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Noninvasive Clinical Model for the Diagnosis of Nonalcoholic Steatohepatitis in Overweight and Morbidly Obese Patients undergoing to Bariatric Surgery

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Rezumat

Model clinic noninvaziv pentru diagnosticul steatohepatitei nonalcoolice la pacienții supraponderali și cu obezitate morbidă supuși chirurgiei bariatrice

Introducere: Biopsia hepatică este o metodă invazivă considerată "gold standard" pentru diferențierea steatohepatitei non-alcoolice (NASH) de alte subtipuri histologice de ficat gras non-alcoholic (NAFLD). Un test noninvaziv pentru diagnosticarea NASH și aprecierea severității NAFLD înainte de intervenția chirurgicală și de asemenea pentru monitorizarea modificărilor hepatice după chirurgia bariatrică reprezintă o importantă provocare medicală.
Scop: A dezvolta un model noninvaziv de biomarkeri pentru diagnosticul NASH la pacienții supraponderali, obezi și cu obezitate morbidă (MOP).
Pacienți și metode: Au fost admiși 60 pacienți (BMI mediu = 47.81kg/m^2) după excluderea altor etiologii de boală hepatică cronică. Au fost prelevate biopsii hepatice în timpul chirurgiei bariatrice sau percutan și stadializate conform scorului Kleiner. Am folosit Testul Wilcoxon rank sum pentru compararea variabilelor continue și regresia logistică pentru predicția NASH.
Rezultate: Analiza de regresie logistică a arătat că valorile BMI, ALT, AST, fosfataza alcalină, (ALP), HOMA-IR, hs-CRP, M30, M65, leptinelor and adiponectinelor au rămas predictori independenți pentru NASH ($p < 0.02$). Folosind analiza AUC, am stabilit următoarele niveluri *cutoff* pentru NASH (egal sau mai mare): BMI $\geq$ 47kg/m^2, ALT $\geq$ 32IU/ml, AST $\geq$ 25UI/ml, ALP $\geq$ 85Iu/ml, HOMA-IR $\geq$ 4, M65 $\geq$ 389U/L, (egal sau mai mic) adiponectine $\leq$ 13.5 mg/L. Scorul NASH, calculat ca suma acestor 7 parametrii, cu un *cutoff* de 4 puncte, poate prezice cu acurateţe NASH (sensibilitate de 90%, specificitate de 93,94% și AUC de 0,957).
Concluzii: Propunem un model noninvaziv pentru diagnosticul NASH care urmează să fie validat prospectiv. Utilizând acest model, putem prezice NASH fără riscurile biopsiei hepatice.

Cuvinte cheie: steatohepatita, markeri noninvazivi, obezitate, chirurgie bariatrică

Abstract

Background: Liver biopsy, an invasive method, is the gold standard for differentiate nonalcoholic steatohepatitis (NASH) from other stages of fatty liver disease. A noninvasive test to diagnose NASH and disease severity before surgery and also for monitoring disease status after bariatric surgery (BS) will be an important medical challenge.
Aim: To create a noninvasive biomarkers model for the diagnosis of NASH in overweight, obese and morbidly obese patients (MOP).
Patients and methods: Sixty patients (mean BMI= 47.81kg/m^2) were admitted after exclusion of other causes of liver disease. Liver biopsies were obtained at the time of the bariatric surgery or by percutaneous liver biopsy and graded using Kleiner score. Continuous variables were compared using Wilcoxon rank sum test and for prediction of NASH we used logistic regression.

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Results: Logistic regression analysis showed that BMI, ALT, AST, alkaline phosphatase (ALP), HOMA-R, hs-CRP, M30, M65, leptine and adiponectine levels remained independent predictors for NASH ($p<0.02$). Using AUC analysis, we established the following cutoff levels being indicative of NASH: BMI ≥ 47 kg/m^2, ALT ≥ 32 IU/mL, AST ≥ 25 IU/mL, ALP ≥ 85 IU/mL, HOMA-IR ≥ 4, M65 ≥ 389 U/L. Adiponectine ≤ 13.5 mg/L. A NASH-score, calculated as the sum of these 7 parameters, at a cutoff level of 4 points, can accurately predict NASH (sensitivity of 90%, specificity of 93.94% and AUC of 0.9576).
Conclusions: We propose a noninvasive model for NASH diagnosis in MOP that should be validated prospectively. Using this noninvasive score, NASH would be predicted without the risks of liver biopsy.

