HVM BIOFLUX
Human & Veterinary Medicine
International Journal of the Bioflux Society

How can we predict steatosis using biochemical markers (SteatoTest and Fatty liver index)?

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Abstract. Objective: The aim of this study was to establish the diagnostic performance of two biological markers - fatty liver index (FLI) and SteatoTest, in identifying hepatic steatosis and assessing its degree. Material and Methods: Seventy seven patients with biopsy-proven non-alcoholic fatty liver disease (NAFLD) were prospectively studied and compared with 18 healthy subjects. The patients were stratified according to the degree of hepatic steatosis. Two biochemical markers, FLI and SteatoTest, were determined. The diagnostic value of each method was assessed using sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and the area under the receiving operating characteristics curve (AUROC). Results: We found that both markers are able to recognize the presence of NAFLD. These are the values obtained for SteatoTest (controls: 0.176±0.0079, NAFLD: 0.744±0.166, p<0.001) and for FLI (controls: 80.29±10.53, NAFLD: 91.1±1.89, p<0.001). The AUROC for the diagnosis of steatosis was 0.811 for SteatoTest (Se: 90.32%, Sp: 67.74%, PPV: 77.8%, NPV: 84.8%) and 0.808 for FLI (Se: 98.33%, Sp: 54.55%, PPV: 94.7%, NPV: 79.7%). We also found SteatoTest and FLI useful for the discrimination between insignificant (S0 and S1) and significant steatosis (S2 and S3) (p<0.001). Conclusion: SteatoTest and FLI are accurate and easy to use markers for the diagnosis of steatosis and they allowed differentiation between mild/moderate and severe steatosis.
Key Words: non-alcoholic fatty liver disease, SteatoTest, non-invasive biomarkers, steatosis.

Rezumat. Obiectiv: Scopul studiului de faţă a fost de stabilirea a performanţei diagnostice, pentru doi markeri biologic neinvazivi - fatty liver index (FLI) şi SteatoTest. Material şi metodă: Au fost studiaţi prospectiv 77 de pacienţi diagnosticaţi cu ficat gras nealcoolic (FGNA) prin biopsie hepatică şi comparaţi cu un lot martor de 18 subiecţi sănătoşi. Pacienţii au fost clasificaţi în funcţie de gradul histologic de steatoză. Au fost utilizate două metode biologice neinvazive FLI si SteatoTest. Valoarea diagnostică a fiecărei metode a fost determinată prin sensibilitate (Se), specificitate (Sp), valoare predictivă pozitivă (VPP), valoare predictivă negativă (VPN) şi aria de sub curbă (AUROC). Rezultate: Valorile obţinute pentru ambii parametrii studiaţi au permis recunoaşterea prezenţei FGNA. Valorile obţinute pentru SteatoTest au fost următoarele: lot control (0,176±0,0079), FGNA (0,744±0,166), p<0,001, iar pentru FLI: lot control (80,29±10,53), FGNA (91,1±1,89), p<0,001. Valoarea curbei AUROC pentru diagnosticul de steatoză a fost de 0,811 pentru SteatoTest (Se: 90,32%, Sp: 67,74%, PPV: 77,8%, NPV: 84,8%) si 0,808 pentru FLI (Se: 98,33%, Sp: 54,55%, PPV: 94,7%, NPV: 79,7%). S-a obţinut de asemenea, cu ajutorul celor doi parametrii o bună departajare între steatoza nesemnificativă (S0 şi S1) şi cea semnificativă (S2 şi S3) (p<0,001). Concluzie: SteatoTest-ul şi indicele FLI au o valoare diagnostică bună pentru diagnosticul steatozei şi permit diferenţierea între steatoza uşoară/moderată şi cea severă.
Cuvinte cheie: ficat gras nealcoolic, SteatoTest, markeri biologici neinvazivi, steatoză.

Introduction. Non-alcoholic fatty liver disease (NAFLD) is a clinico-histopathological entity with histological features that resemble alcohol-induced liver injury, but by definition, it occurs in patients with no history of alcohol consumption. It encompasses a histological spectrum that ranges from fat accumulation in hepatocytes without concomitant inflammation or fibrosis (simple hepatic steatosis) to hepatic steatosis with a necroinflammatory component (steatohepatitis). The latter condition, referred to as non-alcoholic steatohepatitis (NASH), may progress to cirrhosis in up to 20% of patients (Matteoni et al 1999; Pais et al 2011; Vernon et al 2011). NASH is now recognized to be a leading cause of cryptogenic cirrhosis.

