

PREVALENCE AND PREDICTORS OF NON-ALCOHOLIC FATTY LIVER DISEASE AS DEFINED BY THE FATTY LIVER INDEX IN A TYPE 2 DIABETES POPULATION

CRISTINA ALINA SILAGHI^{1,2}, HORATIU SILAGHI³,
HORATIU ALEXANDRU COLOSI⁴, ANCA ELENA CRACIUN^{5,6},
ANCA FARCAS⁷, DANIEL TUDOR COSMA⁸, NICOLAE HANCU⁶,
RALUCA PAIS⁹, CARMEN EMANUELA GEORGESCU²

¹County Emergency Hospital, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

²Endocrinology Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

³5th Surgery Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁴Medical Informatics and Biostatistics Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁵Diabetes, Nutrition and Metabolic Diseases Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁶Diabetes, Nutrition and Metabolic Diseases Department, Regina Maria Clinic, Cluj-Napoca, Romania

⁷1st Internal Medicine Department, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁸Diabetes, Nutrition and Metabolic Diseases Department, County Clinic Emergency Hospital, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania

⁹Service Hépatogastroentérologie, Hôpital Pitié - Salpêtrière, Université Pierre et Marie Curie, INSERM UMRS 938, Paris, France

Abstract

Background and aims. We aimed to study the prevalence and the predictive factors of non-alcoholic fatty liver disease (NAFLD) defined by the fatty liver index (FLI) in type 2 diabetic patients (T2DM).

Methods. Three hundred and eighty-one T2DM outpatients who regularly attended a Consulting Clinic in Cluj were retrospectively included. FLI, a surrogate steatosis biomarker based on body mass index (BMI), waist circumference (WC), triglycerides (TGL) and gammaglutamyl-transferase (GGT) was used to assess NAFLD in all patients. Anthropometric and biochemical parameters were measured. Hepatic steatosis (HS) was evaluated by ultrasonography.

Results. NAFLD-FLI (defined as FLI>60) was correlated with HS evaluated by ultrasound ($r=0.28$; $p<0.001$). NAFLD-FLI was detected in 79% of T2DM. The prevalence of obesity in NAFLD-FLI patients was 80%. Of the patients with normal alanine aminotransferase (ALAT), 73.8 % had NAFLD. At univariate analysis, NAFLD-FLI was correlated with age ($r= -0.14$; $p=0.007$), sex ($r=0.20$; $p<0.001$), LDL cholesterol ($r=0.12$; $p=0.032$), HDL cholesterol ($r= -0.13$; $p=0.015$), ALAT ($r=0.20$; $p<0.001$) and ASAT ($r=0.19$; $p<0.001$). At multiple regression analysis, sex, ALAT and LDL-cholesterol were independent predictors of NAFLD-FLI. After logistic regression model, ALAT, LDL-cholesterol, HOMA-IR were good independent predictors of NAFLD-FLI.

Conclusions. *NAFLD-FLI could be useful to identify NAFLD in T2DM patients. Subjects with T2DM had a high prevalence of NAFLD-FLI even with normal ALAT levels. Our findings showed that sex, ALAT, LDL cholesterol and IR were significant and independent factors associated with the presence of NAFLD in T2DM subjects.*

Keywords: nonalcoholic fatty liver disease, fatty liver index, risk factors, type 2 diabetes

Background and aims

Non alcoholic fatty liver disease (NAFLD) is the most frequent chronic liver disease in Western countries affecting approximately 15-30% of the general population [1,2]. NAFLD prevalence is growing up to 75-90% when NAFLD is associated with type 2 diabetes mellitus (T2DM) and obesity [3]. The disease spectrum includes nonalcoholic fatty liver, nonalcoholic steatohepatitis (NASH), liver cirrhosis, and hepatocellular carcinoma. NAFLD has become a major and emerging cause of liver disease in worldwide, responsible for increased overall and liver-related mortality and significant increase in the health care costs [4,5]. It will represent the major underlying etiology for liver transplantation in Western countries by 2020. NAFLD is frequently associated with visceral obesity, dyslipidemia, insulin resistance (IR), and T2DM and may represent another component of the metabolic syndrome (MetS) [6]. Presence of MetS and obesity were independent factors associated to NASH. Leptin levels and body mass index (BMI) were higher in patients with advanced hepatic fibrosis [7].

