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Ultrasonographic fatty liver indicator detects mild steatosis and correlates with metabolic/histological parameters in various liver diseases

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ABSTRACT

Background and aims. Fatty liver is a common feature of different types of liver diseases. The sensitivity and specificity of ultrasonography for diagnosing fatty liver are variable. A semi-quantitative ultrasound score, i.e., the ultrasonographic fatty liver indicator (US-FLI), is closely associated with metabolic/histological variables in patients with nonalcoholic fatty liver disease (NAFLD). The main aims of this study were to assess the diagnostic performance of US-FLI in detecting varying degrees of histological steatosis, and to examine the association of US-FLI with metabolic/histological parameters in 352 biopsied patients with various chronic liver diseases (173 with hepatitis C [HCV], 23 with hepatitis B [HBV], 123 with NAFLD and 33 with other etiologies).

Results. US-FLI accurately detected mild steatosis (minimum amount 10% on histology) with a cut-off value ≥2 (sensitivity 90.1%, specificity 90%), moderate steatosis (≥30%) with a cut-off value ≥3 (sensitivity 86.4%, specificity 92.5%) and severe steatosis (>66%) with a cut-off value ≥5 (sensitivity 88.5%, specificity 87%). US-FLI was correlated with steatosis percentage in each liver disease group as well as with lobular inflammation, ballooning, portal fibrosis, grading and staging in patients with NAFLD or HCV. US-FLI was also correlated with waist circumference, body mass index and insulin resistance both in the whole sample and in each liver disease group.

Conclusions. US-FLI accurately identifies histological severity and is correlated with metabolic parameters in patients with various steatogenic liver diseases. US-FLI is an easy and versatile tool for the screening of steatosis and the metabolic health of these patients.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; AUROC, area under the receiver operating characteristic; BMI, body mass index; CI, confidence intervals; FLI, fatty liver index; FT3, triiodothyronine; FT4, free thyroxine; GGT, gamma-glutamyltransferase; HBV, hepatitis B virus; HCV, hepatitis C virus; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment of insulin resistance; IR, insulin resistance; LAP, lipid accumulation product; LDL, low-density lipoprotein; MetS, metabolic syndrome; NAFLD, nonalcoholic fatty liver disease; NAS, NAFLD activity score; NASH, nonalcoholic steatohepatitis; NPV, negative predictive value; PPV, positive predictive value; ROC, receiver operating characteristic; SUA, serum uric acid; TC, total cholesterol; TG, triglycerides; TSH, thyroid stimulating hormone; US-FLI, ultrasonographic fatty liver indicator; WC, waist circumference.

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1. Background

Fatty liver is a common condition that may result from metabolic, viral, alcoholic and other etiologies [1]. Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease in Western countries and represents an increasing healthcare issue in many parts of the world [2,3]. NAFLD is closely and bi-directionally related to insulin resistance (IR) and metabolic syndrome (MetS) features [4–6]. Fatty liver in patients with chronic hepatitis C virus (HCV) results from a complex interplay between host and viral factors, including visceral adiposity, IR, MetS, lifestyle habits and HCV genotype [7], while in those with hepatitis B virus (HBV) fatty liver is more strongly associated with host metabolic factors rather than with viral factors [1]. Finally, fatty liver is also the hallmark of the alcohol-induced liver disease [1].

The severity of fatty liver is associated with an increased risk of progressive steatohepatitis (NASH) and with an atherogenic lipoprotein profile among patients with NAFLD [6,8]. Conversely, HCV-related fatty liver is a risk factor for accelerated fibrogenesis, impaired response to traditional antiviral treatments and development of hepatocellular carcinoma [7].

Liver biopsy is the reference standard for the diagnosis of fatty liver and concurrent necro-inflammatory and fibrotic changes; however, this procedure is not free of acute complications, and should be reserved only to patients at risk of progressive liver disease [9,10].

Liver ultrasound being safe, well tolerated, inexpensive and widely available is currently the accepted first-line imaging technique for the diagnosis of fatty liver in both clinical and epidemiological settings [9]. The reported sensitivity and specificity of ultrasound in detecting fatty liver are variable and may be influenced by the operator, the extent of steatosis and the coexistence of obesity [9]. Traditionally, ultrasound is deemed to have a poor sensitivity in identifying fatty liver infiltration when less than 30% of hepatocytes are steatotic [11]. In contrast, recent studies reported that this technique is sensitive for steatosis extent as low as 10–20% [12,13]. Moreover, semi-quantitative ultrasound scores [14–16], such as the Ultrasonographic Fatty Liver Indicator (US-FLI) recently developed by our group [16], are strongly correlated with both anthropometric/biochemical data and liver histology in patients with NAFLD. However, it is currently uncertain if these findings can be also extended to patients with fatty liver of other etiologies.

Thus, the main aim of this study was to assess the diagnostic performance of US-FLI in detecting different degrees of histologically confirmed steatosis in a large series of biopsied patients with liver diseases due to various etiologies. In particular, we attempted to ascertain whether US-FLI may accurately detect mild steatosis. In addition, we assessed the association between US-FLI and anthropometric, metabolic or histological parameters of these patients.

2. Patients and Methods

In this retrospective study we enrolled all patients consecutively submitted to liver biopsy at our Institution over the years 2001 and 2014. Liver biopsy was a part of the diagnostic work-up of abnormal liver tests, suspected liver diseases or grading/staging of known liver diseases, and was invariably preceded by the assessment of the US-FLI [16]. The present study includes all the 53 patients enrolled in our previous study [16]. All enrolled patients were interviewed regarding their familial and personal medical history notably including alcohol consumption. All patients underwent complete physical examination and routine blood sampling for laboratory tests (such as detailed below). Liver biopsy was performed following signature of consent. Criteria for the exclusion from the study were the presence of advanced cirrhosis and either primary or metastatic liver cancer. The Ethical Committee of Modena approved the study protocol and the research was performed according to the Declaration of Helsinki. All patients signed an informed consent form to participate in this study.

NAFLD diagnosis was based on the presence of ultrasonographic evidence of fatty liver in the absence of excessive alcohol consumption (defined as daily alcohol intake >20 g in both sexes) and other competing etiologies of liver disease.

The diagnosis of HCV infection was confirmed by polymerase chain reaction testing in HCV antibody-positive patients, and after excluding competing etiologies of liver disease. HCV was also genotyped.

