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PROGRESSION TO FIBROSIS IN PATIENTS WITH NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) – THE VALUE OF NONINVASIVE MARKERS

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The nonalcoholic fatty liver disease (NAFLD) represents a group of conditions that range from simple steatosis to non-alcoholic steatohepatitis(NASH). Because steatohepatitis can progress to fibrosis and cirrhosis – liver failure, it is beginning to be recognized as an important cause of liver-related morbidity and mortality. Liver biopsy, which is considered an invasive procedure, is the gold standard for assessing histologic lesion in NAFLD. The aim of our study was to evaluate the biological and clinical parameters correlated with NAFLD and the non invasive markers that can be predictors of fibrosis in these patients. 51 patients admitted to the University Hospital Bucharest during 1 year with NAFLD were included. Histological diagnoses used Kleiner et al's scoring system. Fibrotest and BARD scores were used. The sensitivity (Se), specificity (Sp), positive and negative predictive values (PPV, NPV) and the area under the ROC curves (AUROC) were assessed. In 14 patients fibrosis was proven histologically and it was statistically more common in patients with diabetes and AST/ALT ratio higher than 0.8. Positive predictive value and negative predictive value for Fibrotest and BARD score were, 93%, 71% and 61%, 84% respectively.

Keywords: nonalcoholic fatty liver disease, liver fibrosis, BARD score, Fibrotest, non invasive markers

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is considered to be, in the Western world, the number one cause of chronic hepatic disease and a progressively recognized cause of liver-related morbidity and mortality[1-3]. It represents a group of conditions that is defined as an accumulation of excessive fat in the liver without alcohol consumption[3-5]. NAFLD ranges from simple steatosis to non-alcoholic steatohepatitis (NASH) – 20-30% of patients, while the first is usually asyptomatic, the second is characterized by apoptosis, inflammation and fibrosis and can develop serious liver sequelae which may progress to cryptogenic cirrhosis and hepatocellular carcinoma[3,6,7]. Because NAFLD affects one of three persons in the developed countries, it has become the third cause of liver transplantation in the US[1,8.] Moreover, the fatty hepatocytes double the risk for type 2 diabetes mellitus and increases the risk for cardiovascular disease[1,9].

Liver biopsy (LB) is currently the gold standard for the diagnosis and staging of NAFLD. Taking into account that it is an invasive procedure and it has several drawbacks - including distress, discomfort, sampling and interpretation errors, a risk of major complications in 1-3%[1,6,10], several noninvasive methods have been developed. Although they are helpful, all the methods have considerable limitations[11]. Some of the noninvasive markers of liver fibrosis are: Fibrotest, BARD score, HAIR score, BAAT score, Palekar's score, Enhanced Liver Fibrosis (ELF) score, Gholam's score, Original European Liver Fibrosis Panel (OEFL) score, Nippon score and the NAFLD fibrosis score[12-15].

The aim of the study was to assess the practicability of Fibrotest and the BARD score in predicting liver fibrosis in Romanian patients with NAFLD. We chose the 2 scores because they include 7 parameters and have a higher probability to be more reliable.

MATERIAL AND METHODS

The study included 51 patients with NAFLD admitted in the University Hospital Bucharest between 2014-2015, 21 females and 30 males. The study population was a homogenous Caucasian group of patients from Romania. The study was conducted prospectively. The patients had metabolic risk factors, abnormal liver function test and/or fatty liver infiltration at ultrasonography. The patients included had no significant alcohol abuse (<20g/day in women and <30g/day in men – confirmed by a family

member), no B or C hepatitis virus, no malignancy, no drug induced or other specific liver disease. According to the American Association for the Study of Liver Disease guidelines, NAFLD is confirmed when the following 4 criteria are simultaneously present: (1) fatty change of the liver; (2) no other factors causing chronic liver disease are present; (3) no other factors inducing fatty change of the liver are present; (4) no significant alcohol abuse is present[16,17]. All patients signed the informed consent before being included in the study. For the metabolic syndrome – according to Adults Treatment Panel III – the patients had three of the five criteria considered: waist circumference (WC) >88cm for women and >102 for men, blood pressure of at least 130/85 mmHg, serum HDl cholesterol <50 mg/dl for women and <40 mg/dl for men, serum triglyceride concentration of at least 150 mg/dl, and plasma glucose concentration of at least 100 mg/dl[18,19].

