DOI:10.33667/2782-4101-2022-2-12-16

NOVEL METHOD FOR QUANTIFYING HEPATIC STEATOSIS IN PATIENTS WITH METABOLICALLY ASSOCIATED FATTY LIVER DISEASE

Alexey Borsukov, Daria Venidiktova, Semen Borsukov

Fundamental research laboratory "Diagnostic researches and minimally invasive technologies" of Smolensk State Medical University, the Ministry of Health of the Russian Federation, Smolensk, Russia

SUMMARY

Introduction. *The article presents an overview of novel complex algorithm based on methods of ultrasound steatometry, combined elastography and dual-energy X-ray absorbtiometry in "Whole body" mode in diagnostic and monitoring of metabolically associated fatty liver disease.*

The purpose of the study: *to assess the possibility of quantitative ultrasound steatometry using in the detection and monitoring of metabolically associated fatty liver disease.*

Material and methods. *157 patients were examined. The main group consisted of 47 patients with liver steatosis; 45 patients with steatohepatitis and clinically insignificant liver fibrosis (F0-F1); 32 patients with steatohepatitis and clinically significant liver fibrosis (F2-F3); 33 patients with focal hepatic steatosis. We used complex algorithm based on methods of questionnaires, laboratory tests, ultrasound steatometry, combined elastography and dual-energy x-ray absorbtiometry in "Whole body" mode, liver biopsy. to determine the severity of steatosis, a scale was used: S0 – no steatosis; <2.19 dB/cm; S1, minimal steatosis, <5% of hepatocytes with steatosis; 2.2–2.29 dB/cm; S2 – moderate steatosis, <6–32% of hepatocytes with steatosis; 2.3–2.9 dB/cm; S3 – severe steatosis, <33–100% of hepatocytes with steatosis; >2.9 dB/cm.*

Results and conclusion. *It is possible to use quantitative ultrasound steatometry for metabolically associated fatty liver disease, as a reference method both for the initial detection of the disease and for monitoring non-drug treatment (sensitivity 90.7%, specificity 92.4%). The optimal complex for the diagnosis and monitoring of non-drug treatment of metabolically associated fatty liver disease includes an assessment of the level of compliance, the use of quantitative ultrasound steatometry and dual-energy X-ray absorptiometry in the «Whole body» mode (sensitivity 92.8%, specificity 92.3%).*

KEYWORDS: *metabolically associated fatty liver disease, ultrasound steatometry, liver biopsy, dual-energy X-ray absorbtiometry.*

CONFLICT of INTEREST. *The authors declare no conflict of interest.*

Funding. *Funding for the study was carried out from the personal funds of the authors.*

Introduction

Currently, the term non-alcoholic fatty liver disease (NAFLD) is understood as an independent nosological unit, the pathogenesis of which is based on the phenomena of insulin resistance and hyperinsulinemia and which includes a spectrum of clinical and morphological changes in the liver parenchyma: hepatic steatosis, steatohepatitis, hepatic fibrosis, hepatic cirrhosis and its complications (including hepatocellular carcinoma) [1]. In 2020, a new adaptive concept was proposed – MAFLD (Metabolically Associated Fatty Liver Disease), which allows emphasizing the systemic and multifactorial nature of the pathogenesis of a unified lesion of the hepatic parenchyma and personalizing the volumes and directions of treatment and diagnostic care in various clinical variants of the disease [2].

In different countries, the incidence of MAFLD varies and averages 6.3–33%, reaching a level of 62–93% in obese patients [2]. In most countries of the world, MAFLD ranks first among liver diseases [3]. These data correspond to the prevalence of the metabolic syndrome and its components [4, 5, 6].

The prognosis of the disease and management tactics are determined primarily by the severity of liver steatosis [7–10], which further affects the timing of the progression of the underlying disease and determines the risk of complications [11–13].