NAFLD is emerging as the most common chronic liver condition in the Western world, affecting 20-40% of the general population (Falck-Ytter et al 2001; Clark et al

2003; Adams et al 2005; Sheth & Chopra 2008; De Lusong et al 2008). The true prevalence of NAFLD and its different stages is unknown. In a hospital-based study that took place in Romania and it encompassed 3005 patients, we found an overall prevalence of NAFLD of 20% (Radu et al 2008). Obesity and its complications, especially type 2 diabetes and hypertriglyceridemia, are likely to be the main causes of the current NAFLD epidemic (Adams et al 2010).

Liver steatosis is often found in association with common cardiometabolic disorders, conditions that may all occur in a shared context of abdominal obesity and dyslipidemia (Perseghin 2011).

Liver biopsy is considered to be the golden standard for diagnosis of NAFLD, but the technique is invasive and usually painful. Consequently, serum liver function tests have been used as indirect or surrogate tests to diagnose NAFLD (Poynard et al 2005; Bedogni et al 2006).

The fatty liver index (FLI) is an algorithm for the prediction of steatosis based on 4 anthropometrical and biochemical factors.

SteatoTest™ (Biopredictive, Paris, France) is a simple blood test, combining 10 blood components, developed in order to provide an estimate of quantitative steatosis in NAFLD (Poynard et al 2005).

The aim of this study was to establish the diagnostic performance of these two biological markers (FLI and SteatoTest) for the identification of hepatic steatosis and assessing its degree.

Material and Methods. Seventy seven patients with biopsy-proven NAFLD were prospectively studied and compared with 18 healthy subjects that did not presented any metabolic or hepatic disease that could induce hepatic steatosis and that had a normal liver on US examination.

The exclusion criteria were: other etiologies of chronic liver disease: B or C viral chronic hepatitis, autoimmune hepatitis, Wilson's disease, history of hepatotoxic or steatosis-inducing drug use, alcohol consumption (>20 g day^{-1} for women, >30 g day^{-1} for men) and personal history of diabetes mellitus.

Each studied patient was submitted to an abdominal ultrasound exam using a GE Logiq 7 device with a 5.5 MHz convex probe, the day prior to the liver biopsy.

Liver biopsy was the main reference factor used to assess the contribution of biochemical markers in the diagnosis of steatosis.

The degree of hepatic steatosis in NAFLD patients was assessed according to the Kleiner criteria: grade 0: fat droplets <5% hepatocytes (S0); grade 1: fat droplets in 5-33% hepatocytes (S1); grade 2: fat droplets in 33-66% hepatocytes (S2) and grade 3: fat droplets in > 66% hepatocytes (S3) (Kleiner et al 2005).

FLI is a composite index which combines 4 parameters: body mass index (BMI), waist circumference (WC), triglycerides and gamma-glutamyl transpeptidase (GGT) in a mathematical algorithm, developed to detect the presence of steatosis.

FLI = [e 0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (GGT) + 0.053*WC - 15.745] / [1 + e 0.953*loge (triglycerides) + 0.139*BMI + 0.718*loge (GGT) + 0.053*WC- 15.745] * 100

The score varies from 0 and 100. A FLI score < 30 rules out NAFLD, while a FLI score ≥ 60 indicates fatty liver disease (Bedogni et al 2005).

SteatoTest™ combines 10 blood components: alpha2 macroglobulin (g L^{-1}), haptoglobin (g L^{-1}), apolipoprotein A (g L^{-1}), total bilirubin (µmol L^{-1}), GGT (IU L^{-1}), ALT(IU L^{-1}), AST(IU L^{-1}), total cholesterol (mmol L^{-1}), triglycerides (mmol L^{-1}), fasting glucose (mmol L^{-1}) with age, gender and BMI. ST uses numerical values that are inserted in the Biopredictive website. ST scores range from 0 to 1.00 with higher scores indicating a greater probability of significant lesions.

SteatoTest™ is part of the more complex FibroMax™ test; all these tests are patented algorithms (Biopredictive, France) and are provided with instructions for use, securing their interpretation (Poynard et al 2005; Munteanu et al 2008).