Key words: steatohepatitis, noninvasive biomarkers, obese, bariatric surgery

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease in the worldwide occurring in 33 % of the general population in the United States and in over 90% of the patients with morbid obesity that are undergoing bariatric surgery (1,2,3,4,5).

According to the World Health Organization, 2.3 billion adults will be overweight and >700 million will be obese by 2015 (6).

In conjunction with the growing epidemics of obesity in adults and children, the prevalence of NAFLD is also increasing (7,8).

NAFLD encompasses a histological spectrum ranging from simple steatosis (NAFL) to nonalcoholic steatohepatitis (NASH) with various degrees of fibrosis that may progress eventually to cirrhosis (9).

Although NAFL typically follows a benign non-progressive clinical course, NASH (which includes necroinflammation, ballooning degeneration and fibrosis) is a potentially serious condition, since as many as 25% of patients may progress to cirrhosis and experience complications of portal hypertension, liver failure and hepatocellular carcinoma (10,11,12,13).

A key issue for the prognosis and management of NAFLD is the differentiation of simple steatosis from NASH.

To date, liver biopsy remains the best gold standard for diagnosing NASH and grading the severity of liver damages (14). Serious complications of liver biopsy have been described, included retroperitoneal bleeding, bile leak and severe pain. The major contraindication to liver biopsy is coagulopathy and relative contraindication is obesity. Nowadays, laparoscopic bariatric surgery for obesity (15,16) has increased the intraoperative liver biopsies in order to assess liver histology. Obvious, is not possible to perform liver biopsies in all patients with NAFLD in order to exclude the severe form of this disease (NASH). Therefore, the limitations of liver biopsy have stimulated the search for noninvasive serum tests, easy to perform, reliable which can differentiate NASH from simple steatosis especially among patients with high risk for NAFLD (morbidly obese patients submitted to bariatric surgery) (17,18).

To date, a number of individual or combination of non-invasive biomarkers has been developed to differentiate between simple steatosis and NASH.

Given the complexity of the pathogenesis of NASH, it is supposed that adipocytokines and typical cytokines like tumor necrosis factor (TNFα), interleukin 6 (IL-6), C- High sensitivity reactive protein (hs-CRP), secreted by inflammatory cells infiltrating adipose tissue, play an important role in the development of NASH (19,20,21,22,23).

Emerging data suggest that hepatocyte apoptosis, a highly organized and genetically controlled form of cell death, may play an important role in liver injury and disease progression in NAFLD (24,25).

Previous study observed that CK18 fragments (M65 antigen, a measurement of overall cell death due to both apoptosis and necrosis and M30 antigen, a specific measurement of apoptosis) released in serum may have value for the non-invasive detection of the necroinflammatory activity during the course of liver injury (26,27,28,29).

Increase in hepatocyte cell death by apoptosis is typically present in NASH patients but absent in those with NAFL (24). Some studies demonstrated the utility of quantification of CK18 for diagnosis of NASH (30,31).

Based on the actual knowledge of the pathogenesis of NAFLD, a panel of serum biomarkers may provide a better means of discriminating NASH from other less progressive subtype of NAFLD and reduce the need for liver biopsy.

We supposed that a combination of anthropometric data with components of the adipocytokines pathway and apoptosis and necrosis markers, may provide a valuable composite diagnostic marker for NASH.

The aims of the study were to appreciate if individual or combination of pro-inflammatory cytokines (hs-CRP, TNFα, IL6), adipocitokines (adiponectin, leptin), fragments of CK-18 and BMI could be a promising biomarkers for distinguish between NASH and simple steatosis in morbidly obese patients undergoing bariatric surgery.

Patients and methods

Patient's selection

We included 60 overweight (BMI ≥ 25kg/m^2), obese (BMI ≥ 30kg/m^2) and morbidly obese patients (BMI ≥ 40 kg/m^2 or ≥ 35kg/m^2) with significant weight-related co-morbidities) admitted in Fundeni Clinical Institute or "Delta" Clinical Hospital between 2009-2010 after exclusion other causes of liver diseases.

Patients with history of alcohol consumption greater than 20g per day for men and 10g per day for women were excluded. Further, patients were excluded if they had any other

concomitant liver disease (viral or autoimmune hepatitis, primary biliary cirrhosis, henochromatosis, and Wilson's disease), infectious diseases, neoplasia, kidney diseases or ingestion of hepatotoxic drugs (amiodarone, tamoxifen, glucocorticoids).

