

Predictive Factors for Nonalcoholic Steatohepatitis (NASH) in Patients with Nonalcoholic Fatty Liver Disease (NAFLD)

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Abstract

Aims: The aim of our study was to assess the clinical and biological parameters associated with Nonalcoholic steatohepatitis (NASH) and to establish the predictors of significant fibrosis in Nonalcoholic fatty liver disease (NAFLD) patients. **Methods:** We correlated clinical and biochemical parameters with histological features (simple steatosis or steatohepatitis) in 97 patients with NAFLD admitted to the University Hospital Bucharest for persistently raised aminotransferase levels. The biochemical parameters included lipid profile, glucose, liver tests and insulin. The Homeostatic Metabolic Assessment (HOMA)-index and the oxidative stress were also evaluated. Factors associated with NASH and severe fibrosis ($F \geq 3$) were identified using the Mann-Whitney U test and multivariate analysis. The overall validity was measured using the area under receiver operating characteristic curve (AUROC) with 95% CI. **Results:** At univariate analysis, age, BMI, splenic longitudinal diameter (SLD), HOMA, gamma glutamyl transpeptidase, C-reactive protein (CRP), albumin and INR were significantly associated with histologically proven NASH. The multivariate analysis identified four independent predictive factors for the presence of NASH: CRP ($p=0.004$), SLD ($p=0.018$), HOMA ($p=0.03$) and albumin level ($p=0.041$). The variables independently associated with severe fibrosis were albumin ($p=0.008$), blood glucose ($p=0.017$) and BMI ($p=0.048$). **Conclusion:** A predictive model that incorporates the clinical and biological parameters may identify at-risk patients with NAFLD, avoiding liver biopsy on a routine basis.

Key words

Nonalcoholic steatohepatitis (NASH) – nonalcoholic fatty liver disease (NAFLD) – predictive factors – fibrosis.

Abbreviations

NASH- Nonalcoholic steatohepatitis; NAFLD- Nonalcoholic fatty liver disease; HOMA- Homeostatic Metabolic Assessment; AUROC- the Area Under Receiver Operating Characteristic Curve; BMI- Body Mass Index; SLD- Splenic longitudinal diameter; CRP- C-Reactive Protein; INR- International Normalized Ratio; WC- Waist Circumference; ALT- Alanine aminotransferase; AST- Aspartate aminotransferase; GGT- γ -glutamyl transferase; MDA- Malondialdehyde; GSH- Serum glutathion; US- Ultrasonography; PPV- Positive Predictive Value; NPV- Negative Predictive Value; SS- Simple Steatosis.

Introduction

Non-alcoholic fatty liver disease (NAFLD) represents a group of conditions ranging from simple liver steatosis, usually asymptomatic, to nonalcoholic steatohepatitis (NASH), which is characterized by the presence of apoptosis, inflammation and fibrosis, and also by a progressive course, evolving to cryptogenic cirrhosis [1].

The goal of diagnostic procedures is to identify the patients with NASH before the stage of advanced fibrosis. Currently, the diagnosis of NASH requires an invasive liver biopsy with drawbacks of sampling and interpretation errors [2]. Hence the need for noninvasive strategies to cover the whole spectrum of NAFLD is a priority. It is also necessary for future research to develop an algorithm for NAFLD patients screening and to identify patients at risk of liver disease progression. Therefore, the aim of our study was to assess the clinical features and risk factors for NASH and to establish the predictors of fibrosis in NAFLD patients.

Methods

Study population

We performed a study on 134 patients with metabolic risk factors (aged 29-71) admitted in the University Hospital Bucharest between 2003-2009 for abnormal liver function tests and fatty liver infiltration at grey-scale ultrasonography (US). Inclusion criteria consisted of the absence of significant

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alcohol abuse (<20g daily, confirmed by at least one family member), no evidence of hepatitis B and C and of drug induced liver disease and no other specific liver diseases: hemochromatosis, α 1 antitripsin deficiency, Wilson's disease or autoimmune liver disease. Ninety seven patients who fulfilled the inclusion criteria were enrolled and underwent clinical evaluation, anthropometric measurements, blood tests and liver biopsy. According to the histological characteristics the patients were divided in two groups: group I: patients with histological proven simple steatosis (SS) and group II: patients with histological proven NASH. We excluded borderline patients, with steatosis associated with mild inflammation or mild fibrosis, not sufficient for NASH diagnosis.