The gold standard technique for identifying NAFLD is liver biopsy but is not feasible to perform an invasive and costly procedure in such a large number of patients. It is important to identify patients with NAFLD using simple methods, in order to be referred for ultrasonography or liver biopsy and to identify those at highest risk of NASH or advanced liver disease. Clinical risk factors, such as the presence of the MetS and its features, as well as emerging biomarkers can help select NAFLD patients. The “fatty Liver index” (FLI) is a surrogate steatosis biomarker developed in a cohort of patients from the general population with ultrasound-diagnosed hepatic steatosis (HS) [8].

FLI is based on aggregate scores from different anthropometric and metabolic parameters: BMI, waist circumference (WC), triglycerides (TGL) and gammaglutamyl-transferase (GGT). FLI represents an accurate and easy to obtain algorithm for NAFLD, useful in retrospective series of patients [9]. It is considered a screening tool to identify NAFLD in patients with cardiometabolic

risk factors where ultrasound is unavailable. FLI is able to discriminate between the absence and the presence of steatosis showing good diagnostic performance in detecting NAFLD in several population studies [9,10,11,12]. The FLI has been associated with reduced insulin sensitivity, risk of type 2 diabetes, accelerated atherosclerosis and cardiovascular risk [13]. It is associated with all-cause and cardiovascular mortality in patients at high risk of coronary artery disease [14]. The adequacy of FLI as a quantitative biomarker of steatosis remains unknown [9].

NAFLD risk factors in non-diabetic patients was largely analyzed. The prevalence of NAFLD and its risk factors in particular high-risk groups of steatosis patients (like T2DM) was poorly evaluated in Romania. The objective of the present study was to establish the prevalence of NAFLD-FLI in our population and to analyze the association between NAFLD-FLI and metabolic risk factors in patients with T2DM. Specifically, we aimed at studying predictors of steatosis in T2DM patients and to analyze the association with anthropometric measurements and biochemical parameters.

Methods

1. Patients and methods

We retrospectively enrolled 381 type 2 diabetic outpatients who regularly attended a Consulting Clinic in Cluj, Romania between 2014-2015. We previously excluded patients who had other causes of chronic liver disease (autoimmune hepatitis, hemochromatosis, Wilson disease, alpha 1 antitrypsin deficiency), viral B and C hepatitis or HIV infection, alcohol consumption greater than 40 g/day. The study protocol was in accordance with the ethical guidelines of the Declaration of Helsinki and approved by the local Ethics Committee.

2. Clinical and laboratory assessment

All 381 patients underwent a complete clinical and anthropometric evaluation, including: age, sex, weight, height, waist and hip circumference, systolic and diastolic blood pressure. Information on medical history, alcohol consumption, smoking and use of medications was obtained from all patients by interviews during medical examinations.

BMI was calculated by dividing weight in kilograms by the square of height in meters. Overweight/obesity was diagnosed when BMI was ≥ 25.0 kg/m², according to the 1999 WHO criteria [15]. Blood pressure was measured at the right upper arm after patient had been seated quietly for at least 5 minutes. Subjects were considered to have hypertension if their blood pressure was $\geq 140/85$ mmHg according to the European Society of Cardiology (ESC) and European Society of Hypertension (ESH) or if they were taking any anti-hypertensive drugs. Venous blood was drawn in the morning after an overnight fast. We determined by standard laboratory procedures liver function tests, total cholesterol, HDL cholesterol, TGL, apo A and apo B, high-sensitivity C-reactive protein, blood creatinine level, fasting blood glucose, and HbA1c. Plasmatic insulin levels were available in only 208 subjects. Insulin resistance was determined by the homeostasis model assessment (HOMA-IR) method using the following formula: $\text{HOMA-IR} = [\text{fasting glucose (mmol/l)} \times \text{fasting insulin } (\mu\text{UI/ml})] / 22.5$. IR was defined as HOMA-IR ≥ 2.7 according to previous publications for European countries [16]. Viral serology for hepatitis C and B, antimitochondrial, antinuclear, and anti-smooth muscle antibodies were assessed in order to exclude other causes of chronic liver disease.

