PREDICTIVE VALUES OF SERUM URIC ACID AND ALANINE-AMINOTRANSFERASE FOR FATTY LIVER INDEX IN MONTENEGRIN POPULATION

PREDIKTIVNE VREDNOSTI MOKRAĆNE KISELINE I ALANIN-AMINOTRANSFERAZE U CILJU DIJAGNOSTIKOVANJA MASNE JETRE U CRNOGORSKOJ POPULACIJI

Aleksandra Klisic[1], Nebojsa Kavaric[1], Ana Ninic[2]

[1]*Primary Health Care Center, Podgorica, Montenegro*
[2]*Department for Medical Biochemistry, University of Belgrade – Faculty of Pharmacy, Belgrade, Serbia*

Summary

Background: Alanine-aminotransferase (ALT) and uric acid cut-off levels used in non-alcoholic fatty liver disease (NAFLD) diagnosis are advised to be lowered. Due to contradictory results on the utility of both these biomarkers for NAFLD screening, we aimed to determine their cut-off levels that can be applied to Montenegrin population with the fatty liver disease.
Methods: A total of 771 volunteers were enrolled. A fatty liver index (FLI) score ≥60 was used as proxy of NAFLD. The receiver operating characteristic curve analysis with the area under the curve (AUC) was used to determine the cut-off values of ALT and uric acid associated with FLI ≥60.
Results: ALT was independent predictor of FLI in both men and women, whereas serum uric acid was its independent predictor only in women. Lower cut-off levels of ALT are associated with the increased prevalence of NAFLD [i.e., ALT was 19 IU/L (AUC=0.746, sensitivity 63%, specificity 72%, P<0.001) in women and 22 IU/L (AUC=0.804, sensitivity 61%, specificity 95%, P<0.001) in men]. The cut-off value for uric acid was 274 µmol/L (AUC=0.821, sensitivity 68%, specificity 82%, P<0.001) in women.
Conclusions: Lower cut-off levels of ALT in both genders, and serum uric acid in females, can be reliable predictors of the FLI.

Keywords: fatty liver, hyperuricemia, inflammation, transaminases, obesity

Kratak sadržaj

Uvod: Preporuka skorijih studija je da cut-off vrednosti alanin-aminotransferaze (ALT) i mokraćne kiseline budu niže u cilju dijagnostikovanja nealkoholne steatoze jetre. S obzirom na postojanje oprečnih rezultata kada su u pitanju ovi biomarkeri u cilju skrininga na nealkoholnu steatozu jetre, cilj ove studije je bio da se odrede cut-off vrednosti koje bi mogle biti primenjive u crnogorskoj populaciji za dijagnostiku ovog poremećaja.
Metode: Istraživanje je obuhvatilo ukupno 771 dobrovoljca. Indeks masne jetre (engl. fatty liver index-FLI) ≥60 je korišten za dijagnostikovanje nealkoholne steatoze jetre. ROC analiza i površina ispod krive (engl. area under the curve-AUC) su korišteni za određivanje cut-off vrednosti ALT i mokraćne kiseline, koji su povezani sa FLI ≥60.
Rezultati: ALT je nezavisan prediktor FLI kod oba pola, dok je mokraćna kiselina nezavisan prediktor masne jetre samo kod žena. Takođe, niže cut-off vrednosti ALT su povezane sa većom prevalencom pojave masne jetre [za ALT kod žena vrednost je 19 IU/L (AUC=0,746, senzitivnost 63%, specifičnost 72%, P<0,001), a kod muškaraca je 22 IU/L (AUC=0,804, senzitivnost 61%, specifičnost 95%, P<0,001)]. Cut-off vrednost za mokraćnu kiselinu za pojavu masne jetre kod žena je 274 µmol/L (AUC=0,821, senzitivnost 68%, specifičnost 82%, P<0,001).
Zaključak: Niže cut-off vrednosti ALT kod oba pola, kao i niže vrednosti mokraćne kiseline kod žena, mogu biti pouzdani prediktori za pojavu masne jetre.

Ključne reči: masna jetra, hiperurikemija, inflamacija, transaminaze, gojaznost

Address for correspondence:

Aleksandra Klisic, MD, PhD
Center for Laboratory Diagnostics, Primary Health Care Center,
Trg Nikole Kovacevica 6, 81000 Podgorica, Montenegro
Phone and Fax: +382 20 481 999
e-mail: aleksandranklisic@gmail.com

Introduction

It is widely recognized that non-alcoholic fatty liver disease (NAFLD) represents the commonest manifestation of chronic liver diseases (1). Its prevalence is rising along with the growing proportion of obesity and diabetes mellitus type 2 (DM2) worldwide. Although it looks like a benign condition, without any symptoms, during a certain time NAFLD increases the risk of cirrhosis and hepatocellular carcinoma (1). Importantly, it is an early predictor of diabetic complications and cardiovascular disease (CVD) (2, 3).

