.0234	3.125-100 μmol/L
HA (ng/L)	261.5326	332.56504	(Mlae "Mean±SD" 42.6±24.6 ng/mL), (Femlae premenopausal "Mean±SD" 20.1±14.3 ng/mL), (Femlae postmenopausal "Mean±SD" 50.3±19.9 ng/mL)
FibroScan (kPa)	15.683	17.4019	< 7 kilo pascals

α₂-MG: Alpha-2-macroglobulin; Apo-A1: Apolipoprotein A1; GGT: gamma glutamyl transpeptidase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; NO: nitric oxide; HA: Hyaluronic acid.

glutamyl transpeptidase (GGT), total bilirubin, international normalized ratio (INR)], total cholesterol, albumin and platelet count (using standard procedures). Serum levels of cancer antigen 19-9 (CA19-9), cancer antigen 125 (CA125), and cancer antigen 15-3 (CA15-3) (using ECLIA/ROCHE Diagnostics.) serum level of nitric oxide (NO) (using Total Nitric Oxide Assay kit, Assay Designs, USA) , Hyaluronic acid (HA) (using TECO Hyaluronic acid ELISA Kit, TECO medical Group, Germany), haptoglobin and serum alpha-2-macroglobulin (α₂-MG) level using an automatic nephelometer (BNII, Dade Behring; Marburg, Germany), serum apolipoprotein A1 (Apo-A1) (using ELISA kits, Roche, Switzerland) were assessed. In patients with HCC the diagnosis was proven either by applying the EASL 2002 criteria^[29] in patients with liver cirrhosis or invasively using liver biopsy and histopathological assessment. FibroTest (FT; Biopredictive, Paris, France) provided a numerical quantitative estimate of liver fibrosis ranging from 0.00 to 1.00, corresponding to the well-established METAVIR scoring system of stages F0 to F4^[30-34].

Abdominal ultrasound and liver stiffness

Abdominal ultrasound (using Philips iU22 xMATRIX Ultrasound system) and liver stiffness measurement using FibroScan (Echosens, Paris, France) were done for all subjects. FibroScan was performed on the right lobe of the liver through the right intercostal spaces at a depth of 25-45 mm from the skin surface with the patients lying in a dorsal decubitus position with the right arm in maximal abduction. The tip of the transducer probe was covered with coupling gel and the obtained elasticity value was determined as the median of 10 measurements. The results were expressed in kilopascals (KPa). Only those procedures with consecutive 10 validated measurements with a success rate more than 60% and the interquartile range less than 30% of the median value were included.

Statistical Analysis

The patients' biochemical characteristics and FibroScan results are given as the mean±SD as appropriate (Table 1). Fibrosis stage was assessed using FibroTest (FT; Biopredictive, Paris, France) and FibroScan (Echosens, Paris, France). The main endpoint was discriminating patients with severe fibrosis (F3, F4) from those with early or no fibrosis (F0, F1, F2) using a combination of relevant biomarkers (variables). Variables that had a significant relation of

frequencies of normal and abnormal values to fibrosis stages were identified by Chi-square tests. The variables that were significant in the univariate analysis ($P \leq 0.05$) were included in a logistic regression analysis to determine the independent predictors of fibrosis stage. The three final panels (Table 2) were determined in different ways: Panel 1 by entering several variables into the logistic regression that were suspected to be predictive, panel 2 by including only one of the favored variables (CA125) and adding others in a stepwise procedure in order of their relevance. And panel 3 was built totally data-driven by a stepwise logistic regression. A cross-validation was done in each case. A predictive index (according to each model) was constructed by using the regression coefficients of the independent variables (tables 3 for the third model). The diagnostic value of the index was assessed by calculating the area under the receiver operating characteristic (ROC) curves (Figure 1) and (Figure 2) for the third panel. Diagnostic accuracy was calculated by sensitivity, specificity, positive and negative predictive values for a chosen cut-off. The cut-offs selected from the ROC curve were those that best discriminate between severe (F3, F4) and early or no fibrosis (F0, F1, F2). A p -value of 0.05 was set to be the level of statistical significance. Statistical analyses were performed with SPSS 17.0 (IBM Corporation, Somers, NY).

Table 2 Statistical models (panels) applied.