The problem of diagnosing MAFLD remains quite relevant at the present stage of medicine, as well as further treatment and monitoring [14–16]. Consequently, the problem is complex, and therefore requires a meaningful algorithm for early diagnosis [17, 18]. The importance of an adequate and timely assessment of the severity of the pathological process in the liver is beyond doubt: it is necessary in clinical practice to determine the stage, prognosis of the disease and the ability to timely adjust the tactics of treating patients [19, 20]. The use of modern instrumental methods has significantly expanded the amount of information received on the detection rate, periods of early development of liver diseases [21–23].

Ultrasound steatometry is in special, yet little-studied niche in gastroenterology and ultrasound diagnostics, which confirms the growing interest and participation of specialists in various scientific forums dedicated to the possibilities of methods for detecting and assessing MAFLD [24–26]. A general methodological view on the role and place of ultrasonic steatometry in a multidisciplinary hospital has not yet been developed [27, 28].

As MAFLD progresses, the process of pathological changes proceeds sequentially through several stages: from steatosis, stromal inflammatory reaction, stepwise necrosis to the de-

velopment of fibrosis and, in the terminal stage, liver cirrhosis with the possibility of neoplasia in the form of hepatocellular carcinoma [29, 30]. It is known that the assessment of the severity of liver steatosis is important for determining the stage of non-alcoholic fatty liver disease and further prognosis [31].

A reliable method for diagnosing liver pathology is morphological verification, i.e. liver biopsy [32]. Unfortunately, this method is associated with many complications and technical difficulties, so the search for highly informative methods for early, non-invasive diagnosis of liver pathology is constantly being carried out [33]. One of these methods, which allows to assess the severity of liver steatosis, is ultrasound steatometry. This method is based on a quantitative assessment of the attenuation coefficient of an ultrasound wave in tissues in dB/cm (or dB/cm/MHz) and has a number of advantages, such as the possibility of an informative assessment of diffuse and focal changes in the liver in real time, good patient tolerance, no complications, cost-effectiveness [34]. Currently, there is a limited number of works on the use of ultrasound quantitative steatometry in non-alcoholic fatty liver disease, despite the fact that this diagnostic method is promising due to the possibility of non-invasively, repeatedly and quantitatively assessing liver steatosis.

Purpose of the study: to assess the possibility of quantitative ultrasound steatometry using in the detection and monitoring of metabolically associated fatty liver disease.

Material and methods

157 people with metabolically associated fatty liver disease were examined based on the Fundamental Research Laboratory «Diagnostic research and minimally invasive technologies». The main group consisted of 47 patients with liver steatosis (MAFLD); 45 patients with steatohepatitis (MASH) and clinically insignificant liver fibrosis (LF) (F0-F1); 32 patients with steatohepatitis and clinically significant LF (F2-F3); 33 patients with focal hepatic steatosis (FLS). Data on the distribution of patients by sex and age are presented in *Table 1.*

Control group 1 (n=102) – patients with a normal level of adipose tissue in the body according to non-invasive bioimpedancemetry, and not suffering from damage to the liver tissue according to clinical, laboratory and instrumental research methods.

Control group 2 (n=44) – patients with MAFLD, who underwent the entire complex of clinical, laboratory and instrumental procedures used in this study, without including ultrasound quantitative steatometry.