Liver biopsy is established as the gold standard for diagnosis of hepatic steatosis (4). However, due to its invasive diagnostic nature it is not suitable procedure in routine everyday praxis. Therefore, it has been replaced with abdominal ultrasonography, as the commonest technique for NAFLD assessment in clinical trials. In line with this, Bedogni et al. (5) derived fatty liver index (FLI), an algorithm based on body mass index (BMI), waist circumference (WC), triglycerides (TG) and gamma-glutamyl transferase (GGT), as a simple and accurate predictor of NAFLD. An FLI score ≥60 has been shown to have good sensitivity and specificity for NAFLD when diagnosed by abdominal ultrasonography, thus making it suitable for assessment hepatic steatosis in general population (5, 6).

Alanine-aminotransferase (ALT) (7, 8), and uric acid (9, 10), are shown to be independently associated with NAFLD. On the other hand, some studies advised that the ALT cut-off level used in NAFLD diagnosis should be revised and lowered (11, 12), since a threshold of 40 IU/L is commonly used in clinical practice (12). However, even with lower cut-off levels, ALT was shown to be a poor marker of NAFLD in some studies (8, 12, 13).

Similarly, discrepant results were observed when examining uric acid in relation to NAFLD, especially focusing on sex-specific differences and obesity status (14–17).

To our knowledge, there are no data on the prevalence of fatty liver in general population in Montenegro. Considering the high prevalence of overweight/obesity (18) and DM2 (19) in Montenegro, the early diagnosis of NAFLD is of great importance. Due to discrepant results on the utility of both these biomarkers for NAFLD screening, we aimed to determine their cut-off levels that can be applied to Montenegrin population with fatty liver disease.

Materials and Methods

Study population

Out of the total number of 1000 participants (397 men and 603 women) who were screened, 771 of them met the inclusion criteria in the current cross-sectional study (249 men and 522 women). Participants were sequentially recruited in the Primary Health Care Centre in Podgorica, Montenegro, during their routine check-up in a period from October 2012 to May 2016. Subjects older than 18 years of age were included in the study. Participants were regarded to have DM2 if they exibited HbA1c ≥ 6.5% measured on two different occasions, or with at least two measurements of fasting glucose levels ≥ 7.0 mmol/L, or with a random plasma glucose level of ≥ 11.1 mmol/L, or a plasma glucose level ≥ 11.1 mmol/L 2 h after an oral glucose tolerance test. Also, participants were regarded to have DM2 if they self-reported DM2, as well as if they were treated with oral hypoglycemic agents or insulin, as described elsewhere (20).

Participants were excluded from the research if they had liver disease other than NAFLD, malignant diseases, renal dysfunction, cardiovascular diseases, ethanol consumption >20 g/day, type 1 diabetes mellitus, high sensitivity C-reactive protein levels (hsCRP) > 10 mg/L, younger than 18 years of age, pregnancy.

Written informed consent was provided by each participant and all procedures performed in the current research were in accordance with the standards of the Ethical Committee of Primary Health Care Centre in Podgorica, Montenegro and with the Declaration of Helsinki.

Anthropometric and blood pressure measurements

Basic anthropometric measurements, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured as described previously (21).

Biochemical analyses

The blood samples were taken in the morning, between 7 and 10 o'clock, after at least 8 hours of fasting. Serum levels of glucose, total cholesterol, high density lipoprotein cholesterol (HDL-c), low density lipoprotein cholesterol (LDL-c), TG, creatinine, uric acid, total bilirubin, aspartate aminotransferase (AST), ALT and GGT, were measured using standardized enzymatic procedures, spectrophotometrically (Roche Cobas 400, Mannheim, Germany). Serum hsCRP levels were determined using a nephelometric assay (Behring Nephelometer Analyzer, Marburg, Germany).

Assessment of non-alcoholic fatty liver disease

Non-alcoholic fatty liver disease is assessed by FLI, using the following algorithm (5):

$$FLI = (e^{0.953 \times \log_e (\text{triglycerides}) + 0.139 \times BMI + 0.718 \times \log_e (GGT) + 0.053 \times \text{waist circumference} - 15.745})/(1 + e^{0.953 \times \log_e (\text{triglycerides}) + 0.139 \times BMI + 0.718 \times \log_e (GGT) + 0.053 \times \text{waist circumference} - 15.745}) \times 100.$$

A FLI score ≥60 has been shown to have good sensitivity and specificity, thus making it a convenient proxy of NAFLD. The 2016 EASL-EASD-EASO NAFLD guidelines are based on the recommendations on the usage of the serum biomarkers as endorsed diagnostic tool with the FLI as one of the best validated steatosis scores for screening studies in large population samples (22).