Statistical analysis. The quantitative data were expressed as mean ± SD and significance was established by Student's t test and ANOVA (one-way analysis of variance, with post hoc test Tukey). The diagnostic value for each method was assessed using sensitivity (Se), specificity (Sp), positive predictive value (PPV), negative predictive value (NPV) and the area under the receiving operating characteristics curve (AUROC). The statistical analysis was performed using the MedCalc® 9.3.9.0. software.

The study was performed in full accordance with the Declaration of Human Rights (Helsinki, 1975) and with its further revisions. A complete, comprehensive and clear informed consent was provided for patients. The study protocol excluded vulnerable persons, prisoners, mentally impaired persons, severely injured patients, with no legal representatives to sign the consent in their place.

The study was approved by the local ethical committee of the Clinical Emergency Hospital "Prof. Dr. Octavian Fodor" Cluj-Napoca. All patients gave prior informed consent for being included in the study.

Results. The characteristics of the control group and patients are shown in Table 1.

Table 1

Clinical and biological features of the patients

Variables	*Controls (n=18)*	*NAFLD (n=77)*
	Mean ± DS	Mean ± DS
Age (years)	38.66±12.67	45.62±10.95
BMI (kg m^{-2})	21.81±2.25	30.09±4.46
Waist circumference (cm)	73.12±5.92	103.5±14.81
GGT (U $L^{-1)}$	24.77±7.96	98.02±101.63
Triglycerides (mg $dL^{-1)}$	104.94±40.86	237.17±158.46
SteatoTest	0.176±0.079	0.744±0.166
FLI	80.29±10.53	91.1±1.89
Male (number and percentage)	4 (6.56%)	57(93.44%)

A significant difference between the mean values was found for each parameter both in controls and in NAFLD patients (Fig. 1).

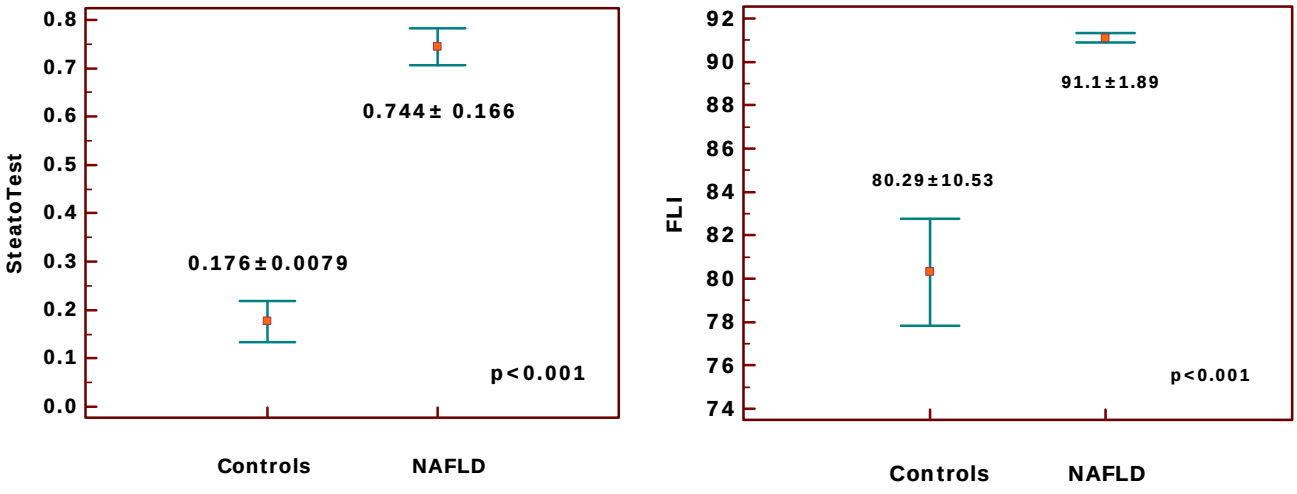

Figure 1. Values of SteatoTest (1a) and FLI (1b) depending on the presence or absence of steatosis (Mean ± SD).

We also obtained a good discriminating value between insignificant (grade 0-1) and significant (grade 2-3) steatosis (see Figure 2).