Clinical evaluation For all the patients we measured their blood pressure three times and the systolic/diastolic blood pressure was considered as the means of the second and third measurement. The body mass index (BMI) was calculated by the formula: weight (kg)/height² (m^2) and the cut-off points were: 25-29,9; 30-34,9; 35-39,9 and >40kg/m^2. The waist circumference was measured between the lower border of the rib cage and the iliac crest, and visceral obesity was considered for values higher than the above mentioned[20].

Serum biochemical markers FibroTest (FT) (Biopredictive, Paris, France, Patented artificial intelligence algorithm USPTO 6, 631, 330) combines five serum biomarkers (α_2-macroglobulin, haptoglobin, apolipoprotein, gama-glutamyl transferase - GGT and total bilirubin) with age and sex of the patient and it generates a quantification of the fibrosis stage[3]. The BARD score ranges from 0 to 4 points, and it comprises 3 variables: a BMI≥28 – 1 point; the presence of diabetes – 1 point; AST/ALT ratio≥0.8 – 2 points [11, 21, 22]. According to Harrison et al, a total of 2-4 points indicates significant fibrosis[23]. The laboratory analysis was determined the day before the liver biopsy and for Fibrotest an outside private laboratory was used.

Histological assessment All liver biopsy specimens were assessed and staged by an expert pathologist, blinded to the clinical results of the patients. To be eligible for evaluation, the liver biopsy had to be at least 1.5 cm in lengths and had to include more than 6 portal tracts. Fibrosis was scored on a 5-point scale suggested by Kleiner et al[24] as follows: stage 0 – no fibrosis; stage 1 – portal or perisinusoidal fibrosis; stage 2 – portal/periportal or perisinusoidal fibrosis; stage 3 – septal or bridging fibrosis; stage 4 – cirrhosis.

Statistical analysis The multivariate data analysis was based on a combination of specific instruments so, for binomial variables we used the phi coefficient and we calculated odds ratio (OR) a with confidence interval of 95%. For each variable, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NVP) were calculated. Validity was measured using the area under the ROC curve (AUROC) with a confidence interval of 95%. For all the tests, the level of significance was established at $p<0.05$.

RESULTS AND DISCUSSIONS

62 patients with fatty liver detected on ultrasonography (US) and metabolic risk factors were included. Out of these patients, 5 refused liver biopsy, 3 were discovered with hepatitis B or C virus, 1 had an alcohol intake higher than 30g/day and 2 were diagnosed with hepato-carcinoma.

The demographic and laboratory characteristics of all the examined patients are presented in Table 1. Twenty patients were women (41.1%), and the average age was 53.16 patients had diabetes (31.3%). 31 patients were obese (BMI≥30) and thirty were hypertensive.

Age (years)*	52.96 (57)
Gender (female %)	21 (41.1 %)
Diabetes %	16 (31.3 %)
BMI (kg/m2)*	32.47 (32)
Waist circumference (cm) *	117.25 (121)
AST (IU/I)*	68.2 (31)
ALT (IU/I)*	91.49 (54)
Triglycerides (mmol/l)*	200.35 (178)
AST/ALT ratio*	0.67 (0.68)

*Means (Median); BMI- body mass index; AST – aspartate aminotransferase; ALT – alanine aminotransferase

Table 1. Demographic and laboratory characteristics of all patients

A comparison of the selected clinical and biochemical features between the groups of patients with no/mild fibrosis (F0-F1) and moderate/advanced fibrosis (F2-F4) was made, but statistically significant differences were found only in the presence of diabetes mellitus and AST/ALT ratio. The results are shown in Table 2.

In comparison with patients with no/mild fibrosis, the ones with moderate/advanced fibrosis were older, had a higher AST/ALT ratio, and a higher percent of them had diabetes.

The fibrotest demonstrated fibrosis in 15 patients, and only in 11 of them fibrosis was confirmed by the liver biopsy. The BARD score demonstrated 13 patients with scores >=2 and 38 patients with low scores. Out of the 13 patients, only in 8 liver biopsy confirmed fibrosis. The accuracy of Fibrotest and BARD score is presented in Table 3. Also, ROC curve was used for Fibrotest and AST/ALT ratio (Figure 1).