NAFLD was defined by a non-invasive marker for liver steatosis, fatty liver index (FLI), an algorithm based on BMI, WC, TGL and GGT (8). $\text{FLI} \geq 60$ indicates the presence of NAFLD. A stratification of NAFLD was made in tertiles I ($\text{FLI}=60-75$); II ($\text{FLI}=75-90$); III ($\text{FLI} > 90$).

3. Hepatic ultrasonography (US)

Hepatic US was performed in all patients after 12 hours fasting, by a single experienced radiologist by a high-resolution B-mode ultrasonography with a 5 MHz transducer (Philips HD11 XE ultrasound system). Each subject was examined in the supine and left lateral positions with the right arm raised above the head, during quiet inspiration. Hepatic steatosis (HS) was diagnosed using well-established criteria, including the hepatorenal echo contrast, liver parenchymal brightness, deep beam attenuation, and vascular blurring.

4. Statistical analysis

Descriptive results have been expressed as means \pm SD. Comparisons between groups according to the presence of NAFLD-FLI, were performed using Student's t-test for normally distributed continuous variables and the Mann-Whitney *U* test for asymmetric continuous variables. Frequency distributions of categorical data have been compared using χ^2 tests and Fisher's exact tests. Correlations between quantitative variables have been investigated using Spearman's correlation coefficient rho. To identify independent variables associated with NAFLD-FLI, we used multiple linear regression analysis. Then, NAFLD-FLI has been dichotomized based on a 60 cut-off

value [8], and multiple logistic regression analyses have been performed using iterative selection of covariates for optimal prediction of NAFLD-FLI being higher than 60. Probability levels lower than 0.05 were considered statistically significant. All analyses have been performed using IBM SPSS 21 MacOS statistical software (Chicago IL).

Results

Three hundred and eighty-one T2DM patients were enrolled in this study (132 females and 249 males), with a mean age of 56.3 years (range, 26-78 years). According to the results obtained by FLI, the 381 patients were divided into 2 groups: with NAFLD-FLI (79%) and without NAFLD-FLI (21%). The prevalence of NAFLD was 79%. Clinical and biochemical characteristics of patients grouped according to NAFLD-FLI status are presented in Table I. We excluded from Table I the parameters used in the FLI algorithm (BMI, WC, TGL and GGT).

NAFLD-FLI was correlated with hepatic steatosis evaluated by ultrasound ($r=0.28$; $p<0.001$) suggesting that FLI had been a good method for evaluating NAFLD in our T2DM population.

The percentage of oral hypoglycemic users was 83.9% and patients treated with insulin was 26.9%. Coronary artery disease (CAD) was present in 26.9%, peripheral artery disease in 19.2% and metabolic syndrome in 85.3.2% of the studied sample. In this population, 38% subjects were treated with statins, and 23.1% were treated with fibrate therapy. NAFLD-FLI was correlated with age ($r=-0.14$; $p=0.007$), sex ($r=0.20$; $p<0.001$), LDL cholesterol ($r=0.12$; $p=0.032$), HDL cholesterol ($r=-0.13$; $p=0.015$), ALAT ($r=0.20$; $p<0.001$) and ASAT ($r=0.19$; $p<0.001$). ALAT was normal (< 35 U in women and < 50 U in men) in 279 patients of 381. Interestingly, we found that 73.8% of patients with normal ALAT had NAFLD (defined as $\text{FLI} > 60$), 60.9% (170 patients) had $\text{FLI} > 75$ and 33% (93 patients) had $\text{FLI} > 90$. In patients with NAFLD-FLI the prevalence of obesity was 80% (307 patients of 381). Table II presents three models of multiple regression analysis, with independent variables that contextually predicted the dependent variable NAFLD-FLI. Sex, ALAT and LDL-cholesterol were independent predictors of NAFLD-FLI. We excluded from our analysis predictors that could have interfered with the parameters used in the FLI score algorithm.

For a subset of 208 patients, the HOMA-IR score was calculated. After iterative selection of covariates for model optimization, our best logistic regression model included ALAT, HOMA and CAD as independent predictors of NAFLD-FLI (Table III). During the model optimization process, other investigated predictors and possible confounders did not significantly contribute to the prediction of a NAFLD-FLI being higher than 60.