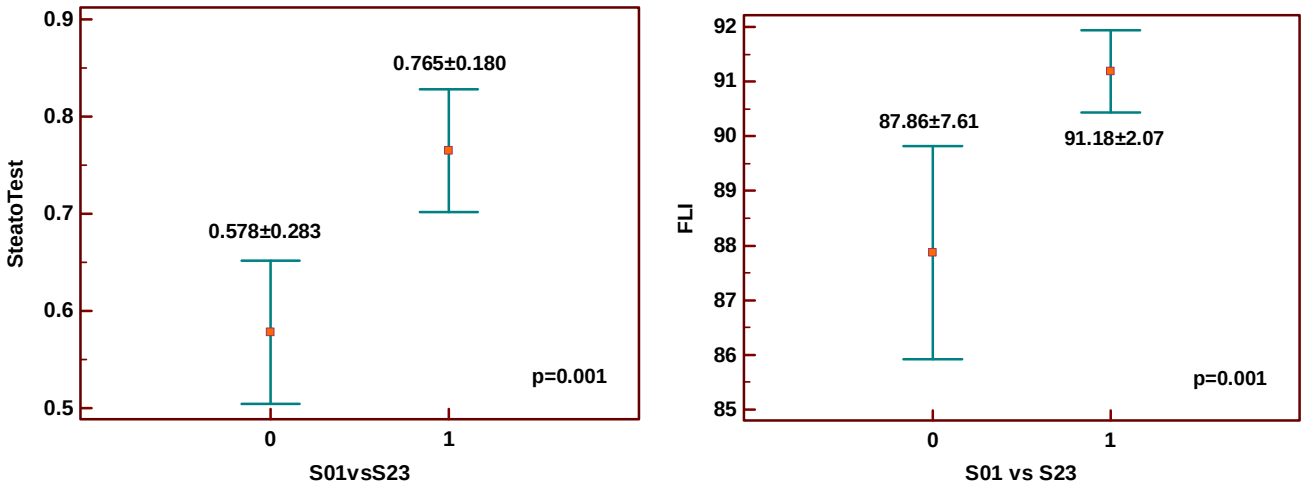

Figure 2. Values of SteatoTest (2a) and FLI (2b) for significant/insignificant steatosis (Mean ± SD).

Median ± SD of SteatoTest and FLI for different grades of steatosis showed a significant difference between steatosis grade 0 and other grades of steatosis (Fig. 3, Table 2).

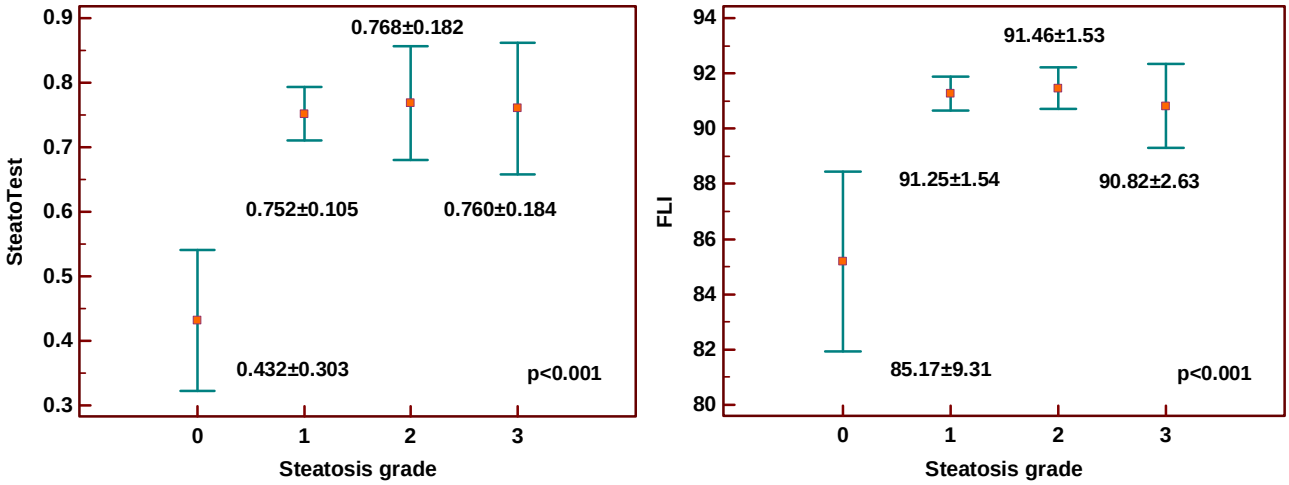

Figure 3. Values of SteatoTest (3a) and FLI (3b) depending on steatosis grade (Mean ± SD).