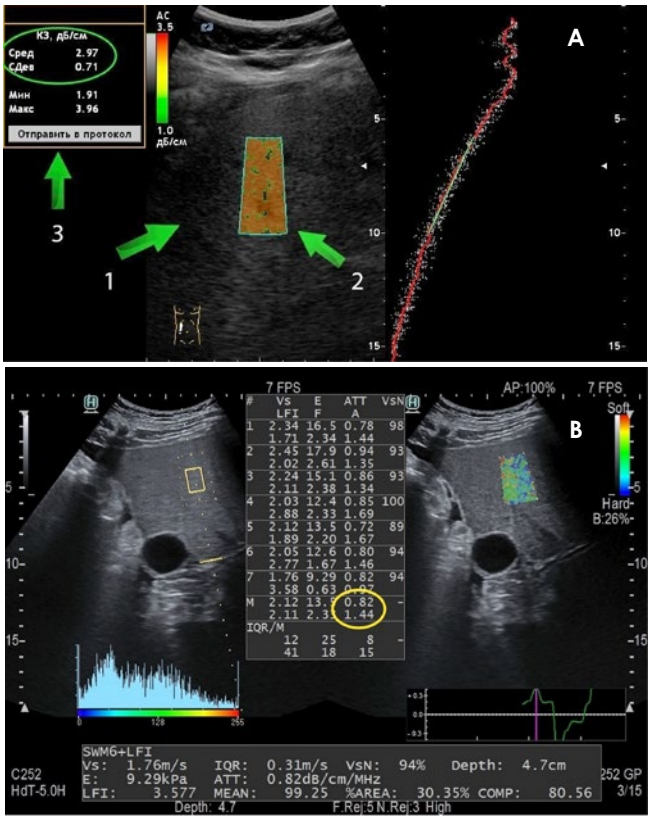

Fig. 1. A. Window of results of ultrasound steatometry: 1 – liver, 2 – active zone of measurement, 3 – short-circuit indicator of the ultrasound wave. B. Window of results of combined elastography: 1 – liver, 2 – active zone of measurement of short-circuit ultrasound wave, 3 – indicator of short-circuit ultrasound wave, quantitative indicator of inflammatory activity (A-index), quantitative indicator of the severity of liver fibrosis (F-index).

The study of patients of the main group (n=157) included:

- Stage 1 – questionnaires (SF-36 V.2., CAGE, AUDIT, DEBQ, CLDQ-NAFLD, IPAQ)
- Stage 2 – clinical and biochemical blood tests (including coagulogram and lipidogram), general urinalysis.
- Stage 3 – liver ultrasound with a quantitative assessment of the ultrasound wave attenuation coefficient (dB/cm), combined elastography of the liver with a quantitative assessment of liver steatosis, activity of the inflammatory process, severity of LF. to determine the severity of steatosis, a scale was used: S0 – no steatosis; <2.19 dB/cm; S1, minimal steatosis, <5% of hepatocytes with steatosis; 2.2–2.29 dB/cm; S2 – moderate steatosis, <6–32% of hepatocytes with steatosis; 2.3–2.9 dB/cm; S3 – severe steatosis, <33–100% of hepatocytes with steatosis; >2.9 dB/cm.

The window of results and the main indicators evaluated during quantitative ultrasound steatometry are shown in *Fig. 1.*

Table 1

Distribution of patients by age and sex

Groups	Total		Men		Women		Average age, year
	n	%	n	%	n	%	
1 gr. MAFLD (n=47)	47	29,9	24	15,3	23	14,6	34,69±1,97
2 gr. MASH with clinically insignificant LF (F0-F1) (n=45)	45	28,7	24	15,3	21	13,4	51,42±1,42
3 gr. MASH with clinically significant LF (F2-F3) (n=32)	32	20,4	18	11,5	14	8,9	49,34±1,91
4 gr. FLS (n=33)	33	21,0	17	10,8	16	10,2	43±2,64
Total (n=157)	157	100	83	52,9	74	47,1	44,62±1,07

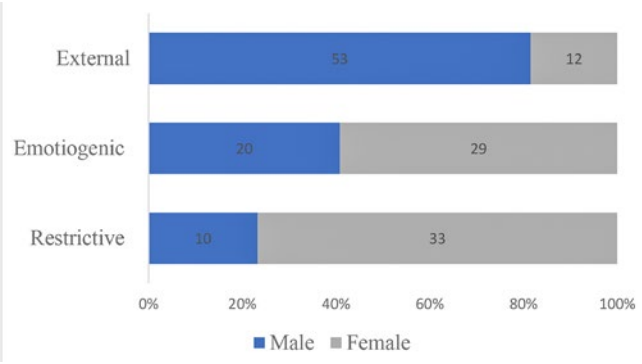

Fig. 2. Distribution of patients according type of eating disorder (DEBQ).