The diagnosis of HBV infection was based on typical serological pattern determined by a standard commercially available enzyme-linked immunosorbent assay.

The diagnosis of alcoholic liver disease was based on clinical data and excessive alcohol consumption (>20 g/day) or abuse.

Other liver diseases (autoimmune or heredo-metabolic) were diagnosed based on clinical data and appropriate testing [17].

2.1. *Laboratory Tests*

Laboratory profile included serum liver tests [alanine and aspartate aminotransferases (ALT, AST); gamma-glutamyltransferase (GGT); alkaline phosphatase and bilirubin]. Moreover, glycolipid, urate and iron metabolic parameters were also assessed [total cholesterol (TC), high-density lipoprotein (HDL)-cholesterol, low-density lipoprotein (LDL)-cholesterol, triglycerides (TG), fasting glucose and insulin, glycated hemoglobin; serum uric acid (SUA); serum iron, transferrin and ferritin]. In addition, complete blood cell count, serum creatinine, total and fractionated proteins, thyroid stimulating hormone (TSH), free thyroxine (FT4) and triiodothyronine (FT3) were all part of the study protocol. Glomerular filtration rate was estimated using the Modification of Diet in Renal Disease study equation [18].

In all patients, the presence of other etiologies of chronic liver disease was ruled out by appropriate testing.

2.2. *Metabolic Parameters*

IR, calculated according to the homeostasis model assessment (HOMA) index, body mass index (BMI), waist circumference (WC), glucose intolerance, diabetes, hypertension and the MetS were defined based on standard criteria [19–22].

2.3. *Abdominal Ultrasound Scanning*

Abdominal ultrasound scanning was always performed after an overnight fasting prior to liver biopsy, by expert physicians (S.B., A.L., D.R.), unaware of the biochemical and histological data of patients, with a 3.5–5 MHz convex probe and a high-resolution B-mode scanner (Siemens AntaresTM Unit, Siemens Sonoline, Germany).

The presence and severity of fatty liver was evaluated according to a semi-quantitative ultrasonographic score, i.e., the US-FLI score that has been recently developed by our group [16]. Specifically, the US-FLI score ranges from 2 to 8. A "*conditio* sine qua non" for the diagnosis of fatty liver is the presence of liver-kidney contrast, graded as mild/moderate (score 2) and severe (score 3). Additional criteria include the presence (score 1 each) or absence (score 0 each) of posterior attenuation of ultrasound beam, vessel blurring, difficult visualization of the gallbladder wall, difficult visualization of the diaphragm and areas of focal sparing. The mean duration of each upper abdominal ultrasound scanning, which included accurate examination of liver, gallbladder, spleen, pancreas, abdominal aorta and kidneys was approximately 10–15 min. The calculation of the US-FLI score was easy and required only 2 min extra time further to a standard abdominal ultrasound scanning, thus carrying no significant extra costs to bear [16]. US-FLI score has shown an "almost perfect"–"substantial" inter-observer agreement (*k* statistics 0.81–0.88) [16,23].

2.4. Histological Evaluation

Histological liver samples were evaluated by a single experienced liver pathologist (L.L.); only biopsy samples at least 15 mm long with at least six portal tracts were considered eligible for analysis.

Steatosis degree was assessed continuously as percentage of the hepatocytes affected. The histologic diagnosis of fatty liver, whatever the etiology, required a 5% minimum amount of fat infiltration.

Biopsy specimens of patients with NAFLD were scored according to Brunt's [24] and Kleiner's criteria [25]. Steatosis was scored 0–3 (0 = <5%; 1 = 5–33%; 2 = 34–66%; 3= >66%); lobular and portal inflammation were graded 0–3 (0 = none; 1 = mild; 2 = moderate; 3 = severe); hepatocellular ballooning was scored 0–2 (0 = absent, 1 = mild/few cells, 2 = prominent/many cells) [24,25]. The necro-inflammatory activity was graded (NASH grading score) according to the Brunt's criteria, and fibrosis staged (stage 0 = none; 1 = perisinusoidal/pericellular or periportal; 2 = perisinusoidal/pericellular plus periportal; 3 = bridging; 4 = cirrhosis) according to the Brunt's and Kleiner's criteria, respectively [24,25]. Perisinusoidal fibrosis was scored 0–3 based on the percentage of zone 3 foci involved [24] and portal fibrosis was scored 0–4 (0 = absent; 1 = enlarged, fibrotic portal tracts; 2 = periportal or portal-portal septa, but intact architecture; 3 = bridging fibrosis with architectural distortion; 4 = cirrhosis) [24,26]. Moreover, the NAFLD activity score (NAS) was calculated according to the Kleiner's criteria [25].

Biopsy specimens of patients with chronic viral hepatitis were scored according to Ishak et al. [27].

Patients with other causes of liver diseases were scored according to Brunt et al. [24] and according to Scheuer [26] as appropriate. In all patients liver fibrosis was considered advanced in the presence of bridging fibrosis or cirrhosis, whatever the staging system adopted.

2.5. Statistical Analysis

The Kolmogorov–Smirnov test was used to assess the normality of variables. The results are shown as means ± SD for continuous variables that were normally distributed and medians (25th–75th percentile) for variables not normally distributed. Categorical variables are shown as relative or absolute proportions. The Spearman's rho correlation was used to test the association between the US-FLI and histological and clinical/metabolic parameters.

The diagnostic performance of the US-FLI and its individual criteria for detecting different histological degrees of steatosis was evaluated by calculating the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy with standard formulas. Moreover, the receiver operating characteristic (ROC) curves with 95% confidence intervals (CI) were also calculated to determine the optimal US-FLI cut-off for detecting the minimum steatosis extent.

A *P*-value <0.05 was considered statistically significant. Statistical analyses were performed by using the statistical software package SPSS, version 17.0 for Windows (SPSS Inc., IL, USA) and STATA version 11 for Windows (StataCorp, College Station, TX).

3. Results

The demographic, anthropometric, metabolic, ultrasonographic and histological characteristics of the study population are shown in Table 1.

Among the 352 biopsied patients enrolled in the study 173 (49.1%) had HCV (120 naïve and 53 treatment-experienced), 23 (6.5%) had HBV (all treatment-naïve), 123 (35%) had NAFLD (70.7% of whom had NASH) and 33 (9.4%) had other liver diseases.