Table 2

Mean difference of SteatoTest and FLI according to steatosis grades

Parameter	*Mean difference*	*p*
SteatoTest		
S0-1	6.18	0.001
S0-2	6.41	0.002
S0-3	5.76	0.015
FLI		
S0-1	0.31	0.000
S0-2	0.33	0.000
S0-3	0.32	0.000

Further on, we tried to compare the AUROCs for these two parameters according to the grade of steatosis (Figs 4-6).

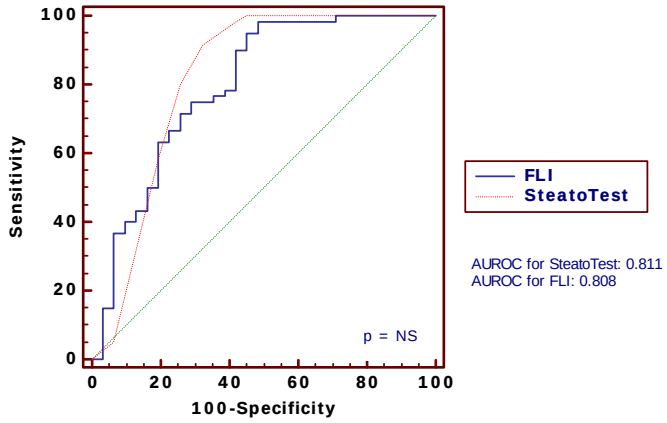

Figure 4. AUROC for attenuation ST and FLI for the diagnosis of hepatic steatosis.

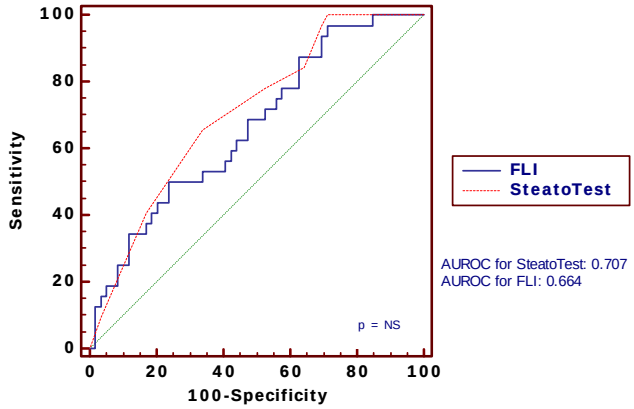

Figure 5. AUROC for ST and FLI for mild steatosis compared with moderate and severe steatosis.

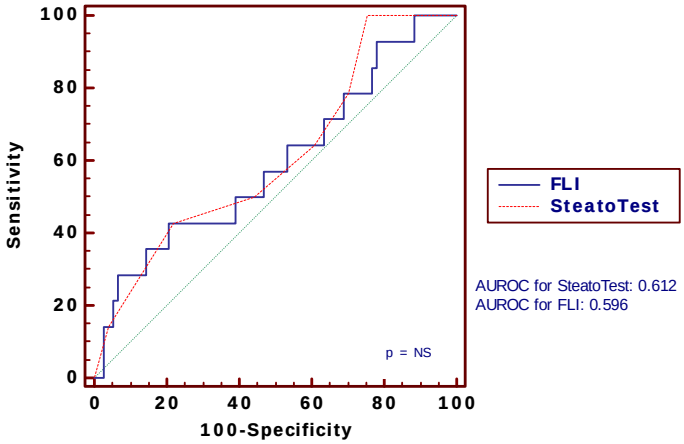

Figure 6. AUROC for ST and FLI for mild/moderate steatosis compared with severe steatosis.

The diagnostic value of the cut-off values of these two parameters is shown in Table 3.

Table 3

Diagnostic value of SteatoTest and FLI in hepatic steatosis

Parameter	*SteatoTest*	*FLI*
Cut-off value	≤ 0.5	86.90
AUROC	0.811	0.808
95% CI	0.717-0.885	0.713-0.882
Se%	90.32	98.33
Sp%	67.74	54.55
PPV%	77.8	94.7
NPV%	84.8	79.7
p	0.0001	0.0001

Discussion and Conclusions. The increasing prevalence of NAFLD in the general population has compelled the research community to identify accessible, cheap, fast and reproducible means for the diagnosis and also for the screening of this condition.

The main screening method is still classical ultrasonography, a widely-spread, accessible, fast method with good diagnostic performance: sensibility 67%, specificity 77% and positive predictive value 67% (Graif et al 2000).