- Stage 4 – determination of complex indices: fatty liver index (FLI), assessment of the state of the liver according to the fibrosis scale in NAFLD (NFS), fibrosis index-4 (FIB-4).
- Stage 5 – dual-energy X-ray absorbtiometry (DXA) in "Whole body" mode.
- Stage 6 – trepan-biopsy of the liver with ultrasound control, with the study of micropreparations on the SAF scale (n=49 (32,7%).

Non-drug measures in all patients were taken according to the general principles of weight loss with its subsequent maintenance: teaching patients a proper lifestyle with a change in eating habits (with the participation of a general practitioner); hypocaloric diet; keeping a food diary; physical exercises.

Statistical processing of the research results was carried out in the Microsoft Excel 2017 database. Data analysis was carried out using descriptive statistics and comparison of samples (using parametric and nonparametric criteria). Pearson rank correlation analysis was used to assess the relationship between two variables. Coefficient r greater than 0 at $p \leq 0.05$ was taken as reliable. Indicators of predictive value (sensitivity and specificity) were determined.

Results

The results of the interpretation of the SF-36 questionnaire at the first visit of patients to the research center showed the results of quality of life slightly below the average levels in the general population of the Russian population. All patients of the control group 1 showed results above the average levels (minimum values for the physical component of health – 79, for the psychological component of health – 72).

The CLDQ-NAFLD (Chronic Liver Disease Questionnaire – Non-Alcoholic Fatty Liver Disease) questionnaire assess the level of quality of life in patients with chronic liver diseases (in particular, NAFLD) on 6 factors: fatigue, anxiety, emotional function, abdominal symptoms, systemic symptoms, activity. Patients of all studied groups assess their physical and psychological state above the average value; high results are noted in groups 1 and 4, which is probably due to the absence of clinical manifestations of NAFLD in most representatives of this group of patients.

The distribution of patients in the main study groups according to the results of DEBQ questionnaire is presented in *Fig. 2*.

In groups of patients 1 and 4, no increase in liver enzymes was recorded. In groups 2 and 3, there was an increase in the level of hepatic transaminases with a predominance of ALT. Additionally, in group 3, there were also changes in the direction of growth of ALT, AST, alkaline phosphatase, total and direct bilirubin.

In patients 1, 2 and 4 of the main study groups, there were no deviations of the coagulogram parameters from normal values. In the majority of patients of the 3rd main study group, at the recruitment stage, a decrease in blood clotting ability (prolongation of prothrombin time) was revealed.

Glucose levels differed significantly in groups 1–4 due to the presence in each of the groups of patients with type 2 diabetes.

In the majority of patients of the main study groups (except for patients of groups 1 and 4), the lipid profile showed a decrease in HDL levels, an increase in TG and LDL. Some of them also had an increase in total cholesterol levels. Increased levels of total cholesterol and LDL have been considered as risk factors for diabetes mellitus and atherosclerosis. a slight increase in triglyceride levels was also detected in patients with type 2 diabetes.

The distribution of patients in the main study groups according to the results of quantitative ultrasound steatometry is presented in *Table 2*.

The coincidence of the data of liver ultrasound quantitative steatometry with the data of combined elastography with the determination of liver steatosis at degrees of liver steatosis S 0 and S 3 was revealed, in the intermediate intervals S 1-S 2 there was a discrepancy in the data per 1 patient in each of the groups. In this group of patients, there was a complete agreement between the data ob-

Table 2

Distribution of patients in the main study groups (n=157) according to the results of quantitative ultrasound steatometry

Liver steatosis (grade)	1 gr. (n=47)		2 gr. (n=45)		3 gr. (n=32)		4 gr.* (n=33)		Total (n=157)	
	n	%	n	%	n	%	n	%	n	%
S 1	27	57,45	18	40	13	40,62	19	57,58	77	49
S 2	13	27,66	16	35,56	10	31,25	13	39,39	52	33,1
S 3	7	14,89	11	24,44	9	28,13	1	3,03	28	17,8