Written informed consent was obtained from all patients before the beginning of the study, in accordance with the principles of the Declaration of Helsinki.

Anthropometric and biochemical evaluation

Anthropometric evaluation included waist, hip to waist ratio, calculation the body mass index (BMI) as weight / height 2 (Kg/m^2). BMI was used as an index for relative weight.

The following biological parameters were measured by standard laboratory technique: aspartate aminotransferase (AST), gammaglutaril-transferase (GGT), alkaline phosphatase (ALP), total cholesterol (TC), HDL cholesterol (HDLc), LDL choresterol (LDLc), triglycerides (TG), fasting glucose (FG), fasting serum insulin (FI).

Homeostasis Model Assessment (HOMA-IR) scores were obtained with HOMA calculator software.

Measurement of adipokines, cytokines, markers of apoptosis and necrosis

One day before liver biopsy a serum samples was stored at -80°C and biomarkers testing was performed when all patients were included in study.

Serum levels of adipokines and cytokines (adiponectin, leptin, h-CRP, IL-6, TNFα) were measured with competitive ELISA assays. Serum levels of CK-18 fragments (M30 and M65) were measured by ELISA assays (PEVIVA AB, Sweden).

Histopathology

Wedge liver biopsy (sample size measures 0,6 cm x 0,5 cm) was obtained from the left liver during bariatric surgery or percutaneous liver biopsy (at lest 2cm in length) perform with ultrasound guidance from right lobe. All biopsies contained a minimum 8 portal tracts and were fixed in formaldehyde, embedded in paraffin and stained with hematoxylin and eosin, Masson trichrome and silver impregnation. Each patient included in study had a liver biopsy. All liver biopsy were read by two hepatopathologists, graded and staging of NAFLD were scored according to the system reported by Kleiner (10).

NAFLD activity score (NAS) represents the sum of scores for steatosis, lobular inflammation and hepatocellular ballooning and ranges from 0 to 8. NAS scores of 0-2 was considered not diagnostic of NASH; scores of 3 to 4 were considered "borderline" and scores of 5 to 8 were considered diagnostic of NASH. (10) Patients were divided into two groups according to their histological diagnosis: NASH (NAS $\geq$ 3) and nonNASH (NAS $<$ 3). Because of small number of patients, in NASH group we additionally included patients with "borderline" NASH.

Statistical analysis

Continuous variables are presented as mean and standard deviation, while categorical variables as percent Univariate comparisons between quantitative variables were performed using Wilcoxon rank sum test, and between categorical variables using Fisher's exact test. We tested separately the association between clinical or biochemical parameters and NASH diagnosis using logistic regression. Crude odds ratios (ORs) were calculated with the corresponding 95% confidence intervals (CIs). The diagnostic performance of each clinical or biological parameter (independent variables) to discriminate about NASH diagnosis was evaluated through receiver operating characteristic (ROC) curve analysis. Area under the ROC curve (AUC) with the corresponding 95% confidence intervals, sensitivity, specificity, positive (PPV) and negative (NPV) predictive values, likelihood ratios positive (LR+) and negative (LR-) were calculated. We chose as cut off value the point on the ROC curve where sensitivity and specificity are almost equal.

All statistical tests were two-sided and a level of $P < 0.05$ was used to indicate statistical significance. We did not use Bonferroni correction adjustment for multiple testing. All statistical analyses were carried out using STATA/SE 11 software (StataCorp, College Station, TX, USA).

Results

The main clinical and laboratory characteristics of the patients included in two groups (NASH and nonNASH) are described in *Table 1*.

The patient's age (mean 45.7 ± 10.3 years vs 44.9 ± 7.6 years) and gender were similar among the two histological groups.

Patients with NASH had significantly greater waist (mean 137 ± 16.91 cm) and BMI (44.9±7.69 kg/m^2) than another group (mean waist 117.84 ± 26.35 cm, mean BMI 39.60±11 kg/m^2).

Significant differences were observed between groups regarding ALT, AST, ALP, FG, HOMA, FI, TG, adiponectin, leptin, hs-CRP, M30 and M60. (*Table 1*)

As expected, according literature, (24) patients with NASH demonstrated significantly statistic lower adiponectin levels (9.25±3.73) compared with patients from nonNASH group (18.57±6.03, p=0.00001).