The study was approved by the local ethics committees and all individuals provided written informed consent prior to enrollment in the study.

Metabolic syndrome was defined according to the revised Adults Treatment Panel III (2001), and three of the five listed criteria were considered: plasma glucose concentration of at least 100 mg/dl, waist circumference (WC) >88 cm for women and >102 cm for men, serum HDL cholesterol <50 mg/dl for women and <40 mg/dl for men, blood pressure of at least 130/85 mm Hg, and serum triglyceride concentration of at least 150 mg /dl [3].

Clinical evaluation

Body mass index (BMI) was calculated as weight (kg) divided by height (m²). Overweight or the degrees of obesity were established on the basis of BMI cut-off points of 25-29.9, 30-34.9, 35-39.9 and >40 kg/m², respectively. Visceral obesity were identified by measuring waist circumference (WC) at the midpoint between the lower border of the rib cage and the iliac crest. Systolic/diastolic blood pressure was defined as the mean of the second and third reading of three consecutive blood pressure measurements.

Biochemical evaluation

Liver function tests were performed prior to the liver biopsy. Blood samples were obtained under fasting conditions and the following tests were performed using standard laboratory methods: alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum albumin, γ -glutamyl transferase (GGT), total bilirubin, alkaline phosphatase, international normalized ratio (INR), glucose, cholesterol, triglycerides, uric acid, serum concentration of iron and ferritin. All were measured using Dade Behring reactants and the Dimension RXL analyzer (Dade Behring, FL, USA). CRP values were determined by the ELISA test and the reference values accepted were between 0.2 and 9 mg/l.

Insulin level was assessed using the method of indirect chemiluminescence (MEIA). Insulin resistance was calculated according to the HOMA index (HOMeostatic Metabolic Assessment): the product between the serum glucose and insulin levels in basal conditions were divided at 22.5. The index of the oxidative stress was evaluated by assessing serum lipid peroxidation product malondialdehyde (MDA), and serum glutathion (GSH). Plasma TBARS (the

condensation product between MDA and TBA-thiobarbituric acid at 95°C) was assessed by using the high performance liquid chromatography (HPLC) method. Serum GSH was measured by the spectrophotometrical Ellman's method.

Ultrasound evaluation

The diagnosis of NAFLD was obtained by US. The measurements were performed using Acuson S2000 Siemens machine. Spleen volume was estimated by splenic longitudinal diameter (SLD). We used the maximum length obtained between the two poles of the spleen. The measurements were performed by postero-lateral scanning. The classification of "bright liver" or hepatic steatosis was based on a four-point scale of hyperechogenicity: 0 = absent, 1 = light, 2 = moderate, 3 = severe, according to the difference between the densities of the liver and the right kidney.

Histological assessment

All the enrolled patients underwent histological assessment by percutaneous liver biopsy. This was performed by senior operators using the Menghini technique with a 1.4-mm-diameter needle (Hepafix; Braun, Germany). The liver samples were fixed in formalin, paraffin embedded and stained with hematoxylin-eosin and Masson's trichrome. All biopsy specimens were analyzed by an expert pathologist (20 year experience) blinded to the patients' clinical results. The length of each liver biopsy was established in millimeters and the number of portal tracts was counted. Only liver fragments of at least 1.5 cm in length, including 8 portal tracts were considered valuable for histological assessment. The diagnosis of NASH was based on the Brunt et al criteria [4], modified by Kleiner et al [5]. The stage of fibrosis was scored based on a five-point scale, as follows: stage 0, absence of fibrosis; stage 1, perisinusoidal or portal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, septal or bridging fibrosis; and stage 4, cirrhosis.