** In group 4 quantitative ultrasound steatometry was performed in a preselected area of interest (in the focus of focal liver steatosis).*

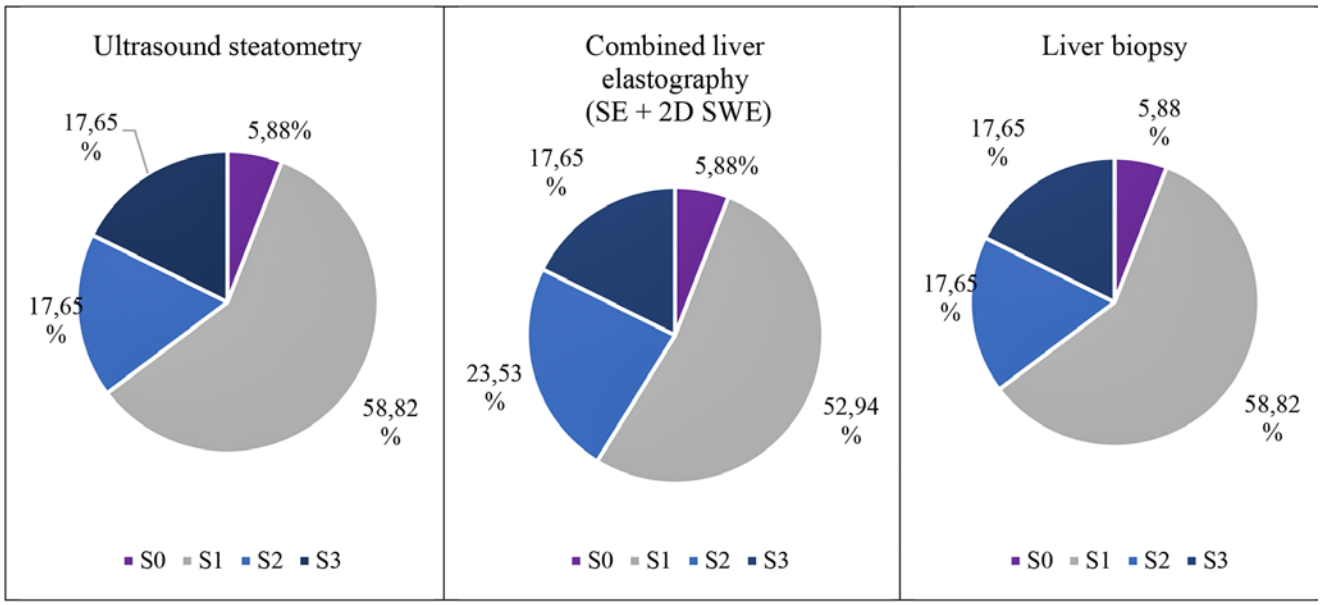

Fig. 3. The ratio of indicators of liver ultrasound steatometry with the determination of the attenuation coefficient of the ultrasound wave (dB/cm), combined elastography with the determination of liver steatosis (dB/cm/MHz) and histological examination of liver biopsy specimens according to the SAF scale in patients (n=17) who agreed to re-biopsy 36 months after the start of the study.

Table 3

Dynamics of changes in the severity of liver steatosis according to the histological examination of liver biopsy specimens (SAF scale) in patients of the main study groups 1–3

	Proportion of patients (%) who agreed to a liver biopsy at baseline (n=49)	Proportion of patients (%) who agreed to a liver biopsy at baseline (n=17)*	Proportion of patients (%) who agreed to a liver biopsy at the end of the study (n=17)
Liver steatosis			
S0	2,04	0	5,88
S1	32,65	29,41	58,82
S2	38,78	41,18	17,65
S3	26,53	29,41	17,65
Activity of the inflammatory process			
A0	32,65	23,53	52,94
A1	14,29	17,65	23,53
A2	24,49	29,41	17,65
A3	16,33	23,53	5,88
A4	12,24	5,88	0
Liver fibrosis			
F0	38,78	35,29	64,71
F1	20,41	23,53	5,88
F2	14,29	23,53	11,76
F3	14,29	11,76	11,76

**The same patients who consented to repeat liver biopsy at the end of the study.*

tained using ultrasound steatometry of the liver with the data obtained from the histological examination of biopsy specimens (*Fig. 3*).