Patients with NAFLD had a higher prevalence of metabolic disorders compared to other forms of liver disease. The prevalence of fatty liver (i.e., steatosis ≥5%) was 64.2% in the whole sample: 45.1% in HCV, 43.5% in HBV, 100% in NAFLD and 45.5% in the "Others" group. This latter group included the following 33 patients: 5 with alcoholic liver disease (4 with steatosis), 5 with autoimmune hepatitis (3 with steatosis), 2 with drug-induced hepatitis, 3 with steatosis due to familial hypobetalipoproteinemia, 1 with hemochromatosis and steatosis, 2 with idiopathic biliary ductopenia (1 with steatosis), 2 with systemic lupus erythematosus and steatosis, 1 with multiple biliary hamartomas, 5 with primary biliary cholangitis (1 with steatosis), 2 with primary sclerosing cholangitis, 1 with systemic sclerosis associated liver disease, 1 with undetermined cholestatic hepatitis, and 3 with serum cholestasis associated to minimal/absent histological alterations. The mean histological extent of steatosis was higher in NAFLD patients and in the "Others" group compared to that in patients with HCV or HBV. The prevalence of cirrhosis was quite similar among the groups while advanced fibrosis was higher in patients with HCV.

3.1. Performance of US-FLI for Detecting Fatty Liver

The diagnostic performance of the US-FLI cut-offs to detect different degrees of steatosis on histology was tested in the whole sample (Tables 2 and S1). US-FLI ≥2 (i.e., the presence of liver–kidney contrast) showed the best diagnostic performance for detecting steatosis extent ≥10% (sensitivity 90.1%, specificity 90%), while US-FLI ≥3 showed the best diagnostic

Table 1 – Characteristics of the study population.

	All (*n* = 352)	HCV (*n* = 173)	HBV (*n* = 23)	NAFLD (*n* = 123)	Others (*n* = 33)
Biometrics					
Age (years)	47.7 ± 11.6	47.9 ± 11.6	45.9 ± 9.5	46.8 ± 12.0	51.2 ± 10.6
Sex (M/F)	220/132	106/67	16/7	89/34	9/24
WC (cm)	96.6 ± 12.7	90.9 ± 9.8	91.1 ± 11.8	101.7 ± 11.6	92.4 ± 17.4
Hypertension (%)	103 (29.3)	44 (25.4)	6 (26.1)	43 (35.0)	10 (30.3)
BMI (kg/m^2)	27.3 ± 4.6	26.0 ± 3.8	25.7 ± 4.6	29.5 ± 4.4	26.4 ± 6.0
Diabetes (%)	57 (16.2)	13 (7.5)	2 (8.7)	36 (29.3)	6 (18.2)
MetS (%)	92 (26.1)	19 (11.0)	4 (17.4)	62 (50.4)	7 (21.2)
Laboratory					
Platelets (×10^3/mm^3)	211.5 ± 61.5	204.3 ± 61.3	210.5 ± 70.2	222.9 ± 59.7	202.7 ± 58.5
Glucose (mg/dL)	95.0 (87.0÷103.0)	93.0 (84.0÷100.0)	93.0 (85.5÷99.5)	97.0 (90.0÷116.0)	95.0 (84.5÷124.8)
Insulin (mIU/L)	10.8 (6.9÷15.8)	9.7 (6.7÷15.9)	10.1 (8.7÷16.2)	11.5 (7.6÷15.7)	9.1 (6.8÷20.0)
HOMA-IR	2.5 (1.5÷4.0)	2.4 (1.5÷3.8)	2.2 (1.4÷2.4)	2.7 (1.7÷ 4.3)	2.0 (1.2÷3.5)
AST (U/L)	40.0 (28.6÷59.0)	46.0 (34.9÷71.0)	28.0 (24.5–39.5)	31.5 (26.0÷49.5)	49.0 (31.0÷86.5)
ALT (U/L)	65.0 (42.0÷110.0)	77.0 (48.0÷132.0)	38.5 (29.3–65.8)	61.0 (41.0÷92.0)	65.5 (42.3÷111.8)
GGT (U/L)	44.3 (27.4÷88.5)	40.0 (25.7÷69.8)	26.0 (16.0÷40.5)	50.0 (32.5÷110.0)	127.0 (70.0÷193.0)
T-BIL (mg/dL)	0.8 (0.6÷1.0)	0.8 (0.6÷1.0)	0.9 (0.7÷1.1)	0.8 (0.6÷1.1)	0.9 (0.7÷1.6)
D-BIL (mg/dL)	0.3 (0.2÷0.3)	0.3 (0.2÷0.3)	0.2 (0.1÷0.3)	0.2 (0.1÷0.3)	0.3 (0.1÷0.3)
TC (mg/dL)	188.5 ± 45.4	171.9 ± 36.5	185.1 ± 51.1	209.1 ± 45.8	197.6 ± 50.4
HDL-C (mg/dL)	46.5 ± 14.1	47.8 ± 14.6	48.4 ± 13.6	44.8 ± 13.3	48.2 ± 17.5
LDL-C (mg/dL)	120.6 ± 39.8	101.3 ± 30.3	111.3 ± 50.0	136.3 ± 39.2	124.3 ± 34.9
TG (mg/dL)	100.0 (70.0÷148.0)	82.0 (62.0÷113.0)	86.0 (58.0÷122.5)	144.5 (91.0÷222.3)	101.0 (68.5÷166.0)
SUA (mg/dL)	5.3 ± 1.4	4.8 ± 1.2	4.9 ± 0.9	6.0 ± 1.4	5.3 ± 1.1
Ferritin (mg/dL)	152.5 (79.0÷257.3)	134.5 (64.5÷264.3)	152.0 (47.0÷179.0)	178.0 (99.5÷279.0)	123.0 (50.5÷364.0)
Ultrasound					
US-FLI ≥2 (%)	197 (56.0)	54 (31.2)	8 (34.8)	118 (95.9)	17 (51.5)
Histology					
Steatosis (%)	226 (64.2)	78 (45.1)	10 (43.5)	123 (100.0)	15 (45.5)
Steatosis extent [a]	38.1 ± 24.8	27.5 ± 22.5	21.3 ± 25.8	45.5 ± 23.5	44.33 ± 22.1
Lobular inflammation	1 (0÷1)	1 (0÷1)	0 (0÷1)	1 (1÷1)	1 (0÷1)
Portal inflammation	1 (1÷2)	2 (1÷2)	1 (1÷1)	1 (1÷1)	1 (1÷2)
Ballooning	0 (0÷1)	0 (0÷1)	0 (0÷0)	1 (0÷1)	0 (0÷1)
Portal fibrosis	1 (1÷2)	1 (1÷2)	1 (1÷1)	1 (0÷1)	1 (1÷2)
Advanced fibrosis (%)	85 (24.1)	59 (34.1)	4 (17.4)	15 (12.2)	7 (21.2)
Cirrhosis (%)	16 (4.5)	8 (4.6)	1 (4.3)	5 (4.1)	2 (6.1)
Grading [b]	–	4 (3÷6)	2 (2÷4)	1 (0÷2)	–
Staging [c]	–	2 (1÷3)	1 (1÷2)	1 (0÷2)	1 (1÷2)