Table I. Clinical and biochemical characteristics of diabetic patients grouped according to NAFLD as assessed by fatty liver index (FLI). Since most variables departed significantly from normal distribution, comparisons between groups based on the presence of NAFLD by FLI, were performed using Mann-Whitney U tests for quantitative variables and Fisher's exact tests for categorical data. Data are presented as means \pm SD or number. $p < 0.05$ were considered significant.

Variable	With NAFLD-FLI mean \pm SD (median)	Without NAFLD-FLI mean \pm SD (median)	P
Age (years)	55.7 \pm 8.9	58.7 \pm 8.5	0.006
Diabetes duration (years)	5.0 (0.38)	5.0 (0.29)	0.22
SBP (mmHg)	140 (100, 230)	150 (100, 200)	0.75
DBP (mmHg)	86 (50, 155)	86 (65, 107)	0.3
TC (mmol/l)	5.09 (2.8, 16.47)	4.68 (2.43, 8.30)	0.006
HDLc (mmol/l)	1.06 \pm 0.43	1.16 \pm 0.46	0.008
LDLc (mmol/l)	3.25 (0.57, 10.30)	3.02 (0.86, 5.07)	0.82
FPG (mmol/l)	157 (78, 476)	162.5 (70, 682)	0.4
HOMA-IR	5.38 (0.94, 135)	2.73 (0.83, 28.92)	0.0001
HbA1C	7.9 (5.1, 15.6)	7.9 (5, 14.4)	0.99
ASAT (units/l)	24.5 (7, 191)	20 (7, 70)	0.0001
ALAT (units/l)	33 (8, 209)	24.6 (11.4, 135)	0.0001
Acid uric (mg/dl)	5.7 \pm 1.65	4.76 \pm 1.37	0.0001

Table II. Multiple regression analysis. Model 1-3: sex, ALAT and LDL-cholesterol are independently correlated with NAFLD.

Model	Independent variable	Non-standardized Coefficients		Standardized Coefficients	t	P
		B	Std Error	Beta		
Model 1 r=0.23 P<0.0001	Age (years)	-0.005	0.002	-0.108	-2.135	0.033
	Sex (male/female)	0.162	0.043	0.190	3.740	0.0001
Model 2 r=0.27 P<0.0001	Age (years)	-0.003	0.002	-0.069	-1.330	0.184
	Sex (male/female)	0.134	0.044	0.157	3.069	0.002
	ALAT (U/l)	0.003	0.001	0.151	2.871	0.004
Model 3 r=0.30 P<0.0001	Age (years)	-0.002	0.003	-0.053	-0.933	0.352
	Sex (male/female)	0.145	0.048	0.165	3.009	0.003
	ALAT (U/l)	0.003	0.001	0.160	2.848	0.005
	LDL- cholesterol (mmol/l)	0.041	0.019	0.117	2.197	0.029

NAFLD = non-alcoholic fatty liver disease, ALAT = alanine aminotransferase, LDL-cholesterol = low density lipoprotein, FLI = fatty liver index.

Table III. Multiple logistic regression analysis: ALAT, HOMA-IR, LDLc and CAD were significant predictors of NAFLD by FLI>60.

Model	Prediction accuracy	Independent variable	B	SE	p	Odds Ratio (OR)	95% CI for OR	
							Lower	Upper
Model 1	84.1 %	ALAT (units/l)	0.42	0.16	0.007	1.043	1.011	1.075
		HOMA-IR	0.135	0.065	0.039	1.144	1.007	1.300
Model 2	84.6%	ALAT (units/l)	0.046	0.016	0.004	1.047	1.015	1.080
		HOMA-IR	0.152	0.067	0.023	1.164	1.020	1.328
		Hypertension (yes/no)	-0.272	0.489	0.578	0.762	0.292	1.987
		CAD (yes/no)	1.476	0.582	0.011	4.377	1.400	13.686
Model 3	85.1%	ALAT (units/l)	0.045	0.016	0.007	1.046	1.012	1.080
		HOMA-IR	0.134	0.065	0.041	1.143	1.005	1.299
		LDLc (mmol/l)	0.420	0.204	0.040	1.522	1.020	2.273

NAFLD = non-alcoholic fatty liver disease, ALAT = alanine aminotransferase, HOMA-IR = homeostasis model assessment insulin resistance, CAD = coronary artery disease, LDLc = low density lipoprotein cholesterol, FLI = fatty liver index.

