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A NEW COMBINED NON-INVASIVE METHOD
FOR ASSESSMENT OF LIVER STEATOSIS AND FIBROSIS
IN NAFLD PATIENTS – PILOT STUDY

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Abstract

The aim of this study was to investigate the clinical efficacy (sensitivity, specificity) of ultrasound-based imaging methods for the assessment of fibrosis and steatosis in patients with non-alcoholic fatty liver disease (NAFLD). Sixty-six patients with NAFLD and 43 healthy volunteers (control group) were tested. Liver biopsy was used as a reference method for the evaluation of NAFLD. Liver stiffness measurement (LSM) with transient elastography (TE) and two-dimensional shear wave elastography (2D-SWE) was used to assess fibrosis. Steatosis was assessed by Controlled Attenuation Parameter (CAP) and EchoLevels (ELs). Using the ELs, two hepato-renal indices were calculated – the hepato-renal index difference (HRI_{diff}) and hepato-renal index ratio (HRI_{ratio}). Additionally, one serum biomarker was calculated – APRI. Cut-offs of cirrhosis (F4) were defined as follows: ≥ 9.64 kPa and ≥ 9.85 kPa for 2D-SWE and TE. Only 2D-SWE had a correlation for significant fibrosis – the cut-off was ≥ 6.50 kPa and for lack of significant fibrosis (F_{<2}) less than 5.26 kPa. Steatosis was diagnosed with a cut-off of 240.50 dB/m for CAP, and HRI_{diff} ≥ 0.39 , and HRI_{ratio} ≤ 0.99 for ELs. Both types of SWE and the two methods for evaluation of ultrasound (US) attenuation have good correlation with fibrosis and steatosis in NAFLD.

Key words: CAP, HRI, steatosis, SWE, fibrosis

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Introduction. NAFLD is a leading cause of liver morbidity and mortality. According to World Health Organization data, in 2016, 1.9 billion were overweight and 650 million were obese. From 2000 to 2015, 23.71% of European citizens were affected by NAFLD. For the period 1988–2016, more than 11.9% of liver transplants were indicated by NASH cirrhosis [1,2].

Between 10 and 30% of NAFLD progress to non-alcoholic steatohepatitis (NASH), and 10–15% of those with NASH develop cirrhosis. The prognosis is determined by the degree of fibrosis. The annual risk of hepatocellular carcinoma (HCC) is 2–3%. The most common cause of death in NAFLD is cardiac and NAFLD patients have a three-fold higher rate of cardiac mortality compared to non-NAFLD population. NAFLD is a consequence of metabolic syndrome. In obesity, the prevalence of NAFLD is higher than 95% and NASH is 15–55%. In patients with type 2 diabetes mellitus (T2DM), NAFLD is 33–66% and NASH is 20–80% [3–6]. NAFLD is predominant in men and adults up to 45–50 years [7].

The gold standard for evaluating NAFLD is a liver biopsy. However, the method has some disadvantages: overall rate of complications (20%), major complications (< 1%), mortality (0.0081–0.03 %), sampling error > 20% and inter-observers discordance > 25%. The complications are less when the biopsy is performed under ultrasound guidance [4,5,8–10].

Due to the disadvantages of liver biopsy, non-invasive evaluation of NAFLD is needed. Most studied methods for fibrosis and steatosis are of two types – serum biomarkers and imaging methods. Biomarkers are widely available, easily applicable, and at low cost, but are less sensitive and specific than the imaging methods. There is currently no reliable biomarker for diagnosis, staging, prognosis, follow-up, and response to NAFLD treatment [4,5,8,9].

Non-invasive imaging methods for the assessment of liver fibrosis are ultrasound and magnetic resonance elastography. The second is of the highest accuracy but expensive and of limited clinical use. Ultrasound-based SWE is more applicable and available. Three types of SWE are used to evaluate liver fibrosis – TE, point shear wave elastography (pSWE), and 2D-SWE. SWE measures liver stiffness in kPa and indirectly assesses fibrosis [8,9,11].

The initial step to evaluate steatosis is B-mode US. However, it is subjective, with low sensitivity and specificity for mild steatosis (<10%) [12]. CAP is a non-invasive technology for quantitative assessment of steatosis. It is based on the attenuation of the ultrasound and is part of FibroScan, with measurement range 100–400 dB/m [4,5,8,9]. Another non-invasive quantitative method for assessing steatosis is EL (General Electric). Using ELs, HRIs are calculated. The difference between ELliver - ELkidney is Hepato-Renal Index difference (HRIdiff), and the ELliver/ELkidney division is the Hepato-Renal Index as a Ratio (HRRatio) [13].