Data are expressed as means (±SD) for continuous variables normally distributed or as medians (25th–75th percentile) for those not normally distributed, and as frequencies (percentages) for categorical variables. ALT: alanine aminotransferases; AST: aspartate aminotransferases; BMI: body mass index; D-BIL: direct bilirubin; GGT; gamma-glutamyltransferase; HBV: hepatitis B virus; HCV: hepatitis C virus; HDL: high-density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; MetS: metabolic syndrome; LDL: low-density lipoprotein; NAFLD: nonalcoholic fatty liver disease; SUA: serum uric acid; T-BIL: total bilirubin; TC: total cholesterol; US-FLI: ultrasonographic fatty liver indicator; WC: waist circumference.

[a] Only in the 226 patients with steatosis.

[b] According to Ishak et al. [27] for patients with HCV or HBV and to Brunt et al. [24] for patients with NAFLD.

[c] According to Ishak et al. [27] for HCV-HBV, to Brunt–Kleiner et al. [24,25] for NAFLD and to Scheuer [26] for the "Others" group.

performance for detecting steatosis extent ≥30% (sensitivity 86.4%, specificity 92.5%) (Table 2). US-FLI ≥ 4 showed the best diagnostic performance for detecting steatosis extent ≥40% (sensitivity 89%, specificity 92.1%) and US-FLI ≥5 for severe (>66%) steatosis extent (sensitivity 88.5%, specificity 87%) (Table S1). The areas under the ROC (AUROC) curves (95% CI) of US-FLI for detecting these different degrees of histological steatosis were high: AUROC 0.934 (0.911–0.958) for steatosis ≥10%, 0.958 (0.939–0.978) for steatosis ≥30% (Fig. 1), 0.965 (0.941–0.982) for steatosis ≥40% and 0.954 (0.932–0.977) for steatosis >66% (Figs. S1 and S2). Furthermore, US-FLI ≥3 had a high specificity for steatosis ≥5–10% (specificity 100%) and ≥20% (specificity 96.8%), while US-FLI ≥ 4 had a high specificity for steatosis ≥20–30% (specificity 99.5–98.1%).

A subgroup analysis was performed to assess the diagnostic performance of US-FLI ≥2 (i.e., liver–kidney contrast positive) for best identifying minimum steatosis percentage threshold in each liver disease group (Table S2, Figs. S3–S7). In patients with HCV, US-FLI ≥2 showed the best diagnostic performance for detecting steatosis ≥20% (sensitivity 92.9%; specificity 88.6%) (AUROC 0.941) while for steatosis ≥10% its sensitivity was lower (76.7%), despite a good specificity (92.9%) (AUROC 0.864) (Table S2, Figs. S3 and S4). In patients with HBV or NAFLD, US-FLI ≥ 2 showed the best diagnostic performance for detecting steatosis ≥10% (sensitivity 85.7%; specificity 87.5%) (AUROC 0.893) and ≥5–10% (sensitivity 95.9–97.5%; specificity 66.7%) (AUROC 0.956), respectively (Table S2, Figs. S5 and S6). In the "Others" group, US-FLI ≥2 showed the best diagnostic

Table 2 – Performance of US-FLI ≥2 (i.e., liver–kidney contrast positive) and US-FLI ≥3 for identifying different histological degrees of steatosis.

Steatosis %	*n*	Se	Sp	PPV	NPV	Acc
US-FLI ≥ 2 (n = 197)						
≥5	226	82.74	92.06	94.93	74.84	86.08
≥10	202	**90.10**	**90.00**	93.91	87.10	**90.06**
≥20	166	95.18	79.03	80.20	94.84	86.65
≥30	140	97.86	71.70	69.54	98.07	82.10
≥40	100	100.00	61.51	50.76	100.00	72.44
≥50	74	100.00	55.76	37.56	100.00	65.06
≥60	62	100.00	53.45	31.47	100.00	61.65
>66	52	100.00	51.67	26.40	100.00	58.81
US-FLI ≥ 3 (n = 137)						
≥5	226	60.62	100.00	100.00	58.61	74.72
≥10	202	67.82	100.00	100.00	69.77	81.53
≥20	166	78.92	96.77	95.62	83.72	88.35
≥30	140	**86.43**	**92.45**	88.32	91.16	**90.06**
≥40	100	96.00	83.73	70.07	98.14	87.22
≥50	74	95.95	76.26	51.53	98.61	80.40
≥60	62	98.39	73.79	44.53	99.54	78.12
>66	52	98.08	71.33	37.23	99.53	75.28

Acc: accuracy; NPV: negative predictive value; PPV: positive predictive value; Se: sensitivity; Sp: specificity; US-FLI: ultrasonographic fatty liver indicator.
Diagnostic performance of other US-FLI cut-off scores is reported in Table S1.

performance for detecting steatosis ≥20% (sensitivity 86.7%; specificity 77.8%) (AUROC 0.896) which was the minimum steatosis extent in that group (Table S2, Fig. S7). However, we point out that these results should be interpreted with some caution, especially in NAFLD patients due to the intrinsic lack of true negative controls (all these patients had steatosis ≥5%) and in HBV and "Others" patients subgroups due to the relatively low number of subjects and the distribution of steatosis percentage in the latter subgroup.