Statistical analysis

The parameter distribution was tested by the Kolmogorov Smirnov test and the data were presented as median (interquartile range). Categorical data were presented as relative frequencies and compared by Chi-square test for contingency tables. Kruskal-Wallis test was employed for comparisons between three groups of patients based on FLI separately in men and women. Correlations between FLI and clinical parameters were tested by Spearman's correlation analysis. Multivariate logistic regression analysis was used to find models consisting of clinical parameters (predictors, independent variables) which significantly influence the variability in the FLI, as dependent variable. The group of patients with FLI <30 was coded as 0, while the group of patients with FLI ≥60 was coded as 1. Variables in the FLI equation (i.e. BMI, WC, TG and

GGT) were excluded from multivariate analyses. Results are presented by odds ratio (OR) with 95% confidence intervals (CI). The explained variation in FLI was given by Nagelkerke R^2 value for single predictors and models. The Hosmer and Lemeshow test was used to examine whether there was a linear relationship between the independent variables and the *log* odds of the dependent variable. The clinical accuracy of the examined parameters and models were assessed by using receiver operating characteristic (ROC) curve analysis. The same analysis was also used to determine the cut-off values of the ALT and uric acid associated with the increase in the prevalence of the fatty liver disease (i.e., FLI ≥60) in both genders. The area under the ROC curve (AUC) between 0.5 and 0.7 suggested the low accuracy of diagnostic test; between 0.7 and 0.8 satisfactory accuracy, between 0.8 and 0.9 good accuracy, while AUC higher than 0.9 suggested the excellent accuracy of diagnostic test (23). Statistical analyses were performed with IBM® SPSS® Statistics version 22 software (USA). In all the analyses, values were considered statistically significant at P < 0.05.

Results

Table I indicates the general characteristics of men according to FLI. As expected, all three FLI

Table I General clinical data of study men's population according to FLI.

	First FLI group (FLI<30)	Second FLI group (FLI ≥30, <60)	Third FLI group (FLI ≥60)	P
N	28	66	155	
Age, years	63.00 (53.00–71.00)	63.00 (52.50–72.00)	61.00 (53.00–67.00)	0.253
BMI, kg/m²	24.08 (23.15–25.25)	26.60 (264.98–27.70)[a]	30.67 28.71–33.00)[a,b]	<0.001
WC, cm	90.00 (85.00–85.00)	98.00 (96.00–101.00)[a]	110.00 (104.00–116.00)[a,b]	<0.001
SBP, mmHg	130.00 (126.00–136.00)	135.00 (127.00–139.00)[a]	134.00 (126.00–144.00)[a]	0.519
DBP, mmHg	80.00 (70.00–90.00)	81.00 (76.00–87.00)[a]	80.00 (74.00–88.00)[a]	0.730
Smokers, n (%)	5 (18%)	20 (30%)	41 (26%)	0.800
Antihyperglycemics, n (%)	11 (39%)	28 (42%)	89 (57%)	0.065
Insulin, n (%)	5 (18%)	10 (15%)	28 (18%)	0.643
Antihyperlipidemics, n (%)	11 (39%)	16 (24%)	65 (42%)	0.012
Antihypertensives, n (%)	13 (46%)	41 (62%)	111 (72%)	0.141
Type 2 diabetes mellitus, n (%)	14 (50%)	30 (45%)	100 (64%)	0.011

Data are presented as median (interquartile range) and compared by Kruskal–Wallis with post *hoc* test.
a – significantly different from the first FLI group, p<0.05
b – significantly different from the second FLI group, p<0.05
BMI – Body mass index; WC – Waist circumference; SBP – Systolic blood pressure; DBP – Diastolic blood pressure

Table II General clinical data of study women's population according to FLI.

	First FLI group (FLI <30)	Second FLI group (FLI ≥30, <60)	Third FLI group (FLI ≥60)	P
N	186	137	199	<0.001
Age, years	56.00 (49.00–61.00)	60.00 (55.00–67.00)[a]	62.00 (56.00–68.00)[a]	<0.001
BMI, kg/m^2	23.59 (21.50–25.07)	27.78 (26.13–29.40)[a]	32.24 (30.18–35.54)[a,b]	<0.001
WC, cm	82.00 (77.00–88.00)	95.00 (92.00–99.00)[a]	107.00 (100.00–112.00)[a,b]	<0.001
SBP, mmHg	129.00 (115.00–140.00)	136.00 (127.00–147.00)[a]	135.00 (126.00–146.00)[a]	<0.001
DBP, mmHg	78.00 (70.00–90.00)	85.00 (76.00–94.00)[a]	81.00 (76.00–90.00)[a]	0.001
Smokers, n (%)	27 (15%)	19 (14%)	25 (13%)	0.800
Antihyperglycemics, n (%)	9 (5%)	28 (20%)	87 (44%)	<0.001
Insulin, n (%)	2 (1%)	7 (5%)	14 (7%)	0.016
Antihyperlipidemics, n (%)	22 (12%)	27 (20%)	66 (33%)	<0.001
Antihypertensives, n (%)	38 (20%)	56 (41%)	134 (67%)	<0.001
Type 2 diabetes mellitus, n (%)	10 (5%)	30 (22%)	101 (51%)	<0.001

Data are presented as median (interquartile range) and compared by Kruskal-Wallis with post *hoc* test.
a – significantly different from the first FLI group, p<0.05; b – significantly different from the second FLI group, p<0.05
BMI – Body mass index; WC – Waist circumference; SBP – Systolic blood pressure; DBP – Diastolic blood pressure

Table III Biochemical analysis of study men's population according to FLI.