The aim of this study was to investigate the clinical efficacy (sensitivity, specificity) of ultrasound-based imaging methods for the assessment of fibrosis and steatosis in patients with NAFLD.

Patients and methods. Sixty-six patients with NAFLD and forty-three healthy volunteers (control group) were studied. All patients signed informed consent for biopsy and non-invasive methods. Both groups had no history of drinking more than 20 and 30 g per day for women and men, respectively.

The control group included healthy individuals aged 36.24 ± 16.03 years, 25 women. Controls were selected according to normal abdominal ultrasound, lack of co-morbidity and medication, negative viral serology (HBV, HCV), BMI < 25 kg/m², normal aminotransferases (AST, ALT), normal cholesterol, triglyceride, uric acid and blood sugar. Liver biopsy was not performed on any of the controls.

The NAFLD group included 66 patients aged 49.08 ± 13.35 years, 47 males. All patients are HBsAg and anti-HCV negative. Biopsy was performed in all patients except four with cirrhosis diagnosed by clinical and laboratory data for portal hypertension – low platelets, enlarged spleen and esophageal varices. The percutaneous biopsy was performed using a Tru-cut automated device under ultrasound guidance and the sample was scored according to SAF scoring system.

APRI was calculated using the following formula: $APRI = (AST \text{ in IU/L}) / (AST \text{ Upper Limit of Normal in IU/L}) / (\text{Platelets in } 10 \text{ G/L})$.

Two types of SWE were used – FibroScan (EchoSens, France) and 2D-SWE (LOGIQ S8, General Electric, USA). We used FibroScan device incorporated in LOGIQ S8, GE – FSMODULE. Liver stiffness was measured in kPa. SWEs were executed by protocol. The elastography was performed in fasting status (at least 4 hours) and after 20 minutes of rest. The patient's position was supine, with elevated and abducted right hand. Measurements were made in the right intercostal space, avoiding vessels. Patients held their breath after exhaling. For 2D-SWE region of interest (ROI) was located 2–6 cm below the capsule. Ten valid measurements were performed for FibroScan and five – for 2D-SWE. The interquartile range (IQR) was below 30%. Figure 1 presents examples of TE and 2D-SWE in cirrhotic patients.

Two methods for quantitative evaluation of ultrasound attenuation were per-

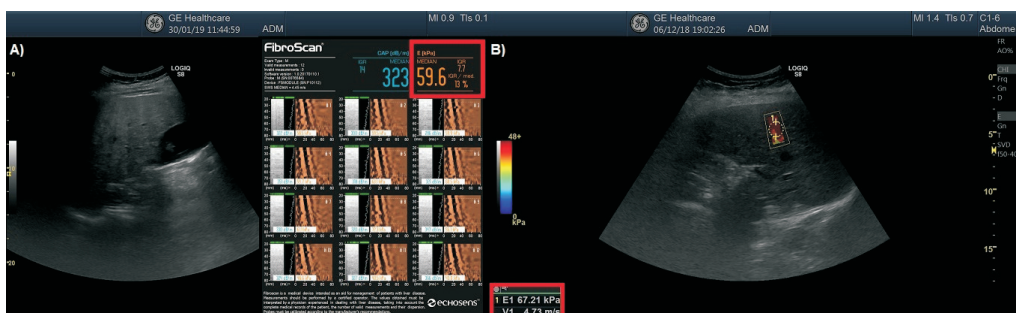


Fig. 1. Liver stiffness of cirrhotic patients with NAFLD – LOGIQ S8, GE, FibroScan (A), 2D-SWE (B)