Model (panel) 1	$\exp(0.065 * CA125 + 0.004 * CA19-9 + 0.104 * CA15-3 + 0.001 * HA - 0.005 * NO - 4.609) / (1 + \exp(0.065 * CA125 + 0.004 * CA19-9 + 0.104 * CA15-3 + 0.001 * HA - 0.005 * NO - 4.609))$
Model (panel) 2	$\exp(0.082 * CA125 + 1.479 * \alpha\text{-MG} + 0.231 * \text{Bilirubin total} - 9.713) / (1 + \exp(0.082 * CA125 + 1.479 * \alpha\text{-MG} + 0.231 * \text{Bilirubin total} - 9.713))$
Model (panel) 3 (Hepa-Index)	$\exp(-0.021 * \text{Platelet} + 1.65 * \alpha\text{-MG} + 0.2 * \text{Bilirubin total} + 0.026 * \text{GGT} - 1.215 * \text{Cholesterol}) / (1 + \exp(-0.021 * \text{Platelet} + 1.65 * \alpha\text{-MG} + 0.2 * \text{Bilirubin total} + 0.026 * \text{GGT} - 1.215 * \text{Cholesterol}))$

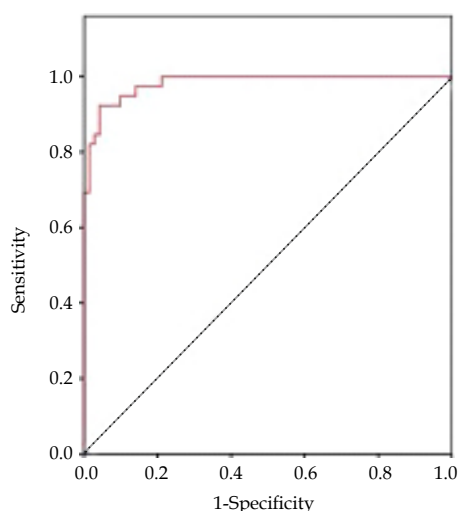


Figure 1 Hepa-Index ROC curve for severe fibrosis (F3-F4) referred to FibroTest.

RESULTS

We studied 118 patients with chronic liver disease of different aetiology. The biochemical characteristics and FibroScan results are shown in Tables 1. FibroTest was done for all cases. FibroScan was possible in only 66 patients (55.93%), 33 patient (27.96%) had severe fibrosis (F3-F4) and 33 patient (27.96%) had early or

no fibrosis (F0-F2). With FibroTest as reference univariate analyses revealed that: alpha-2-macroglobulin, total bilirubin, GGT, total cholesterol, CA125, CA19-9, CA15-3, platelet count, hyaluronic acid and nitric oxide were the most relevant variables associated with severe fibrosis. Relevant variables were combined in partial stepwise logistic regression analyses to create several panels to predict severe LF. We developed 3 different panels (Table 2):

Panel 1: composed of CA125, CA19-9, CA15-3, hyaluronic acid and nitric oxide.

Panel 2: composed of CA125, alpha-2-macroglobulin, total bilirubin.

Panel 3: composed of platelet count, alpha-2-macroglobulin, total bilirubin, GGT and total cholesterol

Panel 1 provided an AUC [95% confidence interval (CI)] for the prediction of severe LF [0.839 (0.743-0.935)] when FibroTest is used as the reference for the fibrosis stage and [0.815 (0.705-0.925)] when FibroScan used as a reference for the fibrosis stage. Panel 2 provided an AUC [95% confidence interval (CI)] for the prediction of severe LF [0.950 (0.912-0.989)] when FibroTest is used as the reference for the fibrosis stage and [0.795 (0.686-0.904)] when FibroScan used as a reference for the fibrosis stage. Panel 3 (Hepa-Index) was the best model which provided a high AUC [95% confidence interval (CI)] for the prediction of severe LF [0.983 (0.964-1.000)] when FibroTest is used as the reference for the fibrosis stage as shown in (Figure 1) and (Figure 2) and [0.869 (0.777-0.960)] when FibroScan used as a reference for the fibrosis stage (Figure 3). A “cut-off” value of 0.2012 predicted severe fibrosis (F3-F4) with a sensitivity of 97.4% and a specificity of 85.9%. Table 3 summarizes the results of the single parameters used for Hepa-Index in the distinct patients groups in different fibrosis stages indicating the positive correlations for bilirubin, alpha-2-macroglobulin, GGT with increasing fibrosis stage and the negative correlation of platelet count and cholesterol.