The severity of steatosis was graded from 1 to 3, according to the percentage of cells with fatty droplets (1: 10-33%; 2: 33-66%; and 3: >66%). The presence of siderosis was evaluated by Perls staining [4, 5].

Statistical analysis

Results were expressed as median and 25th- to 75th-percentile values for continuous variables with non-normal distribution (compared by Mann-Whitney U test); for categorical variables were used frequencies (compared by Fisher's exact test). Variables significant in univariate analysis were used in multivariable analysis (logistic regression) and odds ratios (OR) with 95% confidence intervals were determined. Two separate logistic regression analyses were performed to assess the variables independently associated with (1) presence of NASH and (2) presence of severe fibrosis (F \geq 3). The overall validity of each predictor was measured using area under the receiver operating characteristic curve (AUROC) with 95% CI. The optimal cutoff was chosen at the highest left point on the curve. Finally, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. For all tests, significance was achieved at $p < 0.05$.

Results

Potentially predictive variables for NASH diagnosis

Ninety-seven patients with persistently raised aminotransferase levels and fatty liver detected on ultrasonography (US) after exclusion of other liver disorders, were prospectively enrolled. The patients were selected out of 134 patients with metabolic risk factors who attended the University Hospital Bucharest for liver involvement. Eighty-four patients (86.5%) had visceral obesity, 36 (37.11%) were diabetic and 21(21.6%) had impaired glucose tolerance (IGT). Insulin resistance was diagnosed in 92 (94.8%) patients. According to the liver pathology, the NAFLD patients were divided in two groups: group I- 42 patients with NASH and group II – 45 patients with simple steatosis (SS). Ten patients with borderline histology were excluded from the study. Demographic, clinical and biochemical variables were compared between groups to identify the predictive factors associated with NASH and also with severe fibrosis ($F \geq 3$).

Demographic, clinical and biochemical characteristics of the patients included in the study are shown in Table I.

Patients with NASH were significantly older, had higher values for BMI, GGT, SLD and insulin resistance (IR) compared to patients with SS.

Table I. Patient characteristics expressed as median (max, min)

Characteristics	SS	NASH	p
Age (years)	52 (27, 72)	58 (29, 69)	0.027
BMI(Kg/m2)	28.5 (27, 35)	34.75 (27, 41.5)	<0.01
WC (cm) ♂	122 (101, 138)	133.5(114, 157)	0.031
WC (cm) ♀	114 (88, 136)	117 (97, 144)	0.194
SLD (cm)	92 (70, 121.5)	120.5 (97.5,185)	<0.001
Glucose (mg/dl)	104 (77, 134)	131.5 (78, 214)	0.258
HOMA	2.9 (1.7, 3.1)	4.2 (2.5, 6.2)	<0.001
Triglycerides (mg/dl)	183 (155, 289)	219.5 (119, 423)	0.473
Cholesterol (mg/dl)	210 (114, 400)	220.5 (120, 423)	0.679
HDL cholesterol (mg/dl)	38 (25, 57)	36.5 (23, 48)	0.641
AST (U/L)	78 (32, 223)	91.5 (52, 141)	0.143
ALT (U/L)	91 (39, 183)	99 (63, 183)	0.069
GGT (U/L)	89 (33, 210)	126 (80, 345)	<0.001
Albumin (g/dl)	3.9 (2.6, 4.8)	3.3 (2.1, 4.5)	<0.001
Ferritin (ng/ml)	145 (117, 216)	150 (92, 264)	0.221
INR	1 (0.8, 1.3)	1.1 (0.8, 1.5)	0.036
Uric acid (mg/dl)	4.7 (2.8, 7.5)	6 (3.5, 9)	0.186
CRP (mg/l)	1.5 (0.65, 5.5)	5.3 (2.7, 8.5)	<0.001
MDA (nmol/ml)	0.94 (0.29, 2.55)	2.7 (2.5, 5.45)	<0.001
GSH (μmol/l)	5.25 (3.5, 6.5)	4.2 (2.5, 5.45)	<0.001