Serum TNFα, IL-6, TC, HDLc, LDLc levels showed no association with NASH.

Logistic regression analysis showed a positive significantly statistic association of ALT, AST, ALP, FG, FI, HOMA,TG, leptin, hs-CRP, M30, M65 with NASH and these parameters should be considered independent predictors for the presence of NASH.

We found that the risk of having histological steatohepatitis increased with increasing BMI (OR 4.67; 95%CI: 1.28-17.05) or waist (OR 4.83; 95%CI:1.11-21.01).

We found that ALT and AST levels were a strong independent predictors for steatohepatitis. (OR 28.29; 95% CI: 5.11-156.45, OR 33.92; 95% CI:5.98-192.48 respectively). On the other hand, adiponectin presented a negative significantly statistic association with NASH (OR=0.03 95%

Table 1. *Demographic, clinical and biological characteristics of patients according to NAFLD groups*

Variables	NonNASH	NASH (N=47)	p value (N=13)
Gender: % Women	70.2	69.2	0.999
Age (years)	45.9±10.6	44.9 ± 9.4	0.7462
BMI (kg/m²)	39.6 ± 11.0	49.4 ± 7.6	0.0014
Waist (cm)	117.8 ± 26.3	137 ± 16.9	0.0175
Hip to waist ratio	0.9 ± 0.1	0.9 ± 0.1	0.5936
ALT (IU/L)	21.3 ± 11.8	42.3 ± 15.2	0.00001
AST (IU/)	18.8 ± 5.4	32.2 ± 13.2	0.00001
GGT (IU/L)	40.4±29.3	46.6±17.6	0.1736
ALP (IU/L)	72.3±35.7	90.6±35.5	0.0455
FG (mg/dl)	99.6±25.3	139.0±49.0	0.0025
FI (μUI/ml)	18.5±48.4	19.7±9.2	0.0035
HOMA index	2.6±3.9	6.6±4.8	0.0004
TC (mg/dl)	200.2±42.6	205.0±33.7	0.6749
HDLc (mg/dl)	48.2±11.8	40.4±8.5	0.087
LDLc (mg/dl)	129.7±35.2	128.4±28.5	0.8443
TG (mg/dl)	117.6±61.4	246.0±278.3	0.0053
Adiponectin (mg/L)	18.5±6.0	9.2±3.7	0.00001
Leptin (ng/ml)	34.1±31.1	59.8±32.9	0.006
Hs-CRP (mg/dl)	1.1±1.5	1.7±1.0	0.006
TNFα (pg/ml)	6.5±1.2	7.2±1.6	0.2447
IL-6 (pg/ml)	7.2±10.1	8.4±12.8	0.3865
M30 (U/L)	134.8±47.3	203.3±106.0	0.0037
M65 (U/L)	282.3±169.9	597.7±427.7	0.0004

Table 2. *Association between clinical or biological parameters and NASH diagnosis*

		Odds ratio		
Variable	N	OR	95%CI	P (OR)
BMI	60	4.67	1.28 - 17.05	0.02
Waist	48	4.83	1.11 - 21.01	0.036
ALT	56	28.29	5.11 - 156.45	0.00001
AST	56	33.92	5.98 - 192.48	0.00001
GGT	50	2.63	0.72 - 9.64	0.145
ALP	51	4.33	1.12 - 16.78	0.034
FG	55	4.14	1.06 - 16.13	0.04
FI	48	5.73	1.25 - 26.28	0.025
HOMA-IR	48	7.52	1.60- 35.26	0.011
TC	56	1.53	0.42 - 5.58	0.516
HDLC	49	0.49	0.13 - 1.83	0.286
LDLC	48	1.41	0.37 - 5.45	0.617
TG	54	4.00	1.03 - 15.6	0.046
Adiponectin	58	0.03	0.02 - 0.16	0.00001
Leptin	57	3.09	0.86 - 11.12	0.00001
Hs-CRP	59	6.40	1.51 - 27.1	0.012
TNFα	39	2.56	0.65 - 10.06	0.178
IL6	47	1.48	0.41 - 5.33	0.551
M30	59	4.22	1.12 - 15.87	0.033
M65	59	5.71	1.49 - 21.84	0.011

Results are expressed as mean ± standard deviation (SD) for quantitative variables and as percent (%) for gender.
p values of Wilcoxon rank sum test and Fisher's exact test, as appropriate.