Parameter	No/Mild Fibrosis n=37	Moderate/Advanced Fibrosis n=14
Age (years) *	51,08 (56)	57,93 (61)
Gender (male	21 (56,75 %)	9 (64,28 %)
Diabetes %	7 (1,.91 %)	9 (64,28 %)
BMI (kg/m2)	32,58 (32)	32,16 (32)
WC (cm) *	117,54 (124)	116,5 (109)
AST (IU/I) *	34 (24)	158,7 (178)
ALT (IU/I) *	53,08 (45)	193 (204)
Trig (mmol/l)	201,65 (178)	196,93 (191)
AST/ALT	0,62 (0,60)	0,81 (0,84)
Fibrotest *	0,24 (0,22)	0,56 (0,62)

n-number of cases; BMI- body mass index; WC –waist circumference; Trig – triglycerides; AST – aspartate aminotransferase; ALT – alanine aminotransferase.

Table 2. Comparison of selected clinical and biochemical parameters between patients with no/mild and moderate/advanced fibrosis

Parameter	FIBROTEST	BARD score
Sensitivity	73 %	57 %
Specificity	92 %	86 %
PPV	93 %	62 %
NPV	71 %	84 %
Phi coefficient	0.66 OR:30.2500 95%CI: 5.8376 to 156.75 P<0.0001	0.45 OR:8.5333 95%CI: 2.0681 to 35.20 P<0.0030

PPV-positive predictive value; NPV negative predictive value; OR-odds ratio; CI-confidence interval

Table 3. Accuracy of Fibrotest and BARD score for predicting fibrosis

Because not all patients have easy access to liver biopsy, some guidelines are needed. In our study, a high AST/ALT ratio (>0.8) was associated with advanced fibrosis. As it is easily accessible, the patients with AST/ALT ratio >0.8 should be considered at risk for advanced fibrosis and should be sent to specialized centers for further investigations.

Our study shows that Fibrotest and BARD scores have high specificity. The specificity of Fibrotest is 92% and of the BARD score is 86% and the sensitivity is 73% and 57 %, respectively. The result for Fibrotest were similar to those in the literature: specificity 92/98% and sensitivity 73/77%[1]. The BARD score on the other hand had very different results: specificity 86/44% and sensitivity 57/89%[22]. Fibrotest has a high PPV (93%), while BARD has a high NPV (84%) and can avoid liver biopsy in a large number of cases.

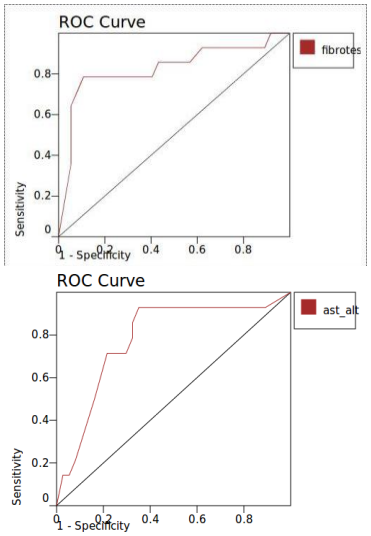

Figure1. Predictive value of Fibrotest and AST/ALT ratio

In order to avoid liver biopsy, an invasive procedure, the search for noninvasive, new and simpler markers of diagnosing liver biopsy is ongoing[11,25]. These markers could substantially reduce the need and number of liver biopsies and it would be more likely to be accepted by patients. The most important advantage of these scoring systems is that they are easily repeatable and derived from accessible clinical and biochemical indices.

The limitations of this study were that we had a small number of patients and we did not have a control group, because healthy people would normally refuse to undergo liver biopsy.

CONCLUSIONS

The data for our study reveals that the Fibrotest can diagnose advanced fibrosis, while the BARD score can reliably exclude advanced fibrosis and reduce the number of liver biopsies in patients with NAFLD. Diabetes mellitus and AST/ALT ratio are considered risk factors of advanced fibrosis in these patients.

The originality of this manuscript is that is a validation of these 2 tests, together, on a romanian population and the patients were included without discrimination (age, sex, comorbidities). Furthermore, in an attempt to use prognostic tests on patients with steatohepatitis, that lead to similar results with liver biopsy, we need multiple validations because all the studies have a relatively small number of patients. The originality lies in the fact that, although the Bard test confirms his prognostic value along with Fibrotest, our data are slightly different from literature, considering that the specificity was higher while the sensitivity was lower.

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