BMI = Body mass index, WC = waist circumference, SLD = Spleen longitudinal diameter HOMA = HOmeostasis Metabolic Assesment, HDL =high density lipoprotein, AST = aspartate transaminase, ALT = alanine transaminase, GGT = γ -glutamyl transferase, INR = international normalized ratio, CRP = C-reactive protein, MDA = malondialdehyde, GSH = glutathion

Variables significantly associated with NASH are shown in Table I. At univariate analysis, age, BMI, SLD, HOMA, GGT, CRP, albumin and INR were significantly correlated with histological proven NASH. Only in men, WC was a significant predictor of NASH.

Values of CRP, MDA, HOMA, GGT, BMI and SLD were higher in patients with NASH, while values of albumin and INR were lower in these patients (Figs. 1, 2).

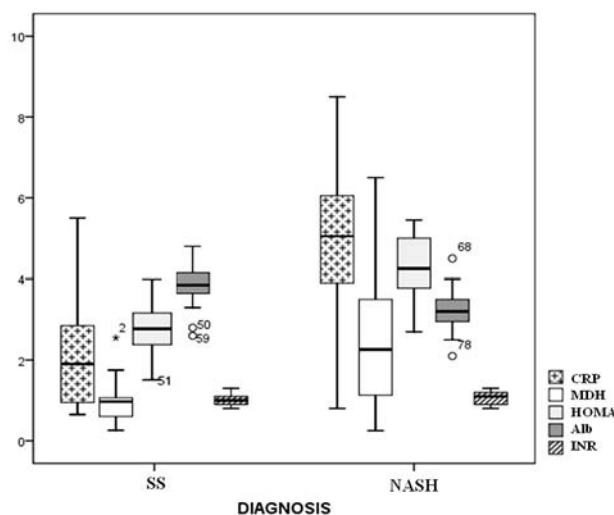


Fig 1. Distribution of CPR, MDH, HOMA, Albumin and INR values according to the histological forms of NAFLD. SS= simple steatosis, NASH= nonalcoholic fatty liver disease, CRP= C- reactive protein, MDA= malonyldialdehyde, HOMA= HOmeostasis Metabolic Assesment, Alb=albumin, INR= International normalized ratio. Top and bottom of boxes represent first and third quartiles, respectively. Length of box represents interquartile range within which 50% of values are located. Thick line through each box represents the median. Error bars mark minimum and maximum values (range). Small circles represent outliers.

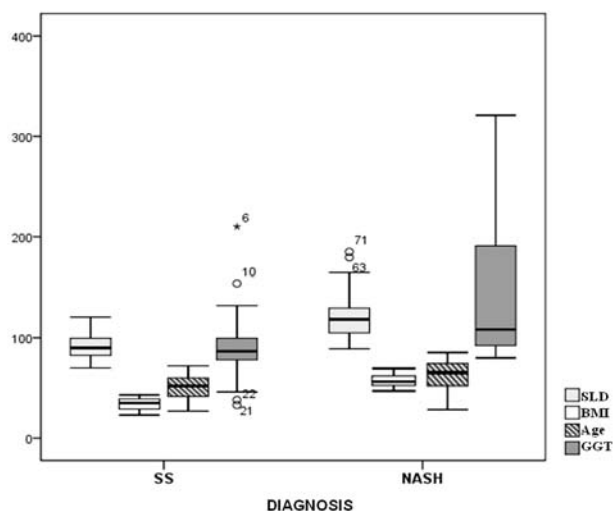


Fig 2. Distribution of SLD, BMI, age and GGT in patients with simple steatosis and in those with NASH. SS= Simple Steatosis, NASH= Nonalcoholic steatohepatitis, SLD= Spleen longitudinal diameter, BMI=Body mass index, GGT= Gamma glutamyl transpeptidase. The accuracy of each parameter for NASH prediction was assessed with ROC Curve Analysis expressed as Area Under ROC (AUC).