CI: 0.02-0.16, p=0.0001). (*Table 2*)

The area under the ROC curve was calculated for establish the statistically performance of each patients' parameters. (*Table 3*)

As an individual parameters, AST and ALT presented an AUC of 0.911 and 0.907 respectively, sensitivity (84.6% and 84.6% respectively), specificity (86.1% and 83.7% respectively) and LR+ (6.06 and 5.20 respectively) for

Table 3. *Receiver operating characteristics (ROC) area under the curve; cutoff values, sensitivity, specificity, Positive and negative likelihood ratios and positive and negative predictive values for discriminate between nonNASH and NASH diagnosis*

AUC	95%CI	Cutoff	Sensitivity	Specificity	LR+	LR-	PPV	NPV	
BMI	0.792	0.677 - 0.907	47	69.2%	72.3%	2.50	0.43	40.9%	89.5%
Waist	0.746	0.606 - 0.886	130	70.0%	71.1%	2.42	0.42	38.9%	90.0%
ALT	0.907	0.831 - 0.983	32	84.6%	83.7%	5.20	0.18	61.1%	94.7%
AST	0.911	0.836 - 0.986	25	84.6%	86.1%	6.06	0.18	64.7%	94.9%
GGT	0.628	0.477 - 0.778	41	61.5%	62.2%	1.63	0.62	36.4%	82.1%
ALP	0.687	0.514 - 0.861	85	69.2%	65.8%	2.02	0.47	40.9%	86.2%
FG	0.788	0.64 - 0.935	103	66.7%	67.4%	2.05	0.49	36.4%	87.9%
FI	0.801	0.661 - 0.942	14	70.0%	71.1%	2.42	0.42	38.9%	90.0%
HOMA-IR	0.866	0.763 - 0.969	4	80.0%	76.3%	3.38	0.26	47.1%	93.5%
TC	0.540	0.371 - 0.709	196	58.3%	52.3%	1.22	0.80	25.0%	82.1%
HDLC	0.666	0.498 - 0.833	43	58.3%	59.5%	1.44	0.70	31.8%	81.5%
LDLC	0.520	0.335 - 0.705	127	54.6%	54.1%	1.19	0.84	26.1%	80.0%
TG	0.766	0.623 - 0.909	140	66.7%	66.7%	2.00	0.50	36.4%	87.5%
Adiponect	0.939	0.848 – 1.000	13.5	92.3%	86.7%	6.92	0.09	66.7%	97.5%
Leptin	0.753	0.615 - 0.89	40	61.5%	65.9%	1.81	0.58	34.8%	85.3%
Hs-CRP	0.759	0.636 - 0.882	1.1	75.0%	68.1%	2.35	0.37	37.5%	91.4%
TNFα	0.615	0.421 - 0.810	6.8	61.5%	61.5%	1.60	0.63	44.4%	76.2%
IL-6	0.578	0.402 - 0.754	2.7	53.9%	55.9%	1.22	0.83	31.8%	76.0%
M30	0.765	0.613 - 0.917	136	69.2%	65.2%	1.99	0.47	36.0%	88.2%
M65	0.824	0.710 - 0.938	389	69.2%	71.7%	2.45	0.43	40.9%	89.2%

Cutoff values correspond to the highest accuracy value (minimal false-positive and false negative results)

prediction histological NASH.

Our analysis revealed that adiponectin had the greatest area under the curve (AUC= 0.939, 95%CI: 0.848 – 1.000), and predicted histological NASH with 92.3% sensitivity and 86.7% specificity.

In the mean time, the M65, HOMA-IR, FI and ALP levels could discriminate NASH from simple steatosis with an AUC > 0.8. (*Table 3*)

Using AUC analysis, we established for these parameters the following cutoff levels being indicative of NASH with the highest accuracy (equal or above): BMI of 47 kg/m^2, ALT of 32 IU/mL, AST of 25 IU/mL, ALP of 85 IU/mL, HOMA-IR of 4, M65 of 389 U/L. For adiponectine, a value equal or below 13.5 mg/L was indicative of NASH.

In order to provide a better statistical accuracy for discriminating NASH from other less progressive subtype of NAFLD, we proposed a predictive model, base on 7 parameters which, all together, provided the greatest AUC. (*Table 3*)

We assigned 1 point for each parameter whose cut-off levels was indicative of NASH, while for those parameters whose cut-off level wasn't indicative of NASH, we attributed 0 points. A NASH-score was calculated as the sum of these 7 parameters, with a total 7 possible points.