	First FLI group (FLI<30)	Second FLI group (FLI ≥30, <60)	Third FLI group (FLI 60)	P
Glucose, mmol/L	5.90 (5.50–6.30)	6.10 (5.40–7.40)	6.70 (5.60–8.67)[a,b]	0.008
Total cholesterol, mmol/L	4.44 (4.12–5.29)	5.09 (4.60–5.78)[a]	5.22 (4.57–6.02)[a]	0.020
HDL–c, mmol/L	1.52 (1.16–1.71)	1.27 (1.03–1.61)	1.01 (0.88–1.26)[a,b]	<0.001
LDL–c, mmol/L	2.82 (1.82–3.39)	2.96 (2.68–3.88)	3.20 (2.49–3.83)	0.055
TG, mmol/L	1.00 (0.84–1.26)	1.48 (1.03–1.80)[a]	2.08 (1.66–2.73)[a,b]	<0.001
AST, IU/L	18.00 (16.00–21.00)	21.00 (18.00–24.00)[a]	20.00 (18.00–25.00)[a,b]	0.219
ALT, IU/L	16.50 (14.00–20.00)	22.00 (18.00–28.00)[a]	24.00 (17.00–30.00)[a,b]	<0.001
GGT, IU/L	13.00 (11.00–17.00)	18.00 (14.00–25.00)[a]	27.00 (20.00–37.00)[a,b]	<0.001
Uric acid, μmol/L	313.00 (250.00–366.00)	300.00 (262.00–364.00)	333.00 (283.00–382.00)	0.084
Total bilirubin, μmol/L	9.25 (5.50–12.70)	8.70 (6.40–11.84)	8.60 (5.95–12.07)	0.978
HsCRP, mg/L	0.73 (0.38–1.58)	0.83 (0.48–1.66)	1.53 (0.84–3.27)[a,b]	<0.001
Creatinine, μmol/L	86.00 (80.00–94.00)	83.00 (75.00–96.00)	84.00 (74.00–97.00)	0.823
FLI	22.00 (15.00–26.00)	48.00 (40.00–54.00)[a]	85.00 (73.00–92.00)[a,b]	<0.001

Data are presented as median (interquartile range) and compared by Kruskal–Wallis with post hoc test.
a – significantly different from the first FLI group, p<0.05; b – significantly different from the second FLI group, p<0.05
HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG – Triglycerides; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; GGT – Gamma-glutamyl transferase; HsCRP – High-sensitivity C-reactive protein; FLI – Fatty liver index

Table IV Biochemical analysis of study women's population according to FLI.

	First FLI group (FLI<30)	Second FLI group (FLI ≥30, <60)	Third FLI group (FLI ≥60)	P
Glucose, mmol/L	5.30 (5.00–5.70)	5.60 (5.10–6.20)[a]	6.50 (5.60–7.70)[a,b]	<0.001
Total cholesterol, mmol/L	5.80 (4.96–6.66)	6.02 (5.25–6.71)	6.13 (5.20–7.06)[a]	0.022
HDL–c, mmol/L	1.76 (1.48–2.02)	1.56 (1.28–1.75)[a]	1.27 (1.10–1.45)[a, b]	<0.001
LDL–c, mmol/L	3.51 (2.83–4.20)	3.74 (2.96–4.41)	3.86 (2.96–4.72) [a]	0.039
TG, mmol/L	1.11 (0.85–1.41)	1.43 (1.03–2.01)[a]	2.27 (1.73–2.91)[a,b]	<0.001
AST, IU/L	18.00 (15.00–21.00)	18.00 (16.00–21.00)	19.00 (17.00–24.00)[a,b]	<0.001
ALT, IU/L	15.00 (12.00–20.00)	19.00 (14.00–23.00)[a]	22.00 (17.00–30.00)[a,b]	<0.001
GGT, IU/L	10.00 (9.00–13.00)	14.00 (11.00–17.00)[a]	19.00 (14.00–25.00)[a,b]	<0.001
Uric acid, µmol/L	220.00 (191.00–261.00)	256.00 (225.00–298.00)[a]	316.00 (254.00–355.00)[a,b]	<0.001
Total bilirubin, µmol/L	7.50 (5.70–9.80)	7.10 (5.80–9.80)[a]	6.60 (5.10–8.60)[a]	0.011
HsCRP, mg/L	0.52 (0.30–1.15)	1.36 (0.89–2.40)[a]	2.16 (1.16–4.24)[a,b]	<0.001
Creatinine, µmol/L	58.00 (53.00–64.00)	63.00 (57.00–71.00)[a]	65.00 (58.00–73.00)[a,b]	<0.001
FLI	12.00 (7.00–21.00)	42.00 (37.00–49.00)[a]	83.00 (69.00–91.00)[a,b]	<0.001

Data are presented as median (interquartile range) and compared by Kruskal-Wallis with post *hoc* test.
a – significantly different from the first FLI group, p<0.05; b – significantly different from the second FLI group, p<0.05
HDL-c-High density lipoprotein cholesterol; LDL-c-Low density lipoprotein cholesterol; TG – Triglycerides; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; GGT – Gamma-glutamyl transferase; HsCRP – High-sensitivity C-reactive protein; FLI – Fatty liver index

Table V Spearman's correlation coefficients of FLI and other clinical parameters in men and women.