The accuracy of each parameter for NASH prediction was assessed by AUROC (Fig. 3).

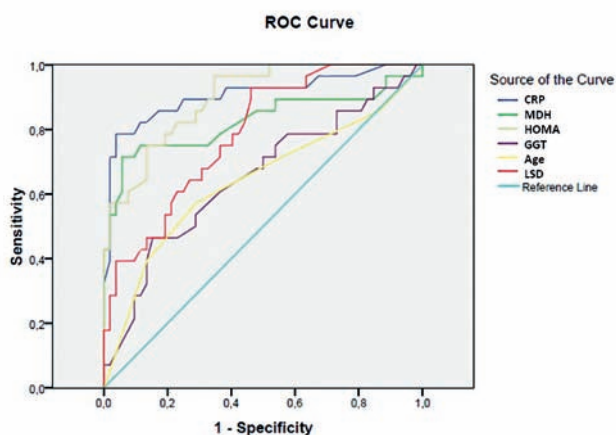


Fig 3. Predictive values of CRP, HOMA, MDA, SLD Age and GGT for distinguishing NASH from SS. CPR=C- reactive protein, MDA= Malonyldialdehyde, HOMA= HOmeostasis Metabolic Assesment, SLD= Splenic longitudinal diameter, GGT= Gamma glutamyl transpeptidase.

Finally, sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated. The optimal cutoff was chosen at the highest left point on the curve (Table II).

Table II. Diagnostic accuracy and optimal cut-off values of each parameter for NASH prediction

Variable	AUC	CI 95%	Cut-off	Sn (%)	Sp (%)	PPV (%)	NPV (%)	+LR
CRP	0.906	0.826 - 0.986	3.5	82	88	79	90	7.9
HOMA	0.850	0.768 - 0.932	2.5	82	69	59	88	2.7
MDA	0.897	0.830 - 0.964	11	60	92	81	81	7.9
LSD	0.825	0.710 - 0.939	1.35	71	94	87	86	12.4
GGT	0.785	0.685 - 0.885	94	75	63	52	82	2

CRP=C- reactive protein, HOMA=HOmeostasis Metabolic Assesment, MDA= Malondialdehyde, SLD= Splenic longitudinal diameter, GGT= Gamma glutamyl transpeptidase; AUC= Area under the ROC curve, Sn= sensitivity, Sp= specificity, PPV= positive predictive value, NPV= negative predictive value, LR= likelihood ratio

Variables significant at univariate analysis were introduced into the logistic regression analysis. The multivariate analysis identified four independent predictive factors for the presence of NASH in NAFLD patients (Table III). Our results showed the highest specificity (94%) of SLD among the other parameters, independently from AUROC data or logistic regression output. Thus, SLD proved to be the best parameter to predict NASH.

Table III. Logistic regression analysis for NASH prediction

Variables	OR	95% CI		p
		Lower	Upper	
C-reactive protein	6.220	1.789	21.629	0.004
Splenic longitudinal diameter	1.186	1.030	1.366	0.018
HOMA	1.141	1.026	1.344	0.030
Albumin	0.049	0.003	0.879	0.041
Constant	0.000			0.123

Potentially predictive variables of fibrosis

Histological characteristics of the two groups are shown in Table IV. No difference in the grade of steatosis was observed between the two groups.