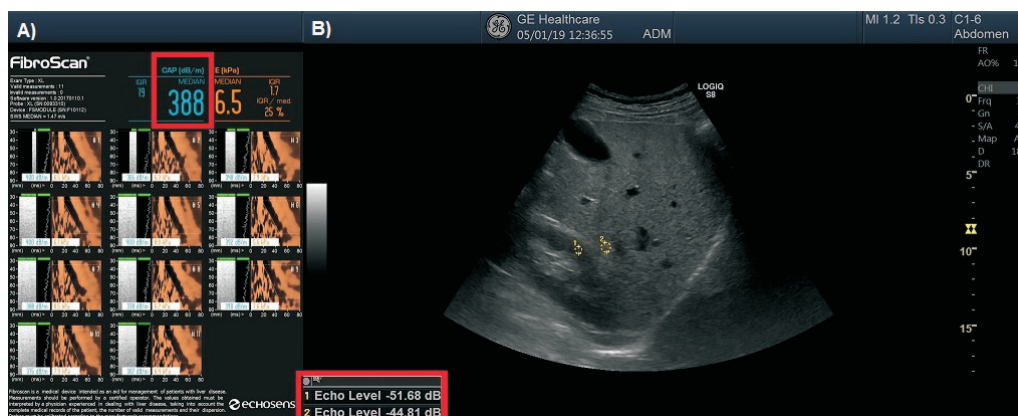


Fig. 2. Liver attenuation measurements in NAFLD group – by CAP (A) and by ELs (B)

formed – CAP and ELs. CAP was measured simultaneously with TE (FibroScan, LOGIQ S8, GE). EL measures the average pixel intensity in the operator-defined ROI. The intensity is higher in fatty liver compared to the parenchyma of the right kidney. EL is measured in dB and is linear in intensity. The zero dB is equal to the maximum intensity, i.e. white with a gray level of 255. The minimum intensity is -99 dB, i.e. black with gray level zero [13]. ELs were measured in the right subcostal space using a convex probe (LOGIQ S8, GE). Measurements were made, within 2 to 7 cm below the liver capsule, at the same depth for the liver and the kidney. The ROIs were approximately 1 cm. HRIdiff and HRIRatio were calculated. Figure 2 shows the measurement of steatosis in NAFLD group by CAP and ELs.

Statistical analysis. The data were analyzed using the IBM SPSS Statistics 19. Descriptive statistics was used. Numerical variables with normal distribution are presented as means \pm standard deviation, while variables with non-normal distribution are presented as median values and range. The Kolmogorov–Smirnov test was used for testing the distribution of numerical variables. Qualitative variables were presented as numbers and percentages. Parametric tests (*t*-tests) were used for the assessment of differences between numerical variables with normal distribution, and nonparametric tests (Mann–Whitney) for variables with non-normal distribution. LSM values were compared and correlated via Wilcoxon signed-rank test. Areas under the receiver-operating characteristic curves (AUROCs) were analyzed to evaluate the cut-offs of APRI, SWEs, CAP, HRIs. The Spearman's correlation was used to test the correlation between non-invasive test and inflammation, ballooning, and steatosis. Ninety-five percent confidence intervals were calculated for each predictive test and $p < 0.05$ was considered significant for each statistical test.

Results. In the NAFLD group, 38.1% had mild steatosis (S1), 28.6% – moderate steatosis (S2), and 33.3% – severe steatosis (S3). Regarding fibrosis,

the patients were staged as follows: F0–58%, F1–6.9%, F2–6.9%, F3–6.9%, F4 (cirrhosis) – 20.7%. No complications occurred after the liver biopsy. None of the cirrhotic patients had ascites. All cirrhotic patients had Child-Pugh A and mean MELD score 8.83 ± 1.72 . Esophageal varices were diagnosed in 83.3% of patients with cirrhosis. 89.6% of patients were obese and 3.4% were overweight. 88.4% of patients had dyslipidemia (34.3% hypertriglyceridemia, 41.2% decreased HDL, 45% took cholesterol-lowering medications), 68% had arterial hypertension, 45.5% had increased waist circumference, 30.5% had T2DM, and HOMA > 2.5 was present in 89.5% of patients without T2DM. In addition, 33.3% had a family history of metabolic disease and 4.8% – of liver disease. 75.5% of patients had hyperuricemia. None of the controls had abnormal metabolic status. The results of NAFLD group and controls are shown in Table 1.