Discussion

The key findings of the current study may be summarized as follows. Firstly we observed a high prevalence of NADLD-FLI (79%) in subjects with type 2 diabetes, with an increase up to 80% in the elderly over 75 years. The prevalence of NAFLD-FLI was higher in males than in females. In our NAFLD-FLI and T2DM patients, 80% were obese (BMI >30 Kg/m²). Compared with patients without NAFLD-FLI, the subjects with NAFLD-FLI were younger, predominantly male (70.5%), and had higher TC, LDLc, uricemia, higher HOMA-IR values, higher ASAT and ALAT and an increased prevalence of the metabolic syndrome but lower HDLc levels. Indeed, the prevalence of NAFLD has increased rapidly in European population and is estimated to be 20% to 30% [17]. Previous studies have reported a prevalence of NAFLD that ranges from 7.27% to 23.4% in the non-obese population [18]. Most studies indicate that NAFLD increases in parallel with that of obesity and diabetes, rising up to 75-90% [2,19]. A strong increase of NAFLD has recently been observed especially in adolescents and in older people. In patients with fatty liver, the prevalence of obesity is between 30% and 100%, and that of type 2 diabetes between 10% and 75% [20].

Secondly, we found that FLI was correlated with HS assessed by US, reinforcing the fact that FLI could be considered a valid tool of NAFLD evaluation in our group of patients. Because of the burden of disease, it is important to identify which patients are most likely to be exposed to NAFLD. It is difficult to perform biopsies on such a large number of patients. For this reason we used FLI for NAFLD diagnosis. In order to enhance the validity of our findings and because our cohort included T2DM patients with traditional coronary risk factors, we used two modalities for diagnosis of HS (US and FLI score for NAFLD).

Thirdly, we found that 73.8% of patients with normal

ALAT had NAFLD (FLI >60), and 33% of them had FLI >90. Obesity had a high prevalence up to 80% in NAFLD patients. Indeed, it has recently been demonstrated that the prevalence of NAFLD and NASH in patients with T2DM and normal ALAT levels is 50% and 55%, respectively. The prevalence of NAFLD is much higher than previously believed in overweight/obese patients with T2DM and normal aminotransferases and many are at increased risk of NASH [21,22]. A recent study indicates that 30% to 60% of patients with biopsy-confirmed NASH have a normal ALAT level [22,23]. Elevated level of ALAT had a sensitivity of 45% and specificity of 85% for diagnosis of NAFLD may correlate with IR [23].

Fourthly, we found that sex, higher HOMA-IR values, higher ALT and LDLc levels were significant and independent factors associated with the presence of NAFLD-FLI in T2DM subjects. Consistent with our findings, previous studies reported that male subjects had higher risk of NAFLD. A possible explanation could be the higher BMI and more accelerating visceral adipose tissue expansion increasing with age. It could facilitate the development of IR and HS by the production of free fatty acid and adipocytokines [24]. Estrogen could suppress visceral adipose tissue and TG accumulation. A recent study reported that estrogen receptor ligands reduced hepatic TG levels through the inhibition of liver X receptor transcriptional activity in a mouse model [25]. Dyslipidemia, hyperinsulinemia and IR are common in subjects with NAFLD independently from BMI. IR was associated with disease progression in NAFLD. FLI was correlated with IR as assessed with HOMA-IR independently from the histological grades of steatosis [26].