Table IV. Liver histology in patients with NAFLD

Histology	SS (45 pts) %	NASH (42 pts) %
Steatosis grade (1/2/3)	27 / 37 / 36	25 / 35 / 40
Necroinflammatory grade (0/1/2/3)	100 / 0 / 0 / 0	0 / 5 / 40 / 55
Fibrosis stage (0/1/2/3/4)	100 / 0 / 0 / 0 / 0	5 / 2 / 48 / 38 / 7

At univariate analysis, variables significantly associated with severe fibrosis ($F \geq 3$) were: blood glucose, albumin, CRP, BMI and triglycerides (Table V). These variables were introduced into the logistic regression analysis to identify the parameters independently associated with severe fibrosis in NASH patients. According to the multivariate analysis, three independent factors were associated with severe fibrosis: albumin level: OR=0.057; 95% CI (0.007-0.477), $p=0.008$; glucose level: OR =1.051; 95% CI (1.009-1.094), $p=0.017$ and BMI: OR =1.266; 95% CI (1.001 -1.618), $p=0.048$. These parameters formed a model able to identify patients with severe fibrosis demonstrated with an AUROC = 0.897, 95% CI=(0.790-1.005), $p<0.001$ (Fig. 4).

Discussion

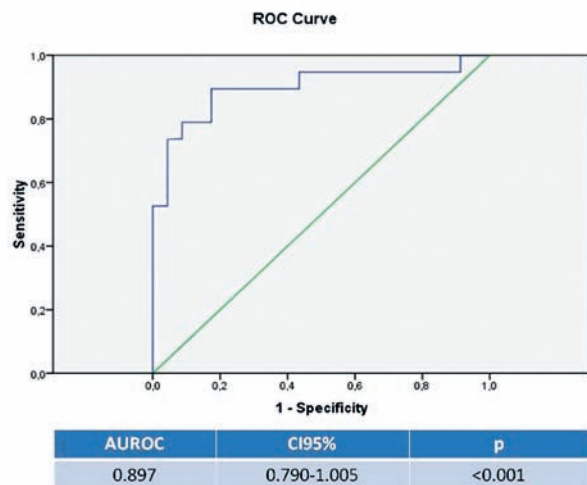
Nonalcoholic steatohepatitis represents the progressive form of NAFLD, with greater potential for progressing to end-stage liver disease. To identify the patients at risk for NASH, 97 patients with persistently raised liver tests and fatty liver detected on US, with exclusion of other liver disorders were prospectively enrolled.

The recognition of insulin resistance (IR) as a major determinant of steatogenesis and possibly of liver disease progression [6] implies that this parameter should be evaluated in all studied patients. Insulin resistance was assessed by fasting serum insulin and glucose and quantified by the HOMA index, a validated method of assessing IR [6, 7]. In our study, NASH group presented higher IR (mean HOMA index, 4.1 vs. 2.7 in patients with SS). The risk of developing NASH was significantly associated with HOMA-IR. At logistic regression analysis, HOMA index was one of the major predictors of NASH.

Table V. The predictive factors for severe fibrosis ($F \geq 3$) in NASH patients

Variables	F0/F1/F2	F3/F4	p
Glucose (mg/dl)	123 (88,164)	139 (85,214)	0.002
Albumin (g/dl)	3.5 (3.0,4.0)	3.1 (2.1,4.5)	0.003
CRP (mg/l)	5.5 (4.5,8.0)	4.8 (2.7,8.5)	0.011
BMI (Kg/m^2)	33 (27,40)	35 (27.5, 41.50)	0.030
Triglycerides (mg/dl)	209 (119,279)	247 (121,423)	0.041
Age (years)	54 (32, 69)	58 (29, 69)	0.502
HOMA	3.9 (2.5,6.5)	4.5 (2.5,6.5)	0.658
Cholesterol (mg/dl)	210(120,423)	221 (132,279)	0.742
HDL cholesterol (mg/dl)	36.5 (25.5,45)	35 (23,48)	0.404
AST (U/L)	92 (52, 141)	90 (62, 137)	0.781
INR	1 (0.8, 1.3)	1 (0.8, 1.3)	0.969
GGT (U/L)	125 (89, 288)	127 (80, 345)	0.667
Uric acid (mg/dl)	6 (3.5, 8)	6.2 (3.5, 9)	0.416
MDA	2.8 (0.4, 5.0)	2.5 (1.0, 4.5)	0.603
ALT (U/L)	99 (63, 137)	99 (87, 183)	0.120
GSH ($\mu mol/l$)	4.1 (2.5, 5.4)	4.3 (3.0, 5.4)	0.810
SLD (cm)	119.9 (101.9, 132)	123.5 (97.5, 185)	0.139