T a b l e 1
Parameters of NAFLD and controls

Parameter	NAFLD group	Control group	<i>p</i> -value
Age (years)	49.08 ± 13.35	36.24 ± 16.03	< 0.0001
Liver size in MCL ¹ (cm)	15.09 ± 1.76	12.78 ± 1.69	< 0.0001
Uric acid (µmol/l)	363.68 ± 86.31	203.60 ± 45.67	< 0.0001
BMI (kg/m ²)	36.32 ± 8.71	21.31 ± 3.62	< 0.0001
HRIdiff	3.34 ± 4.10	-3.79 ± 2.90	< 0.0001
HRIratio	0.93 ± 0.07	1.07 ± 0.06	< 0.0001
CAP (dB/m)	299.18 ± 59.59	190.11 ± 41.53	< 0.0001
AST (IU/l)*	27.00 (16–98)	18.00 (11–29)	< 0.0001
ALT(IU/l)*	41.00 (13–248)	16.00 (10–34)	< 0.0001
APRI*	0.3 (0.1–1.7)	0.2 (0.1–0.4)	0.022
2D-SWE (kPa)*	5.56 (3.8–62.5)	4.54 (3.7–5.7)	< 0.0001
TE (kPa)*	5.10 (2.5–59.6)	3.55 (2.5–5.5)	< 0.0001

¹MCL – midclavicular line *These parameters have abnormal distribution

The Wilcoxon signed-rank test shows that there is no statistically significant difference between the TE and 2D-SWE values.

Using AUROC analysis we defined the cut-off for cirrhosis – TE ≥ 9.20 kPa (Se 50%, Sp 95%), and 2D-SWE ≥ 9.64 kPa (Se 80%, Sp 100%). 2D-SWE had a correlation for significant fibrosis if LSM ≥ 6.50 kPa (AUROC 0.852, Se 75%, Sp 100%). TE did not define significant fibrosis. Cut-off by 2D-SWE for F <2 is 5.26 kPa. APRI ≥ 0.6 defines cirrhosis with 66.7% sensitivity and 96% specificity.

SWEs and APRI do not correlate with inflammation and ballooning, SWEs do not correlate with steatosis. Only APRI shows correlation with steatosis.

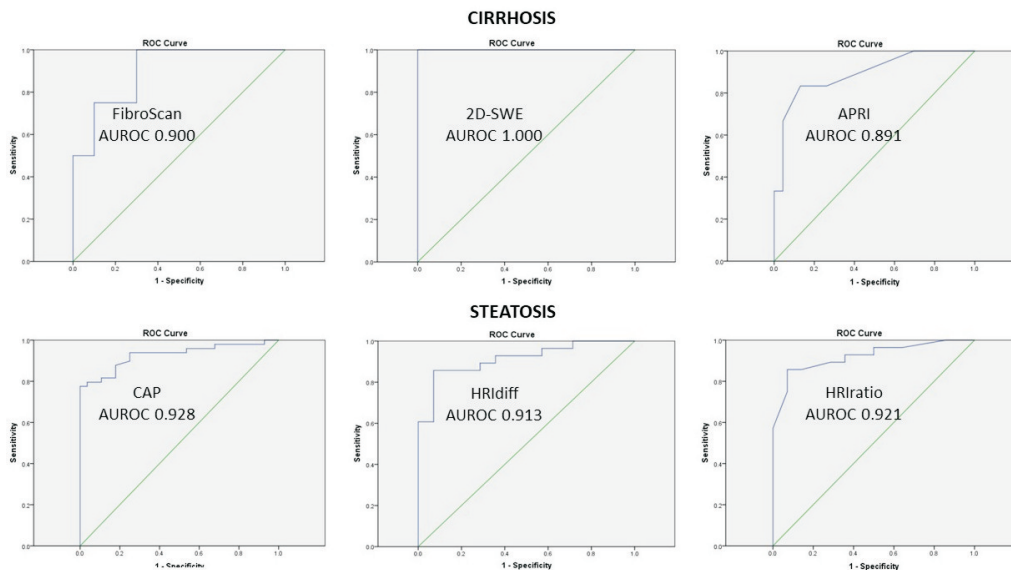


Fig. 3. AUROCs of TE, 2D-SWE, APRI for cirrhosis, and CAP, HRIdiff and HRRatio for steatosis

We found a cut-off for steatosis – CAP ≥ 240.5 dB/m (Se 79.6%, Sp 89.3%), HRIdiff ≥ 0.39 (Se 85.7%, Sp 92.9%) and HRRatio ≤ 0.99 (Se 85.7%, Sp 92.9%). Steatosis can be ruled out by CAP ≤ 213.5 dB/m (Se 75.0%, Sp 93.9%), HRIdiff ≤ -0.5 (Se 85.7%, Sp 85.7%) and HRRatio ≥ 1.01 (Se 85.7%, Sp 85.7%). Figure 3 presents AUROCs of non-invasive methods for the definition of cirrhosis and steatosis.