The presence of liver–kidney contrast (mild–moderate to severe) was also observed in 10 out of 352 patients (2.8%) without steatosis on histology: 5 patients with HCV (3 with stage 1–2 and 2 with stage 3 fibrosis), 1 with HBV with stage 1 fibrosis, 1 with systemic sclerosis associated liver disease (stage 1 fibrosis), 1 with autoimmune hepatitis (stage 1 fibrosis) and 2 with undetermined elevated serum liver enzymes without any evidence of liver fibrosis. Conversely, 39 patients out of 352 (17.3%) with any degree of steatosis did not show positive liver–kidney contrast (19 patients with 5–9% steatosis, 12 with 10–15% steatosis, 5 with 20–25% steatosis and 3 with 30–35% steatosis, respectively).

The diagnostic performance of the individual criteria of the US-FLI to detect different degrees of steatosis on histology in the whole sample of patients is shown in Table S3. The presence of marked liver–kidney contrast as well as the posterior attenuation of the ultrasound beam showed the best diagnostic performance for detecting steatosis ≥40%, while difficult visualization of diaphragm and gallbladder wall had the highest accuracy rate in detecting steatosis >66%.

The diagnostic performance of US-FLI ≥2 for detecting steatosis ≥5% (Table S4) and also higher steatosis thresholds (data not shown) was much better as compared to some surrogate steatosis indices, such as fatty liver index (FLI) and lipid accumulation product (LAP) [28,29].

The diagnostic performance of US-FLI to detect steatosis was also evaluated according to serum aminotransferases levels and fibrosis staging in the whole sample of patients (Table S5) and also according to the genotype status in patients with HCV (Table S6). The diagnostic accuracy of US-FLI was not remarkably changed in patients with and without elevated serum aminotransferases. The sensitivity seemed reduced in patients with advanced liver fibrosis. In addition, the sensitivity seemed also increased in genotype 3 HCV patients, however this result should be interpreted with some caution due to the low number of patients.

3.2. *Correlations Between US-FLI and Metabolic and Histological Parameters*

In the whole series US-FLI was positively correlated with WC, BMI, number of MetS features, GGT, fasting glucose, insulin, HOMA-IR, SUA, serum lipids and ferritin, while it was negatively correlated with HDL-C and direct bilirubin (Table 3). The strongest correlations were observed between US-FLI and WC, BMI, SUA and the number of MetS features. Interestingly, US-FLI was correlated with WC, BMI, HOMA and the number of MetS features in all patient groups, with the only exception of number of MetS features in patients with HBV. Moreover, US-FLI was correlated with ALT in patients with HCV and in those with NAFLD.

Regarding the correlations between US-FLI and histological parameters (Table 4), US-FLI was strongly correlated with steatosis extent in the whole sample (Spearman's rho coefficient = 0.883; $p < 0.001$) and in each group of patients. US-FLI was mildly correlated with lobular inflammation severity in all patients (rho = 0.380; $p < 0.001$) and in those with HCV (rho = 0.380; $p < 0.001$), and moderately in those with NAFLD (rho = 0.490; $p < 0.001$). US-FLI was strongly correlated with ballooning degeneration in all patients (rho = 0.619; $p < 0.001$) and in those with HCV (rho =0.615; $p < 0.001$) and moderately in those with NAFLD (rho = 0.485; $p < 0.001$). US-FLI was strongly correlated with the Brunt's inflammatory grading in patients with NAFLD (rho = 0.622; $p < 0.001$) and less with the Ishak's histological criteria (rho = 0.271; $p < 0.001$) in those with HCV. Finally, US-FLI was mildly correlated with portal fibrosis and fibrosis staging in patients with HCV but only weakly in those with NAFLD. However, these results should be interpreted with some caution especially for patients with HBV, due to the relatively low number of these patients.

4. Discussion

The novel findings of this study are that the US-FLI can accurately detect mild–moderate hepatic steatosis (minimal amount 10% on histology) and is significantly correlated with histological and metabolic parameters in liver diseases of different etiology. In particular, US-FLI ≥2 showed the best diagnostic performance to detect the minimum steatosis amount of 10% on histology with a high sensitivity (90.1%) and specificity (90%), while US-FLI ≥3 showed the best sensitivity (86.4%) and specificity (92.5%) for detecting a moderate steatosis ≥30%.

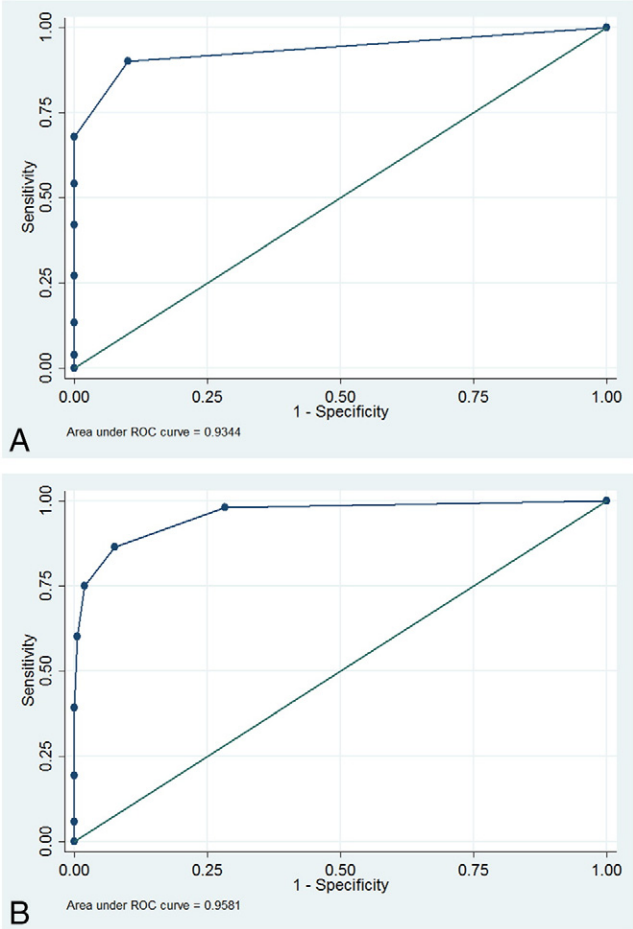

Fig. 1 – ROC curve of US-FLI to best predict minimum liver steatosis percentage thresholds. Panel A. Histological steatosis ≥ 10%. AUROC (95% CI) of US-FLI to detect steatosis ≥10% was 0.934 (0.911–0.958); the best US-FLI cut-off was 2 (sensitivity 90.1%; specificity 90%). Panel B. Histological steatosis ≥ 30%. AUROC (95% CI) of US-FLI to detect steatosis ≥30% was 0.958 (0.939–0.978); the best US-FLI cut-off was 3 (sensitivity 86.4%; specificity 92.5%).