We found that a cutoff level of 4 points can accurately predict NASH in overwight, obese or morbid obese patients (AUC of 0.9576, sensitivity of 90%, and specificity of 93.94%, positive and negative predictive values of 81.82% and 96.88%, respectively, correctly classified 93.02% of morbid obese patients). (*Fig. 1*)

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According to our results, we proposed a model of non-invasive markers with higher diagnostic accuracy for the prediction of NASH (AUC 0.957) than each parameter alone.

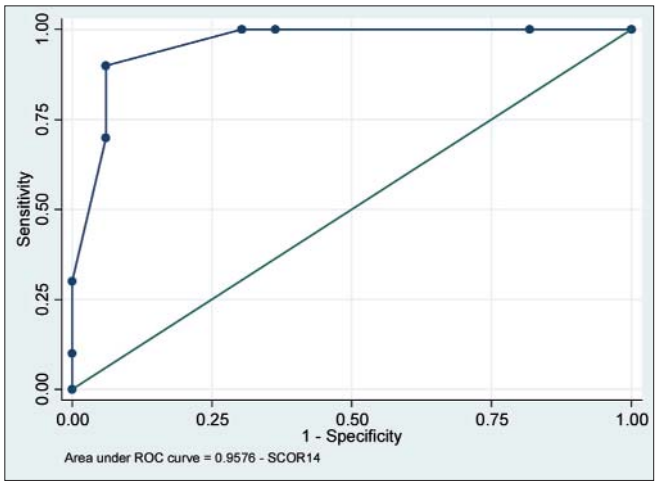


Figure 1. *ROC curve of noninvasive parameters model consisting of: BMI, ALT, AST, ALP, HOMA-IR, M65 and adiponectin levels which accurately diagnosis NASH in morbidly obese patients undergoing bariatric surgery*

Discussion

Nowadays, obesity is a common and well-demonstrated risk factor for NAFLD. In patients with morbid obesity undergoing bariatric surgery, the prevalence of NAFLD is above 90% and up to 5% of patients may have cirrhosis (32,33,34,35).

It is generally agreed that the patients with simple steatosis have very slow, if any, histological progression, while patients with NASH the disease can progress to cirrhotic stage and they have an increased liver-related mortality rate (32,33,34).

Liver biopsy is considered the best gold standard for the assessment of liver fat, inflammation and fibrosis, but because its complications and morbidities, there is a need for less invasive procedures.

Given the increasing incidence and prevalence of NAFLD in our society, there is necessary to develop such noninvasive tests that aid physicians for identification of patients with more severe subtype of liver disease (e.g. NASH), but also allow for noninvasive monitoring of disease, prognosis and response to therapy.

In our study we evaluated some anthropometric data and selected serological parameters base on the actual knowledge about pathogenesis of NAFLD and the results from previous study. We analyzed the predictive values of previously demonstrated markers of apoptosis and necrosis, inflammatory cytokines, adipocitokin, simple laboratory parameters and demographic data for distinguish between NASH and simple steatosis in overweight, obese or morbidly obese patients undergoing bariatric surgery.

In agreement with other studies, (36,37) in our study BMI (OR: 4.67, 95%CI: 1.28 - 17.05, $p<0.02$) and waist circumference (OR: 4.83, 95%CI: 1.11 - 21.01, $p<0.036$) remained independent risk factors for the presence of NASH in our studied patients.

Routine biochemical tests (considering the markers of liver damages) ALT, AST and ALP were correlated to the severity of the hepatic damages. (31)

In our study, as individual parameters, AST and ALT (of cutoff levels 25IU/mL, 32IU/mL respectively) had greater accuracy for prediction histological NASH, with high specificity (86.1% and 83.7% respectively) sensitivity (84.6% and 84.6% respectively) and LR+ (6.06 and 5.20 respectively).

We found significant statistically differences between patients with NASH and nonNASH, regarding glucose tolerance status. Fasting glucose, insulin levels and insulin resistance, assessed by HOMA index, had higher values in NASH in comparison with nonNASH patients ($p= 0.0025$, $p= 0.0035$, $p= 0.0004$). According another study (Shimada et al), HOMA index could be an individual predictor for steatohepatitis in morbidly obese patients (38). In our study, HOMA index of a cutoff level over 4, shown an AUC of 0.866 with a specificity of 76.3% and sensitivity of 80% for detecting NASH.