	Men		Women	
Variable	ρ	P	ρ	P
Age, years	0.165	0.009	0.315	<0.001
BMI, kg/m^2	0.839	<0.001	0.894	<0.001
WC, cm	0.815	<0.001	0.904	<0.001
SBP, mmHg	0.097	0.131	0.243	<0.001
DBP, mmHg	0.030	0.643	0.148	0.001
Glucose, mmol/L	0.194	0.002	0.547	<0.001
Total cholesterol, mmol/L	0.112	0.080	0.101	0.022
HDL-c, mmol/L	-0.397	<0.001	-0.562	<0.001
LDL-c, mmol/L	0.071	0.267	0.089	0.042
TG, mmol/L	0.593	<0.001	0.654	<0.001
AST, IU/L	0.120	0.060	0.165	<0.001
ALT, IU/L	0.322	<0.001	0.395	<0.001
GGT, IU/L	0.564	<0.001	0.580	<0.001
Uric acid, µmol/L	0.186	0.003	0.529	<0.001
Total bilirubin, µmol/L	-0.041	0.522	-0.146	0.001
HsCRP, mg/L	0.347	<0.001	0.571	<0.001
Creatinine, µmol/L	-0.027	0.676	0.291	<0.001

Data are presented as correlation coefficient Rho (ρ)
BMI – Body mass index; WC – Waist circumference; SBP – Systolic blood pressure; DBP – Diastolic blood pressure; HDL-c – High density lipoprotein cholesterol; LDL-c – Low density lipoprotein cholesterol; TG –Triglycerides; AST – Aspartate aminotransferase; ALT – Alanine aminotransferase; GGT – Gamma-glutamyl transferase; HsCRP – High-sensitivity C-reactive protein

Table VI Odds ratios (OR) after univariate and multivariate logistic regression analysis for ALT and UA predicting abilities towards fatty liver disease in men and women.

Predictors Men	Unadjusted OR (95%CI)	P	Nagelkerke R^2
ALT, IU/L	1.178 (1.079–1.287)	<0.001	0.247
Uric acid, mmol/L	1.004 (0.998–1.010)	0.170	0.021
Model 1	Adjusted OR (95%CI)	P	Nagelkerke R^2
ALT, IU/L	1.313 (1.135–1.518)	<0.001	0.554
Uric acid, mmol/L	1.008 (0.999–1.017)	0.089	
Predictors Women	Unadjusted OR (95%CI)	P	Nagelkerke R^2
ALT, IU/L	1.126 (1.090–1.162)	0.001	0.253
Uric acid, mmol/L	1.020 (1.015–1.029)	<0.001	0.383
Model 2	Adjusted OR (95%CI)	P	Nagelkerke R^2
ALT, IU/L	1.167 (1.080–1.261)	<0.001	0.797
Model 3			
Uric acid, mmol/L	1.014 (1.007–1.021)	<0.001	0.791
Model 4			
ALT, IU/L	1.170 (1.078–1.270)	<0.001	0.818
Uric acid, mmol/L	1.014 (1.006–1.022)	<0.001	

Model 1: Age, Glucose, HDL-c, uric acid, hsCRP, ALT (all continuous variables) and antihyperlipemics and type 2 diabetes mellitus (all categorical variables)

Model 2: Age, Glucose, total cholesterol, HDL-c, LDL-c, ALT, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)

Model 3: Age, Glucose, total cholesterol, HDL-c, LDL-c, uric acid, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)

Model 4: Age, Glucose, total cholesterol, HDL-c, LDL-c, ALT, uric acid, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)

groups had significantly different BMI and WC because those variables were used for FLI calculation. The second and the third FLI group had significantly higher SBP and DBP than the first FLI group. Also, the third FLI group had significantly higher percentage of subjects on antihyperlipidemic therapy. The same percentages of men with DM2 were included in the first and in the third group, and both being higher than those in the second FLI group.

It was evident in *Table II* that significantly higher percentage of women on antihyperglycemic, insulin, antihyperlipidemic and antihypertensive therapies and women with DM2 were in the third FLI group (i.e., group with fatty liver disease). Women in the second and in the third FLI group were older and had significantly higher DBP than women in the first group. Furthermore, BMI, WC and SBP were significantly different between all three groups, being higher in the second and the third group than in the first one.

Beside TG concentration and GGT, ALT activity was different between all men's FLI groups, being the highest in men with fatty liver disease (i.e., FLI ≥60).