Median (min, max); Mann-Whitney U test

**Fig 4.** Predictive value of logistic regression model for severe fibrosis ($F \geq 3$) in NASH.

Visceral obesity is one of the most important risk factors for NASH, which is usually associated with impaired insulin activity, overflow of portal triglycerides and overproduction of inflammatory cytokines [8].

Even though many surrogate parameters have been proposed as markers of visceral adiposity, WC remains the simplest and most widely used. In this direction, Singh et al identified WC as an independent predictor of the degree of liver necroinflammation [9]. Park et al used the clinical BMI value to differentiate the simple steatosis from NASH,

establishing a threshold value for BMI as indicator for NASH [10].

Stranges et al demonstrated that abdominal height, a simple anthropometric index of abdominal visceral adipose tissue accumulation, was consistently better correlated to GGT levels than BMI, in both sexes [11]. In our study, almost all patients (87) had visceral obesity. We evaluated the predictive role of BMI, WC and GGT levels for NASH diagnosis. Our results showed that the best correlation with NASH was obtained for GGT levels ($p < 0.001$). Patients with elevated GGT levels had the greatest likelihood of having NASH. Nevertheless, GGT values (Fig. 2) showed some overlapping between the highest quartile in the SS group and the lowest quartile in the NASH group. Furthermore, in the NASH group there was a large dispersion of values. This observation reduces the discriminatory power of this parameter. In fact, GGT is a clue of oxidative stress and can vary according to metabolic conditions, specifically IR [12].

Body mass index values were significantly associated with NASH diagnosis, having a lower predictive power ($p = 0.01$). Regarding WC, only in men was this parameter a significant predictor of NASH ($p = 0.031$). However, at logistic regression analysis all these parameters (GGT, BMI and WC) were not significant predictors. Our data confirmed the observation of Franczani et al who demonstrated a significant correlation between steatosis and visceral adiposity but failed to demonstrate a significant correlation between WC and NASH at multivariate analysis. It is indeed possible, as suggested by the authors, that once NAFLD appears, visceral fat is no longer a major determinant of the severity of liver damage [13].

Insulin resistance, oxidative stress, and an inflammatory cascade are believed to play integral roles in the pathogenesis and progression of NAFLD. A “multi-hit” (formerly “double-hit”) hypothesis has been used to describe the pathogenesis of NAFLD. Inflammatory mediators, reactive oxygen species and abnormal apoptotic mechanisms serve as “second hits” that result in superimposed inflammation (NASH) [14, 15].

The origin of oxidative stress in patients with NASH is represented by mitochondrial dysfunction, followed by increased reactive oxygen species (ROS) production, which, in turn, initiates a positive feedback with organelle damage. ROS damage mitochondrial DNA (mtDNA) and activate cellular lipid peroxidation, generating malondialdehyde and 4-hydroxynonenal [16-18]. In our study, plasma concentration of MDA was lower in subjects with steatosis versus NASH patients ($p < 0.001$). Increased oxidative stress in patients with NASH produced a decrease of antioxidant capacity of the liver expressed by reduced serum GSH. NASH patients had serum glutathione significantly lower than patients with steatosis ($p < 0.001$). Despite their contribution to NASH pathogenesis, the traditional parameters of oxidative stress (MDA and GSH) were not significant predictors of NASH at logistic regression analysis.