The distribution of the results of liver biopsy of patients who consented to repeat biopsy according to the severity of steatosis (S0-S3), the activity of the inflammatory process (A0-A4) and fibrosis (F0-F3) are presented in *Table 3*.

Based on the results of the primary DXA study, the results shown in *Table 4* were obtained.

Table 4

Distribution of patients of the main group based on the fat mass index according to DXA data in the «Whole Body» mode

Fat mass index	1 gr. (n=47)		2 gr. (n=45)		3 gr. (n=32)		4 gr. (n=33)	
	n	%	n	%	n	%	n	%
Excess	13	27,66	13	28,89	8	25	19	57,58
Obesity 1 grade	16	34,04	18	40	9	28,13	12	36,36
Obesity 2 grade	14	29,79	13	28,89	14	43,75	2	6,06
Obesity 3 grade	4	8,51	1	2,22	1	3,12	0	0

Conclusions

1. It is possible to use quantitative ultrasound steatometry for metabolically associated fatty liver disease, as a reference method both for the initial detection of the disease and for monitoring non-drug treatment (sensitivity 90.7%, specificity 92.4%).
2. The optimal complex for the diagnosis and monitoring of non-drug treatment of metabolically associated fatty liver disease includes an assessment of the level of compliance, the use of quantitative ultrasound steatometry and dual-energy X-ray absorptiometry in the «Whole body» mode (sensitivity 92.8%, specificity 92.3%).

References

1. *Ivashkin VT, Maevskaya MV, Pavlov CS, et al. Clinical guidelines for the diagnosis and treatment of non-alcoholic fatty liver disease of the Russian Society for the Study of the Liver and the Russian Gastroenterological Association. Russian Journal of Gastroenterology, Hepatology, Coloproctology.– 2016.–T. 26.–No. 2.–P. 24–42. (In Russ.).*
2. *Lazebnik LB, Golovanova EV, Turkina SV, et al. Non-alcoholic fatty liver disease in adults: clinic, diagnosis, treatment. Recommendations for therapists, third edition. Experimental and clinical gastroenterology. 2021; 185(1): 4–52. DOI: 10.31146/1682–8658-ecg-185–1–4–52 (In Russ.).*
3. *Zhou, J. Epidemiological Features of NAFLD From1999 to 2018 in China / J. Zhou // Hepatology.– 2020.–V. 71.–No. 5.–P. 1851–1864.*