Also, HDL-c was the lowest in men with fatty liver disease (i.e., FLI ≥60). Vice versa was established for hsCRP levels, which were the highest in men with FLI ≥60. Total cholesterol concentration was the lowest in men without fatty liver disease (i.e., FLI<30), (Table III).

As in men, TG concentration and GGT activity, were significantly different between all three FLI groups, being the highest in women with fatty liver. The same was found for glucose, HDL-c, uric acid, hsCRP, creatinine concentrations and ALT activity. Total cholesterol and LDL-c were higher in women with fatty liver disease than in those who did not have it. Total bilirubin concentration was the highest in women without fatty liver (i.e., FLI <30) and AST activity was the highest in women with fatty liver (i.e., FLI ≥60), (Table IV).

Bivariate associations were revealed by Spearman's correlation analysis. In men, FLI was highly associated with all the parameters used for its calculation (BMI, WC, TG and GGT). Also, significant positive associations were established between FLI and age, glucose, ALT, uric acid, hsCRP and signifi-

Table VII ROC analysis for single parameter discriminatory abilities towards fatty liver disease development in men and women.

Predictors	AUC(95% CI)	SE	Sensitivity (%)	Specificity (%)	P
Men					
ALT, IU/L	0.804 (0.719–0.890)	0.044	61	95	<0.001
Uric acid, µmol/L	0.576 (0.500–0.650)	0.067	74	46	>0.05
Model 1	0.932 (0.891–0.974)	0.021	74	100	0.001
Women					
ALT, IU/L	0.746 (0.698–0.794)	0.025	63	72	<0.001
Uric acid, µmol/L	0.820 (0.779–0.858)	0.021	65	84	<0.001
Model 2	0.965 (0.948–0.982)	0.009	90	91	<0.001
Model 3	0.965 (0.948–0.982)	0.009	88	92	<0.001
Model 4	0.971 (0.956–0.986)	0.008	92	90	<0.001

Model 1: Age, Glucose, HDL-c, uric acid, hsCRP, ALT (all continuous variables) and antihyperlipidemics and type 2 diabetes mellitus (all categorical variables)
Model 2: Age, Glucose, total cholesterol, HDL-c, LDL-c, ALT, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)
Model 3: Age, Glucose, total cholesterol, HDL-c, LDL-c, uric acid, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)
Model 4: Age, Glucose, total cholesterol, HDL-c, LDL-c, ALT, uric acid, creatinine, hsCRP, SBP, DBP, AST, total bilirubin (all continuous variables) and gender, antihyperglycemics, insulin, antihyperlipidemics, antihypertensives, type 2 diabetes mellitus (all categorical variables)

cant negative association between FLI and HDL-c. In women, FLI correlated positively with all the examined parameters, except with HDL-c and total bilirubin. FLI correlated highly negatively with HDL-c and total bilirubin. Correlation coefficients (ρ) and P values were presented in *Table V*.

Further statistical analysis included binary logistic regression in order to determine whether measurement of ALT and uric acid which significantly correlated with FLI could have a potential predictive role on fatty liver occurrence. The first FLI group (FLI <30) was selected as a reference group and coded as 0 while the third FLI group (FLI ≥60) was selected as group of patients with fatty liver disease and coded as 1. Coding was performed separately in men and women. Unadjusted analysis indicated that in men ALT was a predictor and in women ALT and uric acid were predictors for fatty liver disease. As ALT activity rose for 1 IU/L, probability for fatty liver disease increased by 17.8% in men (*Table VI*). The un-adjusted Nagelkerke R^2 for FLI of 0.247 means that 24.7% of variation in FLI was caused by ALT in men. As ALT activity rose for 1 IU/L and uric acid concentration rose for 1 µmol/L, probability for fatty liver disease in women increased by 12.6% and 2%, respectively (*Table VI*). In women, the unadjusted Nagelkerke R^2 for FLI of 0.253 and 0.383 means that 25.3% and 38.3% of variations in FLI were caused by ALT and uric acid, respectively.

Thereafter, we constructed logistic regression models to further test the potential independent associations of the ALT and uric acid with fatty liver disease in men and women. The best models for each

of the investigated clinical parameter are presented in *Table VI*. In men the Model 1 incorporated adjustments for all the clinical parameters significantly correlated with FLI as continuous variables, and therapy usage and DM2 presence differently distributed among FLI groups as categorical variables. In women models 2, 3 and 4 incorporated adjustments for all the clinical parameters significantly correlated with FLI as continuous variables and therapy usages and DM2 presence differently distributed among FLI groups as categorical variables. ALT kept its independent predictive power on fatty liver disease in men [OR (95% CI) = 1.313 (1.135–1.518), P < 0.001]. The level of influence of the Model 1 on variation of FLI was 55.4% (*Table VI*). Also, ALT kept its independent prediction in Model 2 and Model 4 on fatty liver disease in women: [OR (95% CI) = 1.167 (1.080–1.261), P <0.001] and [OR (95% CI) = 1.170 (1.078–1.270), P <0.001], respectively. Uric acid was independent predictor of fatty liver disease only in women: the Model 3 [OR (95% CI) = 1.014 (1.007–1.021), P <0.001] and the Model 4 [OR (95% CI) = 1.014 (1.006–1.022), P < 0.001]. The levels of influence of the Models 2, 3 and 4 on variation of FLI were 79.9%, 79.1% and 81.8%, respectively.