These findings are consistent with the results of a recent meta-analysis by Hernaez et al. [30] who reported that among patients with NAFLD the overall sensitivity and specificity of ultrasonography for the detection of moderate-to-severe hepatic steatosis, compared to histology, were approximately 85% and 95%, respectively. Moreover, in accordance with two studies using semi-quantitative ultrasonographic scores [13,14], but at variance with some previous studies [11,12,31] suggesting that ultrasound was not accurate for detecting steatosis <20–30%, we found that the minimum steatosis threshold for the detection by ultrasound could be lowered to 10%, maintaining an adequate accuracy. Our US-FLI ≥2 showed much higher sensitivity (90.1% vs. 70%) but lower specificity (90% vs. 100%) than the US fatty liver score ≥ 6 proposed by Bril et al. [13], while it showed quite similar sensitivity (90% vs. 91.7%) but lower specificity (90% vs. 100%) than the Hamuguchi's score ≥ 2 [14]. Recently, Dasarathy et al. [12] also reported a higher sensitivity (96.4%) and specificity (97.8%) of the bright liver echo pattern/liver–kidney contrast for detecting steatosis ≥20% compared to those of our US-FLI for steatosis ≥10%, while the sensitivity of US-FLI for steatosis ≥20% was similar. Again, Palmentieri et al. [31] showed a diagnostic performance of the bright liver echo pattern for detecting a 30% steatosis quite similar to that of US-FLI for steatosis ≥10%. Moreover, US-FLI ≥2 had a diagnostic performance for detecting the presence of steatosis of any extent (≥5%) which was better than that found in other studies [31,32].

The high heterogeneity of the study populations might partly account for the wide inter-study variability of the reported specificity. The two studies reporting maximum specificity were conducted one in patients with NAFLD [13] and another one in patients with NAFLD and liver diseases other than viral or alcoholic [14]. Other studies, like the present one, included a mixed liver disease population [12,31,32]. However, Dasarathy et al. [12] reported high sensitivity and specificity of ultrasound only for detecting macrovesicular steatosis, while the accuracy of ultrasound significantly fell for microvesicular steatosis. In our study, the vast majority of patients presented a mixed steatosis pattern. Three published studies that examined the accuracy of ultrasound in predicting steatosis among HCV patients reported a fair number of false positive cases, suggesting that a high necro-inflammatory grading, but not fibrosis stage, might generate a bright liver pattern beside steatosis [33–35]. Conversely, Palmentieri et al. [31] reported only two false positive HCV patients and showed histological steatosis as the strongest predictor of 'bright liver' on ultrasound. In our study, we found five false positive patients with HCV and other five with different liver diseases, almost all with a low level of hepatic inflammation and fibrosis on histology. Nevertheless, in our study a subgroup analysis in patients with HCV (Table S2 and Fig. S3) showed a performance of US-FLI in detecting steatosis that was better than that reported in other ultrasound studies of HCV patients [32–37].

Our study showed for the first time a semi-quantitative ultrasonographic score cut-off (US-FLI ≥5) for the detection of severe (>66%) liver steatosis. The positivity of individual US-FLI criteria may also aid to identify the degree of steatosis: marked liver–kidney contrast and posterior ultrasound beam attenuation are strongly associated to moderate steatosis (≥40%) while difficult diaphragm and gallbladder wall visualization are more strongly associated to severe (>66%) steatosis. Palmentieri et al.[31] previously reported that the posterior attenuation was found exclusively in patients with steatosis ≥30%.

Significant associations between semi-quantitative ultrasonographic steatosis indices and histological/metabolic parameters have been previously reported in patients with NAFLD [9,14,16]. Our study confirms and extends this finding by showing that these associations are valid not only in patients with NAFLD but also in those with other liver diseases. US-FLI is highly correlated with anthropometric parameters and HOMA-IR in each liver disease group, thus highlighting the strong association between fatty liver, obesity and IR across different liver disease etiologies. Moreover, US-FLI is strongly correlated with histological steatosis extent both in all patients and in each group of liver disease. The present study confirmed the existence of strong correlations between US-FLI and histological

Table 3 – Univariate correlations between US-FLI and anthropometric and metabolic parameters.

	US-FLI				
	All (n = 352)	HCV (n = 173)	HBV (n = 23)	NAFLD (n = 123)	Others (n = 33)
WC (cm)	0.536†	0.382**	0.797**	0.355†	0.483*
BMI (kg/m^2)	0.447†	0.257**	0.510*	0.300**	0.424*
Number of MetS traits	0.382†	0.161*	0.380	0.179*	0.399*
Platelets (×10^3/mm^3)	0.109	−0.038	0.438	0.050	-0.060
AST (U/L)	-0.092	0.171*	−0.039	0.159	-0.027
ALT (U/L)	0.053	0.206**	0.067	0.267*	-0.067
GGT (U/L)	0.121*	0.100	0.198	−0.103	-0.030
T-BIL (mg/dL)	0.063	0.179*	0.206	0.086	-0.812**
D-BIL (mg/dL)	−0.151**	−0.106	−0.017	−0.067	−0.143
Glucose (mg/dL)	0.258†	0.115	0.469	0.108	0.323
Insulin (mIU/L)	0.265†	0.128	0.147	0.419†	0.480
HOMA-IR	0.314†	0.240*	0.777*	0.354†	0.587*
SUA (mg/dL)	0.408†	0.161*	−0.248	0.357†	0.057
TC (mg/dL)	0.188**	−0.148	0.308	−0.120	-0.058
HDL-C (mg/dL)	−0.152*	−0.051	0.123	−0.176	-0.442
LDL-C (mg/dL)	0.212**	−0.043	0.444	−0.101	−0.167
TG (mg/dL)	0.297†	−0.029	0.153	0.041	0.161
Ferritin (mg/dL)	0.264†	0.176*	0.177	0.290**	0.450

Values are Spearman's rank correlation coefficients.
ALT: alanine aminotransferase; AST: aspartate aminotransferase; BMI: body mass index; D-BIL: direct bilirubin; GGT: gamma-glutamyltransferase; HBV: hepatitis B virus; HCV: hepatitis C virus; HDL: high-density lipoprotein; HOMA-IR: Homeostasis Model of Assessment-Insulin Resistance; LDL: low-density lipoprotein; MetS: metabolic syndrome; NAFLD: non-alcoholic fatty liver disease; SUA: serum uric acid; T-BIL: total bilirubin; TC: total cholesterol; TG: triglycerides; WC: waist circumference.
* $p < 0.05$.
** $p < 0.01$.
† $p < 0.001$.