The Hepa-Index is also correlated with FibroScan results (Figure 2). A “cutoff point” of 0.2012 predicted severe fibrosis (F3-F4) with a sensitivity of 78.8% and a specificity of 90.9%. Cross-validation results were less than 5% different.

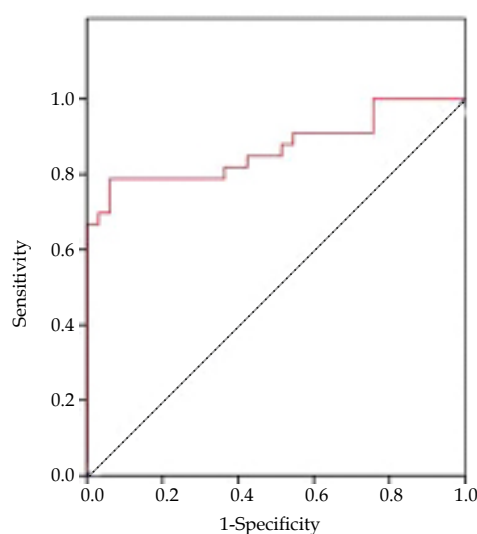


Figure 2 Hepa-Index ROC curve for severe fibrosis (F3-F4) referred to FibroScan.

DISCUSSION

In our study we assessed a set of 17 serum parameters that predict LF (alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin,

Table 3 Mean values±SD of variables included in Model 3 (*Hepa-Index*) in different fibrosis stages.

Grade of Fibrosis according to FibroTest	F0 n=44, 37.3%	F0-F1 n=9, 7.6%	F1 n=5, 4.2%	F1-F2 n=10, 8.5%	F2 n=10, 8.5%	F3 n=11, 9.3%	F4 n=29, 24.6%
Variables							
α -MG (g/L)	1.94±0.48	1.88±0.45	2.45±0.68	3.39±0.94	3.33±0.77	3.16±1.07	3.32±1.16
Total bilirubin (μ mol/L)	7.83±3.96	12.03±6.09	9.16±5.55	9.05±4.65	9.10±4.39	10.81±4.98	25.54±22.27
Cholesterol (mmol/L)	5.43±1.23	4.82±0.90	5.85±1.31	5.88±1.14	4.80±1.07	4.79±1.02	4.13±1.06
GGT (IU/L)	34.19±26.53	83.27±82.75	106.92±105.35	47.4±58.46	37.32±22.59	84.66±57.63	211.1±208.42
Platelet (Cpt/L)	300.6±78.89	258.4±95.46	237.2±33.14	244.9±67.90	172.4±51.03	160.5±70.29	138.2± 66.47
Hepa-Index	0.0059±0.02277	0.0947±0.11402	0.1054±0.11503	0.0869±0.10951	0.3385±0.34195	0.5779±0.31953	0.9549±0.11235

α -MG: Alpha-2-macroglobulin, GGT: gamma glutamyl transpeptidase.

GGT, ALT, total cholesterol, AST, albumin, CA19-9, CA125, CA 15-3, INR, platelet count, hyaluronic acid and serum level of nitric oxide) in different types of chronic liver diseases. Among the 17 serum parameters we identified five (alpha-2-macroglobulin, total bilirubin, GGT, platelet count and total cholesterol) as most relevant predictors of hepatic fibrosis in the studied patients. The findings are concordant with results of Naveau *et al* who found that increased α -macroglobulin has a significant diagnostic value for staging hepatic fibrosis in patients with alcoholic liver disease^[35,36] and Azer *et al.* who reported that progression of fibrosis lead to an increase of bilirubin as a result of impaired hepatic excretion and enterohepatic circulation which is attributed to portal systemic shunting^[37]. Also GGT has previously been found to be correlated with liver fibrosis among patients infected with hepatitis B and C^[6,38-40]. More over Wai *et al*^[5] proposed a simple and elegant model of AST-to-platelet ratio index (APRI), which predicted bridging fibrosis as determined by the Ishak scoring system, with an AUC of 0.80-0.88. Another model, developed by Forns *et al*^[6], included the routinely measured variables of GGT, cholesterol, platelet count, and prothrombin time in combination with age.