Furthermore, we performed the ROC analysis in order to test the clinical accuracy of ALT in men and ALT and uric acid in women towards the presence of fatty liver disease (*Table VII*). The calculated AUC for ALT as a single parameter indicated its good clinical accuracy (23): AUC (95% CI) = 0.804 (0.719–0.890), P <0.001 in development of fatty liver
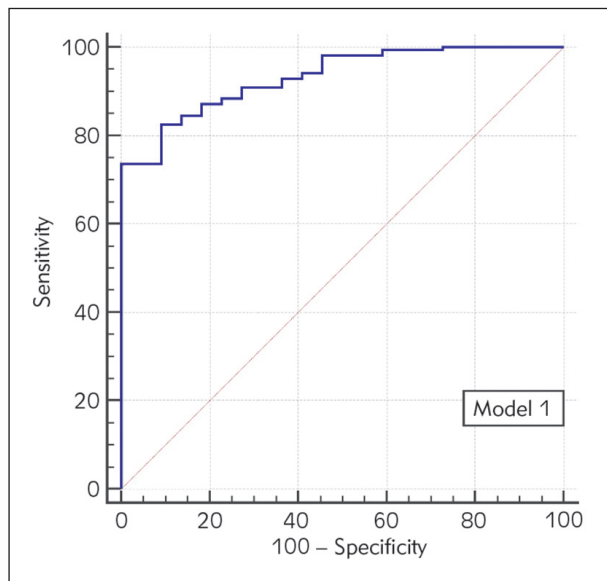
Figure 1 ROC curves for models' discriminatory abilities towards fatty liver disease in men.
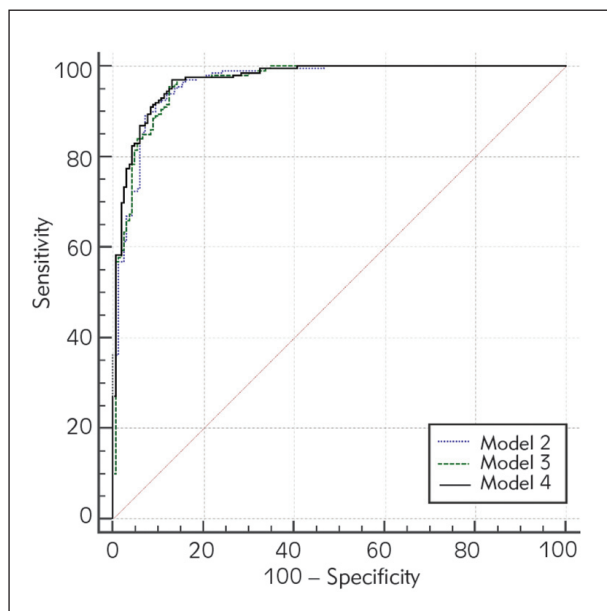

Figure 2 ROC curves for models' discriminatory abilities towards fatty liver disease in women.

disease in men. The ROC-analysis-estimated clinical accuracy of uric acid in men as a single parameter did not show any significant discriminatory ability towards fatty liver disease: AUC (95% CI) = 0.576 (0.500-0.650), P>0.05. The calculated AUC for ALT and uric acid in women as single parameters revealed that only uric acid had a good clinical accuracy for fatty liver development: AUC (95% CI) = 0.820 (0.779-0.858), P<0.001 (*Table VII*).

Additionally, we constructed four models using predictive probabilities generated by logistic regression analysis. *Figure 1* and *Table VII* showed

ROC curve for the Model 1 in men. The AUC of this model was 0.932 indicated excellent discriminatory capability towards fatty liver development. The same was found for models 2, 3 and 4 generated in women (*Figure 2* and *Table VII*). All of them indicated excellent discriminatory capability towards fatty liver development. The pair-wise comparisons of ROC curves between Models 2 and 4 (AUC difference =0.006, P=0.073) and Models 3 and 4 (AUC=0.006, P=0.061) showed that improvement with ALT and uric acid together in the same model was not statistically significant in discriminatory capability towards fatty liver disease (data not presented). Also, a pair-wise comparison of Model 2 and Model 3 ROC curves (AUC difference=0.000, P=0.956) was not significant in discriminating patients with and without fatty liver disease. ALT in the Model 2 and uric acid in the Model 3, both with the same other clinical parameters could be considered to have the same clinical accuracy for fatty liver disease development.