parameters of NAFLD (steatosis, lobular inflammation, ballooning, NASH grade and NAS) we also found in our previous study [16]. In addition, a mild correlation between US-FLI and portal fibrosis and fibrosis staging was also found. Interestingly, US-FLI showed a stronger correlation with steatosis percentage than that reported for other ultrasonographic scores [13–15]. US-FLI correlated with hepatic inflammation, ballooning severity and, to a lesser extent, with fibrosis mainly in patients with NAFLD or HCV. A previous study also showed a positive correlation between an ultrasonographic steatosis score and fibrosis severity [15], although the severity of fibrosis does not reflect the echogenicity of liver parenchyma [31,38].

Table 4 – Univariate correlations between US-FLI and features of liver histopathology.

	US-FLI				
	All (n = 352)	HCV (n = 173)	HBV (n = 23)	NAFLD (n = 123)	Others (n = 33)
Steatosis	0.883†	0.754†	0.723†	0.797†	0.805†
Lobular inflammation	0.380†	0.380†	0.102	0.490†	0.228
Ballooning	0.619†	0.615†	–	0.485†	0.183
NAS	–	–	–	0.721†	–
Portal fibrosis	−0.125*	0.278†	−0.336	0.177*	0.059
Inflammatory grading	–	0.271†	0.100	0.622†	–
Fibrosis staging	–	0.311†	0.328	0.185*	0.059

Values are Spearman's rank correlation coefficients.
NAS: Non-Alcoholic Fatty Liver Disease Activity Score.
* $p < 0.05$.
† $p < 0.001$.

The performance of our US-FLI is much better than the surrogate FLI and LAP indexes for detecting steatosis ≥5%: 82.74% sensitivity and 92.06% specificity for our US-FLI vs. 62.00% sensitivity and 85.71% specificity for FLI index ≥60 cut-off [28]; 62.26% sensitivity and 83.72% specificity for LAP score ≥ 40 [29]. The diagnostic performance of these two surrogate indexes of steatosis in our series was quite similar to that found in a previously published study [39].

A recent study showed that a quantitative diagnostic index derived from a computerized analysis on texture, backscattering and attenuation features of ultrasound imaging was able to distinguish severe NAFLD and a normal liver from mild NAFLD, and it was significantly correlated with metabolic risk factors [40]. Evidence also suggests that the ultrasound severity of NAFLD can predict the presence and severity of coronary heart disease as well as the incidence of future cardiovascular events [9,41,42].

Collectively, these findings suggest that US-FLI may help to identify a subgroup of patients who are more likely to develop advanced liver disease (cirrhosis) and future cardiovascular events.

Possible limitations of our study are its single-center, retrospective, cross-sectional design, the relatively low number of patients submitted to liver biopsy for liver diseases other than NAFLD and HCV and the operator dependency of the ultrasound technique, although we demonstrated a very good inter-observer agreement for US-FLI measurements (k statistics 0.81–0.88) [16,23].

In conclusion, our study shows that US-FLI is indeed significantly correlated with both metabolic parameters and histological features of steatogenic liver disease owing to various etiologies. Our findings fully support the use of US-FLI in routine clinical practice as an easy and versatile tool to screen for hepatic steatosis and the metabolic health of patients.

Authors' Contributions

Guarantor of the article: Stefano Ballestri MD, PhD.

Authors contributions: S. B. and A. L. conceived the study design. S. B. analyzed the data. S. B. and F. N. collected the data. S. B., G. T. and A. L. wrote the first draft of the manuscript, and all the authors contributed to the analysis of data and preparation of the final version of the manuscript.

All authors approved the final version of the manuscript.

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Conflict of Interest

The authors do not have any disclosures to report.

Appendix A. Supplementary Data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.metabol.2017.04.003.

REFERENCES

[1] Loria P, Marchesini G, Nascimbeni F, Ballestri S, Maurantonio M, Carubbi F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. Atherosclerosis 2014;232:99–109.

[2] Non-alcoholic fatty liver disease (NAFLD) study group, Lonardo A, Bellentani S, Argo CK, Ballestri S, Byrne CD, Caldwell SH, et al. Epidemiological modifiers of non-alcoholic fatty liver disease: focus on high-risk groups. Dig Liver Dis 2015;47:997–1006.

[3] Fazel Y, Koenig AB, Sayiner M, Goodman ZD, Younossi ZM. Epidemiology and natural history of non-alcoholic fatty liver disease. Metabolism 2016;65:1017–25.

[4] Ballestri S, Zona S, Targher G, Romagnoli D, Baldelli E, Nascimbeni F, et al. Nonalcoholic fatty liver disease is associated with an almost two-fold increased risk of incident type 2 diabetes and metabolic syndrome. Evidence from a systematic review and meta-analysis. J Gastroenterol Hepatol 2016;31:936–44.

[5] Ballestri S, Nascimbeni F, Romagnoli D, Lonardo A. The independent predictors of non-alcoholic steatohepatitis and its individual histological features: insulin resistance, serum uric acid, metabolic syndrome, alanine aminotransferase and serum total cholesterol are a clue to pathogenesis and candidate targets for treatment. Hepatol Res 2016;46:1074–87.

[6] Katsiki N, Mikhailidis DP, Mantzoros CS. Non-alcoholic fatty liver disease and dyslipidemia: an update. Metabolism 2016;65:1109–23.

[7] Lonardo A, Adinolfi LE, Restivo L, Ballestri S, Romagnoli D, Baldelli E, et al. Pathogenesis and significance of hepatitis C virus steatosis: an update on survival strategy of a successful pathogen. World J Gastroenterol 2014;20:7089–103.

[8] Chalasani N, Wilson L, Kleiner DE, Cummings OW, Brunt EM, Unalp A, NASH Clinical Research Network. Relationship of steatosis grade and zonal location to histological features of steatohepatitis in adult patients with non-alcoholic fatty liver disease. J Hepatol 2008;48:829–34.

[9] Ballestri S, Romagnoli D, Nascimbeni F, Francica G, Lonardo A. Role of ultrasound in the diagnosis and treatment of nonalcoholic fatty liver disease and its complications. Expert Rev Gastroenterol Hepatol 2015;9:603–27.