However, classical ultrasonography can only provide an approximate assessment of the degree of fatty liver infiltration.

Liver biopsy has long remained the "gold standard", other investigations are referred to for the diagnosis of this condition, despite its invasiveness, morbidity and mortality risks, high cost and sampling errors.

Predicting the degree of fatty infiltration of hepatocytes is of high importance for the evolution of NAFLD, due to the physiopathologic link between fatty infiltration (lipotoxicity) and evolution towards fibrosis, based on insulin resistance (IR) as the initiating factor. The progression of IR is the "first hit" and induces simple liver steatosis; the "second hit" factors (reactive oxygen species, mitochondrial dysfunction, endotoxemia) lead to the establishment of NASH, fibrogenetic response and potential evolution towards cirrhosis (Day 2002; Browning & Horton 2004).

Steatosis can be assessed both through pathologic examination (with all its possible drawbacks) and through the use of non-invasive biological tests.

In order to avoid the pathologic examination, a computerized system allowing a quantitative estimation of steatosis regardless of its origin (NAFLD, viral chronic hepatitis, alcoholic liver disease) was developed. The grade of steatosis is expressed in numbers between 0-1 and it combines 10 biological parameters: alfa2 macroglobulin, haptoglobin, apolipoprotein A1, bilirubin, GGT, ALT, AST, fasting glucose, triglycerides and cholesterol with age, gender and BMI.

Using the system patented by Biopredictive.com, a satisfactory estimation of the degree of steatosis can be obtained (Table 4).

Table 4

Conversion from SteatoTest to steatosis grade

SteatoTest	*Steatosis percentage*
0.69-1.00	S:>32%
0.57-0.68	S:6-32%
0.38-0.56	S:1-5%
0.00-0.37	S:0%

The results obtained in this study indicated a significant low score of SteatoTest for controls. The SteatoTest AUROC for the prediction of steatosis had a good value (0,811) with a cut-off value (0.5) of high sensitivity, specificity, PPV and NPV. Our data showed a better value for AUROC for the detection of steatosis and a higher cut-off value compared to data found by the original authors (AUROC 0.800, cut-off value 0.38) (Poynard et al 2005; Munteanu et al 2008).

We also found a good SteatoTest value for the discrimination between insignificant (S0 and S1) and significant steatosis (S2 and S3). The AUROC value in this case was 0.707.

In the present study we were not able to differentiate between grade 1, 2 and 3 of steatosis by means of ST and the AUROC for discrimination between mild/moderate and severe steatosis only reached 0.612.

The fatty liver index, calculated with the above-mentioned formula, has been proposed in the published literature for the diagnosis of steatosis; the authors proposed a ≥ 60 value for the prediction of NAFLD (Bedogni et al 2006).

The present study group yielded significantly higher values for NAFLD patients and for discrimination between grade 0 and grade 1, 2 and 3. The cut-off value obtained in our study was 89.6-fold higher than the previously published one (≥ 60).

The index had a good discriminating value, however, for the differentiation between insignificant (S0-1) and significant (S2-3) steatosis – AUROC 0.664. Recently, the same authors, based on this index, developed another one: the lipid accumulation product (LAP) cardiometabolic disorders (Bedogni et al 2010).

A limitation of the study is the relatively small number of patients included. However, it is important to stress-out that all these patients were biopsied.

This study is important because externally validates the two noninvasive scores in patients with biopsy proved NAFLD.

In conclusion, our findings suggest that the use of non-invasive methods for diagnosis of NAFLD is adequate and accurate. SteatoTest and FLI are precise and easy to use for diagnosis of steatosis and for differentiation between mild/moderate and severe steatosis. FLI contains routine lab tests, is cheaper and has similar efficiency to SteatoTest for detection of steatosis and its degree. The complementary use of two biological methods could improve the diagnostic accuracy.

In practice, the two indices could be used for the detection of steatosis patients in order to offer nutrition and lifestyle counselling, as well as for the detection of severe steatosis patients requiring medical supervision and therapy.

Acknowledgements. This study was supported by the Romanian National Authority for Scientific Research as a part of the CEEX program FINALISM 94/2006-2008.

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Received: 02 May 2011. Accepted: 16 June 2011. Published online: 06 August 2011.
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How to cite this article:
Radu C. I., Crişan D. A., Grigorescu M., 2011 How can we predict steatosis using biochemical markers (SteatoTest and Fatty liver index)? HVM Bioflux **3**(2):89-96.