Finally, ROC analysis was used to estimate cut-off values for ALT in men and ALT and uric acid in women in our study population associated with increase in the prevalence of the fatty liver disease. The cut-off value for ALT was 19 IU/L (AUC=0.746, sensitivity 63%, specificity 72%, P<0.001) in women and 22 IU/L (AUC = 0.804, sensitivity 61%, specificity 95%, P<0.001) in men. The cut-off value for uric acid was 274 μmol/L (AUC=0.821, sensitivity 68%, specificity 82%, P<0.001) in women.

Discussion

The findings of the current study reveal that ALT was independent predictor of the fatty liver disease as determined by FLI in both men and women, whereas serum uric acid was its independent predictor only in women (*Table VI*). Moreover, unlike commonly used threshold of 40 IU/L in clinical practice (12), we have shown that lower cut-off levels of ALT activity in our study population are associated with the increased prevalence of the fatty liver disease. Namely, the cut-off values in our study are as follows: ALT was 19 IU/L (AUC=0.746, sensitivity 63%, specificity 72%, P<0.001) in women and 22 IU/L (AUC=0.804, sensitivity 61%, specificity 95%, P<0.001) in men. Our results are in line with some previous reports that suggest the ALT cut-off level used in NAFLD diagnosis should be downward in order to improve the sensitivity of the method and to better identify individuals being at risk of NAFLD, as well as to prevent its progression (11, 12). Namely, Miyake et al. (11) have reported ALT activity to be 17 IU/L for females and 25 IU/L for males, as the cut-off levels used for NAFLD diagnosis. Epidemiological studies have also recommended lower cut-off levels (i.e., 19 IU/L for females and 30 IU/L for males) to be accepted as a normal upper limit level (12).

However, despite suggested lower ALT cut-off levels, there are studies that reject that ALT is a reliable marker of NAFLD (8, 12, 13). In line with this, van den Berg et al. (8) have shown that even 80.3% of individuals that were classified into the group with NAFLD had normal ALT, when using the upper limit of normal levels of this enzyme. Similarly, another study has confirmed that normal ALT activities were observed in 79% of subjects with hepatic steatosis (24). Although ALT exists in two isoforms in humans (25, 26), the ALT2 isoform makes the main contribution to homeostasis of free fatty acids (FFA), being highly expressed in adipose tissue of obese individuals (25). In addition, increased lipid peroxidation can cause the ALT to leak more easily out of the hepatocytes. Moreover, it is assumed that increased ALT activity in circulation may also be a result of the compensatory response to the impaired hepatic insulin signalling (25).

We failed to confirm the independent relationship between uric acid and FLI in males, but confirmed only in females. In addition, the cut-off value for uric acid was 274 μmol/L (AUC=0.821, sensitivity 68%, specificity 82%, P<0.001) in women. However, it is questionable whether hyperuricemia raises the risk for fatty liver occurrence in females more than in males since controversy exists in literature concerning the relationship between serum uric acid and NAFLD (14–17). A study conducted by Wu et al. (15) in a large population group showed that the relationship between uric acid and NAFLD was significantly stronger in females than in males. Similar observations were reported by Yang et al. (16) in a four-year retrospective cohort study in Chinese population. The independent effect of hyperuricemia (i.e., defined as serum uric acid level level of >360 μmol/L in females) on NAFLD was stronger in females than in males, but it was found only in non-obese subjects (16). To make this question more complicated, Fan et al. (14) reported that an increase in serum uric acid was independently associated with the higher risk of NAFLD only in males, but not in females in a study that encompassed exclusively individuals with DM2.

On the contrary, recent large meta-analysis (17) has reported that increased risk for NAFLD occurrence is significantly associated with hyperuricemia in both males and females. Moreover, uric acid was found to directly inhibit insulin signalling and induce insulin resistance, which is considered to be the underlying mechanism of hepatic steatosis (14). Importantly, Lanaspa et al. (27) reported that serum uric acid could directly stimulate hepatic fat synthesis.

However, due to gender-difference observed in relation with serum uric acid and hepatic steatosis in many studies, it may be assumed that uric acid acts on fatty liver development through different mechanisms in males and females. Future studies are needed to elucidate this assumption.

The main disadvantage of the current study is its cross-sectional design, which does not allow making a cause-effect inference. Moreover, we have not evaluated hepatic steatosis directly by ultrasound, computed tomography, or liver biopsy, but with FLI. Nevertheless, we followed the 2016 EASL-EASD-EASO NAFLD guidelines recommendations on the usage of the serum biomarkers as a preferred diagnostic tool with the FLI as one of the best validated steatosis scores for screening studies in large population samples (22). The strength of the current study, and advantage over our previous studies (20, 21) is the fact that we included a large sample size of population of Montenegro. Therefore, we assume that lower cut-off levels of ALT in both males and females, as well as serum uric acid levels in females only, can be reliable predictors of FLI, which can facilitate identifying individuals having a great risk of fatty liver disease development. Additional studies are needed to confirm our results, as well as to make a comparison between these data (i.e., fatty liver assessed with FLI algorithm) and fatty liver as assessed with ultrasound in relation to ALT and uric acid levels.

Conflict of interest statement

The authors stated that they have no conflicts of interest regarding the publication of this article.

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