[10] Alkhouri N, Feldstein AE. Noninvasive diagnosis of nonalcoholic fatty liver disease: are we there yet? Metabolism 2016;65:1087–954.

[11] Saadeh S, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, et al. The utility of radiological imaging in nonalcoholic fatty liver disease. Gastroenterology 2002;123:745–50.

[12] 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;51:1061–7.

[13] Bril F, Ortiz-Lopez C, Lomonaco R, Orsak B, Freckleton M, Chintapalli K, et al. Clinical value of liver ultrasound for the diagnosis of nonalcoholic fatty liver disease in overweight and obese patients. Liver Int 2015;35:2139–46.

[14] Hamaguchi M, Kojima T, Itoh Y, Harano Y, Fujii K, Nakajima T, et al. The severity of ultrasonographic findings in nonalcoholic fatty liver disease reflects the metabolic syndrome and visceral fat accumulation. Am J Gastroenterol 2007;102:2708–15.

[15] Liang RJ, Wang HH, Lee WJ, Liew PL, Lin JT, Wu MS. Diagnostic value of ultrasonographic examination for nonalcoholic steatohepatitis in morbidly obese patients undergoing laparoscopic bariatric surgery. Obes Surg 2007;17:45–56.

[16] Ballestri S, Lonardo A, Romagnoli D, Carulli L, Losi L, Day CP, et al. Ultrasonographic fatty liver indicator, a novel score which rules out NASH and is correlated with metabolic parameters in NAFLD. Liver Int 2012;32:1242–52.

[17] Morisco F, Pagliaro L, Caporaso N, Bianco E, Sagliocca L, Fargion S, et al. Consensus recommendations for managing asymptomatic persistent non-virus non-alcohol related elevation of aminotransferase levels: suggestions for diagnostic procedures and monitoring. Dig Liver Dis 2008;40:585–98.

[18] Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D, Modification of Diet in Renal Disease Study Group. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Ann Intern Med 1999;130:461–70.

[19] Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma

glucose and insulin concentrations in man. Diabetologia 1985;28:412–9.
[20] Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. American Diabetes Association: clinical practice recommendations. Diabetes Care 2002;25: S1–S147.
[21] Mancia G, Fagard R, Narkiewicz K, Redon J, Zanchetti A, Böhm M, et al. Guidelines for the management of arterial hypertension: the task force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). Eur Heart J 2013;34:2159–219.
[22] Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JI, Donato KA, et al. Harmonizing the metabolic syndrome: a joint interimstatement 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;120:1640–5.
[23] Ballestri S, Nascimbeni F, Romagnoli D, Lonardo A. Do ultrasonographic semiquantitative indices predict histological changes in NASH irrespective of steatosis extent? Liver Int 2015;35:2340–1.
[24] Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
[25] Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–21.
[26] Scheuer PJ. Classification of chronic viral hepatitis: a need for reassessment. J Hepatol 1991;13:372–4.
[27] Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
[28] 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.
[29] Cuthbertson DJ, Weickert MO, Lythgoe D, Sprung VS, Dobson R, Shoajee-Moradie F, et al. External validation of the fatty liver index and lipid accumulation product indices, using 1H-magnetic resonance spectroscopy, to identify hepatic steatosis in healthy controls and obese, insulin-resistant individuals. Eur J Endocrinol 2014;171:561–9.
[30] Hernaez R, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, et al. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. Hepatology 2011;54:1082–90.
[31] Palmentieri B, de Sio I, La Mura V, Masarone M, Vecchione R, Bruno S, et al. The role of bright liver echo pattern on ultrasound B-mode examination in the diagnosis of liver steatosis. Dig Liver Dis 2006;38:485–9.
[32] Soresi M, Giannitrapani L, Florena AM, La Spada E, Di Gesaro V, Rappa F, et al. Reliability of the bright liver echo pattern in diagnosing steatosis in patients with cryptogenic and HCV-related hypertransaminasaemia. Clin Radiol 2009;64:1181–7.
[33] Hepburn MJ, Vos JA, Fillman EP, Lawitz EJ. The accuracy of the report of hepatic steatosis on ultrasonography in patients infected with hepatitis C in a clinical setting: a retrospective observational study. BMC Gastroenterol 2005;5:14.
[34] Perez NE, Siddiqui FA, Mutchnick MG, Dhar R, Tobi M, Ullah N, et al. Ultrasound diagnosis of fatty liver in patients with chronic liver disease: a retrospective observational study. J Clin Gastroenterol 2007;41:624–9.
[35] Chen CH, Lin ST, Yang CC, Yeh YH, Kuo CL, Nien CK. The accuracy of sonography in predicting steatosis and fibrosis in chronic hepatitis C. Dig Dis Sci 2008;53:1699–706.
[36] Loy M, Serra G, Chessa L. The prevalence of bright liver echo pattern in patients with chronic hepatitis C: correlation with steatosis and fibrosis. J Ultrasound 2014;19:91–8.
[37] Macaluso FS, Maida M, Cammà C, Cabibi D, Alessi N, Cabibbo G, et al. Body mass index and liver stiffness affect accuracy of ultrasonography in detecting steatosis in patients with chronic hepatitis C virus genotype 1 infection. Clin Gastroenterol Hepatol 2014;12:878–84.
[38] Mathiesen UL, Franzen LE, Aselius H, Resjo M, Jacobsson L, Foberg U, et al. Increased liver echogenicity at ultrasound examination reflects degree of steatosis but not of fibrosis in asymptomatic patients with mild/moderate abnormalities of liver transaminases. Dig Liver Dis 2002;34:516–22.
[39] 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;40:1209–22.
[40] Liao YY, Yang KC, Lee MJ, Huang KC, Chen JD, Yeh CK. Multifeature analysis of an ultrasound quantitative diagnostic index for classifying nonalcoholic fatty liver disease. Sci Rep 2016;6:35083.
[41] Targher G, Byrne CD, Lonardo A, Zoppini G, Barbui C. Non-alcoholic fatty liver disease and risk of incident cardiovascular disease: a meta-analysis. J Hepatol 2016;65:589–600.
[42] Lonardo A, Sookoian S, Pirola CJ, Targher G. Non-alcoholic fatty liver disease and risk of cardiovascular disease. Metabolism 2016;65:1136–50.