This study has some limitations that should be kept in mind. First, this study has a retrospective design that could introduce a bias concerning the selection of patients for hepatic US. Second, NAFLD was defined

using FLI, a steatosis biomarker validated against liver US as a simple and performant tool for the detection of fatty liver and the prediction of IR [9]. A recent study in T2DM patients showed that the performance of FLI (and others surrogate steatosis markers) for the prediction of steatosis was very low [27]. FLI seems to be insufficiently accurate for the quantification of steatosis and has a limited value for monitoring changes in steatosis induced by pharmacological and nonpharmacological measures. FLI is prone to substantial confounding due to steatohepatitis and advanced fibrosis and had a significant positive but weak-to-moderate correlation with HOMA-IR [9]. Third, the reference for NAFLD-FLI was considered HS evaluated by US. Hepatic US is widely used in evaluation of NAFLD, because it is safe, noninvasive, and widely available. It is an operator-dependent procedure, with an acceptable sensitivity in relation to the detection of HS (60–94% sensitivity and 66–97% specificity) [28,29]. US has a low sensitivity especially for mild steatosis as it detects the steatosis when present in more than 20–30% of hepatocytes [29,30,31]. According to available study data, the positive predictive value in mild steatosis is only 67% at best [29]. Additionally, it cannot reliably quantify steatosis, an important drawback for the ability to monitor dietary and pharmacological interventions. Liver biopsies (LB) were not available in our subjects. LB is invasive and may result in severe complications but is considered the gold standard technique for identifying NAFLD [32]. Fourth, we could not completely exclude secondary causes of liver disease and this limitation could have increased the number of included patients.

Conclusions

Our results are in line with those of other studies suggesting that FLI could be useful to identify NAFLD in T2DM patients when US is unavailable. Subjects with T2DM had a high prevalence of NAFLD-FLI even when ALAT levels were normal. Our findings showed that sex, ALAT, LDL-cholesterol and IR were significant and independent factors associated with the presence of NAFLD in T2DM subjects.

Acknowledgement

This paper was published under the frame of European Social Found, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/138776.

References

1. Tilg H, Kaser A. Treatment strategies in nonalcoholic fatty liver disease. *Nat Clin Pract Gastroenterol Hepatol.* 2005;2:148-155.
2. Williams CD, Stengel J, Asike MI, Torres DM, Shaw J, Contreras M, et al. Prevalence of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis among a largely middle-aged population utilizing ultrasound and liver biopsy: a prospective

- study. *Gastroenterology.* 2011;140:124–131.
3. Gupte P, Amarapurkar D, Agal S, Baijal R, Kulshrestha P, Pramanik S, et al. Non-alcoholic steatohepatitis in type 2 diabetes mellitus. *J Gastroenterol Hepatol.* 2004;19:854–858.
4. Musso G, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med.* 2011;43:617–649.
5. Baumeister SE, Völzke H, Marschall P, John U, Schmidt CO, Flessa S, et al. Impact of fatty liver disease on health care utilization and costs in a general population: a 5-year observation. *Gastroenterology.* 2008;134:85–94.
6. Kotronen A, Yki-Järvinen H. Fatty liver: a novel component of the metabolic syndrome. *Arterioscler Thromb Vasc Biol.* 2008;28:27-38.
7. Aller R, Izaola O, Ruiz-Rebollo L, Pacheco D, de Luis DA. Predictive factors of non-alcoholic steatohepatitis: relationship with metabolic syndrome. *Nutr Hosp.* 2015;31:2496-2502.
8. Bedogni G, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, et al. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* 2006;6:33.
9. Fedchuk L, Nascimbeni F, Pais R, Charlotte F, Housset C, Ratziu V, et al. Performance and limitations of steatosis biomarkers in patients with nonalcoholic fatty liver disease. *Aliment Pharmacol Ther.* 2014;40:1209-1222.
10. Kim JH, Kwon SY, Lee SW, Lee CH. Validation of fatty liver index and lipid accumulation product for predicting fatty liver in Korean population. *Liver Int.* 2011;31:1600–1601.
11. Ruhl CE, Everhart JE. Fatty liver indices in the multiethnic United States National Health and Nutrition Examination Survey. *Aliment Pharmacol Ther.* 2015;41:65-76.
12. Yang BL, Wu WC, Fang KC, Wang YC, Huo TI, Huang YH, et al. External validation of fatty liver index for identifying ultrasonographic fatty liver in a large-scale cross-sectional study in Taiwan. *PLoS One.* 2015;17;10:e0120443.
13. Calori G, Lattuada G, Ragona F, Garancini MP, Crosignani P, Villa M, et al. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology.* 2011;54:145–152.
14. Lerchbaum E, Pilz S, Grammer TB, Boehm BO, Stojakovic T, Obermayer-Pietsch B, et al. The fatty liver index is associated with increased mortality in subjects referred to coronary angiography. *Nutr Metab Cardiovasc Dis.* 2013;23:1231–1238.
15. World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. *World Health Organ Tech Rep Ser.* 2000;894:1–253.
16. Bugianesi E, Pagotto U, Manini R, Vanni E, Gastaldelli A, de Iasio R, et al. Plasma adiponectin in nonalcoholic fatty liver is related to hepatic insulin resistance and hepatic fat content, not to liver disease severity. *J Clin Endocrinol Metab.* 2005;90:3498–3504.
17. Blachier M, Leleu H, Peck-Radosavljevic M, Valla DC, Roudot-Thoraval F. The burden of liver disease in Europe: a review of available epidemiological data. *J Hepatol.* 2013;58:593–608.
18. Kim NH, Kim JH, Kim YJ, Yoo HJ, Kim HY, Seo JA, et al. Clinical and metabolic factors associated with development and regression of nonalcoholic fatty liver disease in nonobese subjects. *Liver Int.* 2014;34:604–611.
19. Chalasani N, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, et al. The diagnosis and management of non-alcoholic fatty liver disease: practice guideline by the American