As in other studies, (30) we didn't find significant differences between patients with NASH and simple steatosis ($p=0.550$, $p=0.172$ respectively) regarding serum IL6 and TNFα.

Similar to other studies has been previously reported, (40,41) our results reveled that higher hs-PCR levels was statistically correlated with the presence of NASH and remain independent predictors for more severe hepatic damages.

According to literature, patients with NASH have a lower adiponectin levels in comparison with patients with simple steatosis (42).

Our results demonstrated that the adiponectin with a cutoff level of 13.5 appears to be more accurate for diagnosis of NASH and provided a greatest AUC (0.939), a sensitivity of 92.3%, a specificity of 86.7% and correctly classified 61.5% of patients.

A recent meta-analysis estimated that serum CK levels have a sensitivity of 78 %, a specificity of 87% and an AUC of 0.82 (95% CI: 0.78-0.88) for NASH in patients with NAFLD (43).

We found that M65 antigen, the CK18 fragment which measure the overall cell death due to both apoptosis and necrosis, had significantly accuracy for discriminating NASH from simple steatosis with an AUC=0.824 (95% CI: 0.710-0.938), a sensitivity of 69.2 % and a specificity of 71.7%. Regarding M30 fragment, which measure only apoptosis, our data shown a sensibility and specificity of 69.2%, 65.2% and an AUC =0.765 (95%CI: 0.613-0.917).

In order to provide a better statistical accuracy for discriminating NASH from other less progressive subtype of NAFLD, we proposed a predictive model, base on seven parameters with a maximum score of 7 points.

The results demonstrate that this multimarkers model appears to be more accurate for diagnosis NASH than using each parameter alone. A cutoff levels of 4 points, provided an AUC (0.958) significantly statistic higher than utilizing each biomarker (BMI, ALT, AST, ALP, HOMA, adiponectin, M65) alone (0.79, 0.90, 0.91, 0.88, 0.75, 0.939 and 0.824 respectively.)

For instance, patients who have BMI $\geq$ 47 kg/m^2, ALT $\geq$ 32 IU/mL, AST $\geq$ 25 IU/mL, ALP $\geq$ 85 IU/mL, HOMA-IR $\geq$ 4, M65 $\geq$ 389 U/L or adiponectine $\leq$ 13.5 mg/L and obtained a NASH score $\geq$ 4, are more likely to have NASH than simple steatosis in a 93.02% of the cases.

Many previous studies on panels of noninvasive biomarkers with significant diagnostic value for NASH have been published.

Younossi et al has proposed a combination set, called NASH Diagnostics Test, based on four parameters (CK 18 fragments: M30 and M65, adiponectin and resistin) that had a sensitivity of 95.45 %, a specificity of 70.21% and an AUC of 0.908 for NASH in obese patients. This Test has been validated by Feldstein at al. (28)

Another noninvasive test based on 13 parameters (NASHTest), proposed by Poynard at al. (44) commercially available, validated by Mathurin at al (45) for patients with morbid obesity, had a good statistic performances for NASH prediction (AUC 0.79, a sensitivity of 29 %, a specificity of 98%).

Other few tests have been already developed for the diagnosis of steatosis and steatohepatitis, but these study presents some limitation related to the reduced number of

patients (Shimada index, Palekar index, Sookoian model) (39,46,47).

As we have been demonstrated, our study proposed a model base on demographic and serological biomarkers for prediction of NASH, with significantly statistically accuracy. Unfortunately, there are some limitation of our statistically analysis. First of all, regarding the relative small number of studied patients and second because its results should be validated prospectively. Finally, we included in a study patients with well characterized phenotype (overweight, obese and morbidly obese patients), so it would be important to validate this model in a general population.

Our results, according to those already published, relieved that using a combination of biomarkers that characterized different pathways involved in already known NAFLD pathogenesis, allowed us a better accuracy for discrimination between steatohepatitis and simple steatosis rather than we used each individual parameter.

Therefore, with this biomarkers model we can "save" and reduce number of liver biopsy at 93 from 100 obese patients. In the mean time, it could provide a noninvasive, reproductible, and accurate method for reduced intraoperative liver biopsies, monitoring and evaluated the effect of bariatric surgery on NAFLD

In the next years, due to the epidemics of obesity and NAFLD, will be an important medical challenge to introduce a new generation of noninvasive biomarkers, as a first line, for accuracy diagnostic of steatohepatitis in order to replaced liver biopsy.

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