Discussion. Our study demonstrates good capabilities of the non-invasive methods for evaluation of cirrhosis and steatosis. 2D-SWE has a better correlation with cirrhosis than TE. Both SWEs have higher AUROC than APRI. The three methods for assessment of ultrasound attenuation have a good correlation with steatosis (CAP – AUROC 0.928, HRIdiff – AUROC 0.913, and HRRatio – AUROC 0.921).

CASSINOTTO et al. [14] compares the three types of SWE in patients with NAFLD with similar utility for the diagnosis of severe fibrosis and cirrhosis. The 2D-SWE and TE cut-offs were very similar: 6.3/6.2kPa for $\geq F2$, 8.3/8.2kPa for $\geq F3$, and 10.5/9.5kPa for F4. The cut-offs of 2D-SWE and TE for $F \geq 2$ have a sensitivity $\geq 90\%$ [14]. In our study, we established similar values for cirrhosis. 2D-SWE defines cirrhosis better than significant fibrosis (AUROC 1.000 vs. AUROC 0.852) and TE is not capable of defining significant fibrosis.

A meta-analysis of 11 cohorts tested the CAP and found AUROC 0.85 for $S \geq 1$ ($> 5\%$ fat accumulation), for $S \geq 2$ ($> 33\%$) – 0.88, and for $S \geq 3$ ($> 66\%$) – 0.87. The cut-offs are 232.5 dB/m, 255 dB/m, 290 dB/m for $S \geq 1$, $S \geq 2$ and

$S \geq 3$, respectively [15]. We found a cut-off of 240.5 dB/m for steatosis. Using ELs, we calculated HRIs and defined cut-offs for steatosis. VON VOLKMANN et al. [13] found that HRIdiff was significantly higher in the NAFLD group compared with controls, 6.2 dB (0.8–11.4) vs. 1.9 dB (0.0–6.1), $p = 0.012$. HRIRatio was significantly lower between the same two groups, 0.9 dB (0.8–1.02) vs. 1.01 dB (0.9–1.12), and $p < 0.0001$. Our data show the same results. In the NAFLD group the HRIdiff is positive and significantly higher than controls ($p < 0.0001$), and the HRIRatio is below 1.0 and significantly lower than controls ($p < 0.0001$). Von Volkman et al. show that TE, ELs and liver size had significant differences between NAFLD patients and healthy controls [13].

The group of our NAFLD patients consisted of elderly people, predominantly men, with metabolic syndrome (increased waist circumference $\geq 102/88$ cm, arterial hypertension, T2DM or HOMA ≥ 2.5 , high triglycerides, low HDL). Recently, ESLAM et al. [16] proposed changing the nomenclature and replacing the term NAFLD with MAFLD (Metabolic Associated Fatty Liver Disease). The criteria for this diagnosis are steatosis with at least two metabolic abnormalities.

Our study is a pilot one. Although the cohort of patients is not large, we were able to define cut-offs of liver stiffness and ultrasound attenuation for non-invasive assessment of fibrosis and steatosis in patients with NAFLD. A weakness of our study is the age difference between the two groups. A disadvantage of non-invasive methods for the evaluation of NAFLD is their inability to evaluate ballooning and activity, and still there is no evidence of feasibility of the methods of treatment follow-up [4,5].

Conclusion. In our study, we found that non-invasive methods have good sensitivity and specificity for assessment of fibrosis and steatosis in NAFLD. To our knowledge, our study is the first to present TE, 2D-SWE, CAP, and ELs simultaneously in biopsy-proven NAFLD. In addition, the methods can be repeated several times for their safety and ease of use and could serve to monitor patients and possibly evaluate therapeutic response. Furthermore, integration of 2D-SWE and ELs, and TE and CAP, into one machine allows comprehensive assessment of NAFLD. More clinical studies are needed to prove the diagnostic accuracy of non-invasive methods for evaluation of NAFLD.

REFERENCES

- [1] YOUNOSSI Z. (2018) The Epidemiology of Nonalcoholic Steatohepatitis, *Clinical liver disease*, **11**(4), 92–94.
- [2] PIMPIN L., H. CORTEZ-PINTO, F. NEGRO, E. CORBOULD, J. V. LAZARUS et al. (2018) Burden of liver disease in Europe: Epidemiology and analysis of risk factors to identify prevention policies, *J. Hepatol.*, **69**, 718–735.