Gastroenterological Association, American Association for the Study of Liver Diseases, and American College of Gastroenterology. *Gastroenterology*. 2012;142:1592–1609.

20. Angulo P. Nonalcoholic fatty liver disease. *N Engl J Med*. 2002;346:1221–1231.
21. Portillo-Sanchez P, Bril F, Maximos M, Lomonaco R, Biernacki D, Orsak B, et al. High prevalence of nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus and normal plasma aminotransferase levels. *J Clin Endocrinol Metab*. 2015;100:2231–2238.
22. Rinella ME. Nonalcoholic fatty liver disease: a systematic review. *JAMA*. 2015;313:2263–2273.
23. Maximos M, Bril F, Portillo Sanchez P, Lomonaco R, Orsak B, Biernacki D, et al. The role of liver fat and insulin resistance as determinants of plasma aminotransferase elevation in nonalcoholic fatty liver disease. *Hepatology*. 2015;61:153–160.
24. Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*. 2009;120:1640–1645.
25. Han SI, Komatsu Y, Murayama A, Steffensen KR, Nakagawa Y, Nakajima Y, et al. Estrogen receptor ligands ameliorate fatty liver through a nonclassical estrogen receptor/Liver X receptor pathway in mice. *Hepatology*. 2014;59:1791–1802.
26. Gastaldelli A, Kozakova M, Hojlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, et al. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. *Hepatology*. 2009;49:1537–1544.
27. Guiu B, Crevisy-Girod E, Binquet C, Duvillard L, Masson D, Lepage C, et al. Prediction for steatosis in type-2 diabetes: clinico-biological markers versus 1H-MR spectroscopy. *Eur Radiol*. 2012;22:855–863.
28. Lupşor-Platon M, Stăfănescu H, Mureşan D, Florea M, Szász ME, Maniu A, et al. Noninvasive assessment of liver steatosis using ultrasound methods. *Med Ultrason*. 2014;16:236–245.
29. Machado MV, Cortez-Pinto H. Non-invasive diagnosis of non-alcoholic fatty liver disease. A critical appraisal. *J Hepatol*. 2013;58:1007–1019.
30. Dasarathy S, Dasarathy J, Khiyami A, Joseph R, Lopez R, McCullough AJ. Validity of real time ultrasound in the diagnosis of hepatic steatosis: a prospective study. *J Hepatol*. 2009;51:1061–1067.
31. Weiß J, Rau M, Geier A. Non-alcoholic fatty liver disease: epidemiology, clinical course, investigation, and treatment. *Dtsch Arztebl Int*. 2014;111:447–452.
32. Pais R, Lupşor M, Poantă L, Silaghi A, Rusu ML, Badea R, et al. Liver biopsy versus noninvasive methods--fibroscan and fibrotest in the diagnosis of non-alcoholic fatty liver disease: a review of the literature. *Rom J Intern Med*. 2009;47:331-340.