The inflammatory status was demonstrated by the significantly higher values of inflammation parameters,

reflected by CRP. For NASH prediction, CRP values delimited an AUROC of 0.906 (CI 95% 0.826–0.986); with 82% sensitivity and 88% specificity. At the same time, CRP values were the most important independent predictive factor for NASH diagnosis. Yoneda et al reported for the first time a significant elevation of CRP in patients with NASH compared with SS. These data suggest that CRP may be a biological feature that distinguishes NASH from simple nonprogressive steatosis and may also indicate the severity of hepatic fibrosis in NASH patients [19]. However, Haukeland et al found no difference between patients with SS and those with NASH regarding the CRP serum levels [20]. Moreover, in obese patients, Zimmermann et al found that CRP may be a marker of steatosis, but not of NAFLD severity [21]. Our study supports the results of Assy et al, who established that high CRP activity was an independent predictor of NAFLD. They suggested the role of CRP in NAFLD progression and proposed its inclusion in NAFLD algorithms [22]. Recently, Malhi et al showed that inflammation and apoptosis are key features of progressive NASH. Apoptosis can be induced by free fatty acids, and this is a possible mechanism in the pathogenesis of NASH [23]. We should mention that CRP increases in every acute inflammation with drawbacks of interpretation errors. On the other hand, in a complex analysis, Tarantino et al found that peripheral IR determines not only increased hepatic synthesis of free fatty acids and triglyceride accumulation but also overproduction of IL-6 leading to low-grade chronic inflammation and spleen enlargement. This feature is explained by the accumulation of activated macrophages inside the spleen due to monocyte chemoattractant protein-1, which is over-expressed in adipose tissue [24, 25]. Our study showed that spleen enlargement, assessed by SLD, was also an independent factor of NASH prediction (Table III).

The prognosis and clinical management of chronic liver diseases are highly dependent on the extent of liver fibrosis. This is particularly true in patients with NASH - the most common cause of chronic liver disease by the end of the last decade - with potential for serious liver damage and liver-related mortality [26]. That is why we proposed to estimate the predictive factors associated with the progressive course of NASH. To achieve this, we correlated the clinico-biological parameters with severe fibrosis ($F \geq 3$). Our results showed a striking relationship between albumin, glucose and BMI and severe fibrosis, according to the multivariate analysis in NASH patients.

Several authors showed that variables related to glucose control (impaired glucose metabolism/diabetes) were more frequently identified as predictors of fibrosis [27, 28]. Severe fibrosis was independently predicted by diabetes presence in Franczani's study [29]. Angulo et al [30] constructed a scoring system for identification of liver fibrosis in NAFLD patients. This noninvasive scoring system included albumin, BMI and glucose concentration as independent indicators of advanced liver fibrosis. Younossi et al [31] also developed a reliable NASH-related fibrosis model which included clinical and biological characteristics: diabetes, gender,

BMI, triglycerides. Our data identified hyperglycemia to be significantly correlated with fibrosis ($p=0.017$), and so we concluded that glucose concentration has an impact on liver fibrogenesis in NASH patients. Also, albumin concentration in our study was a strong predictor for fibrosis in the NASH group (Table III). Another parameter correlated with fibrosis was BMI, with a lower predictive value. In our study, the NASH-related advanced fibrosis model had good performance characteristics according to the AUROC ($p < 0.001$) and could also become useful in the management of patients with NAFLD.

Our study has some limitations. Even though HOMA is a very good index to assess IR, some authors emphasize its daily variability related to the type of ingested food, mainly in obese or diabetic patients [32]. The CRP is an acute reactant phase protein and in order to use it as a reliable marker of NASH diagnosis, other inflammatory conditions should be excluded.

In **conclusion**, the goal of our study was to establish a clinico-biological strategy to identify at risk patients with NAFLD without using liver biopsy on a routine basis. Can we identify the patients at risk? And is liver biopsy the only predictor? We are beginning to find answers. A predictive model that incorporates the independent risk factors may allow identification of NASH among patients with suspected NAFLD and may one day provide the best diagnostic approach for patients with NAFLD. Further testing and validation are needed for these noninvasive strategies to refine their role in clinical practice and supplant the need for liver biopsy in most NAFLD patients.

Conflicts of interest

Nothing to declare.

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