4. O'Neill, S. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies / S. O'Neill, L. O'Driscoll // Obesity reviews.– 2015.– V. 16.– No. 1.– P. 1–12.
5. Sheveleva MA, Hudyakova NV, Shishkin AN, et al. Non-alcoholic fatty liver disease and metabolic syndrome. Health is the basis of human potential: problems and ways to solve them.– 2019.– T. 14.– No. 2.– P. 632–642. (In Russ.).
6. Godoy-Matos, A. F. NAFLD as a continuum: from obesity to metabolic syndrome and diabetes / A. F. Godoy-Matos, W. S. S. Júnior, C. M. Valerio // Diabetology & Metabolic Syndrome.– 2020.– V. 12.– No. 1.– P. 1–20.
7. DamLarsen, S. Long – term prognosis of fatty liver: risk of chronic liver disease and death / S. DamLarsen, M. Franzmann, V. Andersen // Gut.– 2004.– V. 53, No. 7 – P. 750–755.
8. EASL-ALEH Clinical Practice Guidelines: Noninvasive tests for evaluation of liver disease severity and prognosis // Journal of Hepatology.– 2015.– V. 63 – No. 1.– P. 237–264.
9. McPherson, S. Evidence of NAFLD progression from steatosis to fibrosingsteatohepatitis using paired biopsies: implications for prognosis and clinical management / S. McPherson, T. Hardy, E. Henderson et al. / Journal of Hepatology.– 2015 – V. 62.– P. 1148–1155.
10. Yakovenko MS. Correction of the metabolic syndrome in patients with non-alcoholic fatty liver disease and eating disorders. Attending doctor.– 2020.– No. 2.– P. 23–27. (In Russ.).
11. Dedov II, Melnichenko GA, Shestakova MV, et al. National clinical guidelines for the treatment of morbid obesity in adults. 3rd revision (treatment of morbid obesity in adults). Obesity and metabolism.– 2018.– T. 15.– No. 1.– P. 53–70. (In Russ.).
12. Ahmedov VA. Non-alcoholic fatty liver disease is a dramatic consequence of obesity. Medical alphabet.– 2019.– T. 3.– No. 20.– P. 37–40. (In Russ.).
13. Shelihovskaya PA, Sabirova AI, Mamytova AB, et al. Nephrotic and cerebrovascular risks in non-alcoholic fatty liver disease. The Scientific Heritage.– 2020.– No. 46–3 (46).– P. 71–76. (In Russ.).
14. Kashenko VA, Micinskaya AI, Sokolov AY, et al. Obesity and non-alcoholic fatty liver disease: therapeutic options. Siberian Medical Review.– 2020.– No. 3 (123).– P. 20–29. (In Russ.).
15. Venidiktova DY. The level of patient compliance as the basis for the appointment of personalized non-drug treatment of non-alcoholic fatty liver disease. Practical medicine.– 2021.– T. 19.– No. 1.– P. 96–105. (In Russ.).
16. Zvenigorodskaya LA, Mkrtumyan AM, Shinkin MV, et al. Clinical significance of key components of the adipo-cardiovascular axis in patients with type 2 diabetes mellitus and non-alcoholic fatty liver disease. effective pharmacotherapy.– 2021.– T. 17.– No. 20.– P. 26–36. (In Russ.).
17. Borsukov AV, Venidiktova DY. Evaluation of the comparative effectiveness of methods for instrumental diagnosis of liver steatosis in patients with metabolic syndrome. Practical medicine.– 2018.– T. 16.– No. 2.– P. 16–21. (In Russ.).
18. Zhirkov II, Gordienko AV, Pavlovich IM, et al. Non-invasive diagnostic methods for steatosis in non-alcoholic fatty liver disease. Experimental and clinical gastroenterology.– 2020.– T. 174.– No. 5.– P. 61–66. (In Russ.).
19. Trofimchuk TA, Fedorova TE, Efimenko NV, Muhotin NA. Modern aspects of etiopathogenesis and treatment of non-alcoholic fatty liver disease. Spa medicine.– 2017.– No. 3.– P. 107–115. (In Russ.).
20. Venidiktova DY, Borsukov AV. Ultrasound quantitative liver steatometry in patients with overweight fat: the possibilities of an improved technique. Radiation diagnostics and therapy.– 2020.– T. 11.– No. 1.– P. 64–69. (In Russ.).
21. Pavlov ChS, Kotovich MM. The place of biopsy and morphological examination of liver tissue in children and adults in the clinician's practice. Clinical medicine.– 2007.– T. 85.– No. 9.– P. 72–77. (In Russ.).