- [3] YOUNOSSI Z. M., A. B. KOENIG, D. ABDELATIF, Y. FAZEL, L. HENRY et al. (2016) Global epidemiology of nonalcoholic fatty liver disease – Meta-analytic assessment of prevalence, incidence, and outcomes, *Hepatology*, **64**, 73–84.
- [4] CHALASANI N., Z. YOUNOSSI, J. E. LAVINE, M. CHARLTON, K. CUSI et al. (2018) The Diagnosis and Management of Nonalcoholic Fatty Liver Disease: Practice Guidance from the American Association for the Study of Liver Diseases, *Hepatology*, **67**(1), 328–357.
- [5] MARCHESINI G., CH. P. DAY, J. F. DUFOUR, A. CANBAY, V. NOBILI et al. (2016) EASL–EASD–EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease, *J. Hepatol.*, **64**, 1388–1402.
- [6] BHALA N., R. YOUNES, E. BUGIANESI (2013) Epidemiology and natural history of patients with NAFLD, *Curr. Pharm. Des.*, **19**(29), 5169–5176.
- [7] YOUNOSSI Z. M. (2019) Non-alcoholic fatty liver disease – A global public health perspective, *J. Hepatol.*, **70**(3), 531–544.
- [8] CASTERA L., H. L. CHAN, M. ARRESE, N. AFDHAL, P. BEDOSSA et al. (2015) EASL–ALEH Clinical Practice Guidelines: Non-invasive tests for evaluation of liver disease severity and prognosis, *J. Hepatol.*, **63**(1), 237–264.
- [9] DIETRICH C. F., J. BAMBER, A. BERZIGOTTI, S. BOTA, V. CANTISANI et al. (2017) EFSUMB Guidelines and Recommendations on the Clinical Use of Liver Ultrasound Elastography, Update 2017 (Long Version), *Ultraschall Med.*, **38**(4), 16–47.
- [10] SIDHU P. S., K. BRABRAND, V. CANTISANI, J. M. CORREAS, X. W. CUI et al. (2015) EFSUMB Guidelines on Interventional Ultrasound (INVUS), Part II. Diagnostic Ultrasound-Guided Interventional Procedures (Long Version), *Ultraschall Med.*, **36**(6), 15–35.
- [11] SIGRIST R. M. S., J. LIAU, A. E. KAFFAS, M. C. CHAMMAS, J. K. WILLMANN (2017) Ultrasound Elastography: Review of Techniques and Clinical Applications, *Theranostics*, **7**(5), 1303–1329.
- [12] PIRMOAZEN A. M., A. KHURANA, A. EL KAFFAS, A. KAMAYA (2020) Quantitative ultrasound approaches for diagnosis and monitoring hepatic steatosis in nonalcoholic fatty liver disease, *Theranostics*, **10**(9), 4277–4289.
- [13] VON VOLKMANN H. L., R. F. HAVRE, E. M. LØBERG, T. HAALAND, H. IMMERMVOLL et al. (2013) Quantitative measurement of ultrasound attenuation and Hepato-Renal Index in Non-Alcoholic Fatty Liver Disease, *Med. Ultrason.*, **15**(1), 16–22.
- [14] CASSINOTTO C., J. BOURSIER, V. DE LEDINGHEN, J. LEBIGOT, B. LAPUYADE et al. (2016) Liver stiffness in nonalcoholic fatty liver disease: A comparison of Supersonic Shear Imaging, FibroScan and ARFI with liver biopsy, *Hepatology*, **63**, 1817–1827.
- [15] SHI K. Q., J. Z. TANG, X. L. ZHU, L. YING, D. W. LI et al. (2014) Controlled attenuation parameter for the detection of steatosis severity in chronic liver disease: a meta-analysis of diagnostic accuracy, *J. Gastroenterol. Hepatol.*, **29**, 1149–1158.
- [16] ESLAM M., P. N. NEWSOME, S. K. SARIN, Q. M. ANSTEE, G. TARGHER et al. (2020) A new definition for metabolic associated fatty liver disease: an international expert consensus statement, *J. Hepatol.*, **73**(1), 202–209.

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