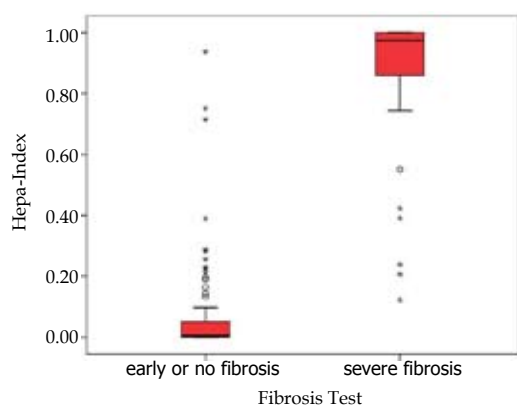


Figure 3 Box plot of Hepa-Index for differentiating severe fibrosis (F3-F4) from early or no fibrosis (F0-F2) in relation to FibroTest.

Hepa-Index provides information for all patients and can easily be applied to clinical routine. Testing for platelet count, total bilirubin, GGT and total cholesterol are routine in most hospitals and laboratories. Alpha-2-macroglobulin is available to any laboratory with a nephelometer. It is therefore less expensive and more convenient to perform Hepa-Index than a liver biopsy or other expensive noninvasive and difficult tests. In chronic viral hepatitis treatment is generally recommended when significant fibrosis has taken place^[41]. A Hepa-Index ≥ 0.2012 could be used as a tool to decide on the indication for antiviral therapy without the requirement for liver biopsy. In addition, the exclusion of advanced fibrosis among patients who have a Hepa-Index < 0.2012 may be particularly useful

in providing prognostic information for patients who are reluctant to undergo biopsy. Another advantage of the Hepa-Index is that it can be used among elderly patients who are unlikely to develop liver-related morbidity or mortality in the absence of advanced fibrosis^[42].

Several models for predicting hepatic fibrosis have been proposed and validated based on a collection of those markers in cohorts of patients with different kinds of chronic liver diseases, mostly in cohorts of patients with chronic viral hepatitis. Some of them are not patented (e.g. PGA, AP, Bonacini, Pohl, Forns, APRI, MP3, FIB-4, FibroIndex), others are patented (FibroTest /FibroSure, FibroSpect II, ELF, FibroMeter and HepaScore)^[5-8,38,43-51]. Although studies demonstrated that FibroTest and other multiparametric panel markers, have some diagnostic limitations and inter-laboratory variations^[52], FibroTest remains the most widely used noninvasive method for the diagnosis of advanced fibrosis with a diagnostic value using standardized area under the ROC curves of 0.84 (95% confidence interval 0.83-0.86) referred to histology, without a significant difference between the causes of liver disease, hepatitis C, hepatitis B, and alcoholic or non alcoholic fatty liver disease. The prognostic value of FibroTest in predicting liver related complications (digestive haemorrhages, ascites and hepatocellular carcinoma) and mortality is at least similar to that of biopsy in HCV, HBV and ALD. Recently, it has been shown to reliably predict the 5-year survival rate of patients with chronic hepatitis C^[53]. Health authorities in some countries have already approved validated biomarkers as the first line procedure for the staging of liver fibrosis^[54].

Among radiologic methods for assessment of hepatic fibrosis, elastographic measurements, either ultrasonography-based (FibroScan, Echosense, Paris, France) or magnetic resonance-based, and magnetic resonance diffusion weighted imaging, show the most promise for accurate staging of hepatic fibrosis^[55].

The index developed in the presented study shows very strong correlation with FibroTest in a prospectively enrolled cohort of patients. The limitations of our study include, the lack of correlation with the currently accepted gold standard reference for hepatic fibrosis which is liver biopsy, therefore Hepa-Index should be validated in comparison to liver biopsy in a prospectively enrolled cohorts of patients of different types of liver diseases. It should be also compared with another "established" panel test, e.g. ELF, APRI, Forns, Fibroindex and Hepa-Score. Another limitation is the cross-sectional character of the study; therefore Hepa-Index should also be longitudinally assessed and validated in treatment contexts in cohorts of patients of distinct chronic liver diseases to evaluate the diagnostic value in special scenarios. Hepa-Index is a promising and useful tool to assess hepatic fibrosis.

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