22. Maevskaya MV, Ivashkin VT. Liver and nutrition. Optimal diet for non-alcoholic fatty liver disease. Russian journal of gastroenterology, hepatology, coloproctology.– 2018.– T. 28.– No. 5.– P. 105–116. (In Russ.).
23. Tarasova LV, et al. Review of laboratory diagnostic methods used in non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) at the present stage. Experimental and clinical gastroenterology.– 2019.– No. 4 (164).– P. 72–77. (In Russ.).
24. Bakulin IG, Sandler UG, Keyan VA, et al. Assessment of liver steatosis using a non-invasive method: myth or reality? Doctor. RU.– 2015.– No. 12.– P. 57–64. (In Russ.).
25. Karlas, T. Non-invasive assessment of hepatic steatosis in patients with NAFLD using controlled attenuation parameter and 1 H-MR spectroscopy / T. Karlas // PloS one.– 2014.– V. 9.– № 3.– P. 91–98.
26. Pu, K. Diagnostic accuracy of controlled attenuation parameter (CAP) as a non-invasive test for steatosis in suspected non-alcoholic fatty liver disease: a systematic review and meta-analysis / K. Pu // BMC gastroenterology.– 2019.– V. 19.– № 1.– P. 1–11.
27. Cosgrove, D. EFSUMB Guidelines and Recommendations on the Clinical Use of Ultrasound Elastography. Part 2. Applications / D. Cosgrove, J. Bamber, C. F. Dietrich et al. // Ultrashal Med.– 2013 – P. 238–253.
28. Borsukov AV, Morozova TG. Complex elastography in the differential diagnosis of diffuse liver diseases. Scientific notes of the Oryol State University.– 2015.– No. 4.– P. 378–383. (In Russ.).
29. Bedossa, P. Current histological classification of NAFLD: strength and limitations / P. Bedossa // Hepatology International.– 2013.– V. 7.– № 2.– P. 765–770.
30. Byrne, C. D. What's new in NAFLD pathogenesis, biomarkers and treatment? / C. D. Byrne, G. Targher // Nature reviews gastroenterology & hepatology.– 2020.– V. 17.– № 2.– P. 70–71.
31. Barigou M. New trends in non-alcoholic fatty liver diseases (NAFLD) / M. Barigou, L. Favre, M. Fraga, F. Artru // Revue medicale suisse.– 2020.– V. 16.– № 687.– P. 586–591.
32. Gerhardt, F. Biopsy rate and nonalcoholic steatohepatitis (NASH) in patients with nonalcoholic fatty liver disease (NAFLD) / F. Gerhardt // Scandinavian Journal of Gastroenterology.– 2020.– P. 1–6.
33. Falck-Ytter, Y. The risks of percutaneous liver biopsy / Y. Falck-Ytter, A. J. McCullough, J. F. Cadranel et al. // Journal of Hepatology.– 2001.– V. 33.– № 1.– P. 764–764.
34. Venidiktova DY, Borsukov AV, et al. Ultrasound steatometry technique for non-alcoholic fatty liver disease: pilot results. Clinical practice.– 2019.– T. 10.– No. 1.– P. 23–29. (In Russ.).

Received 26.06.2022
Revised 18.07.2022
Accepted 24.07.2022

INFORMATION ABOUT AUTHORS

***Borsukov Alexey Vasilyevich**, MD, Professor, the Head of Fundamental research laboratory "Diagnostic researches and minimally invasive technologies", Smolensk State Medical University, the Ministry of Health of the Russian Federation, 214019, Smolensk, 28 Krupskoy str., ORCID: 0000-0003-4047-7252. Tel. +79036492210; e-mail: bor55@yandex.ru*

***Venidiktova Daria Yuryevna**, Junior scientist of Fundamental research laboratory "Diagnostic researches and minimally invasive technologies", Smolensk State Medical University, the Ministry of Health of the Russian Federation, 214019, Smolensk, 28 Krupskoy str., ORCID: 0000-0001-5497-1476. Tel. +79107850371; e-mail: 92darv@gmail.com*

***Borsukov Semen Alekseevich**, the 5-th grade student of Smolensk State Medical University, the Ministry of Health of the Russian Federation, 214019, Smolensk, 28 Krupskoy str., ORCID: 0000-0002-5747-6048. Tel. +79056953120; e-mail: semen.borsukov99@gmail.com*