https://doi.org/10.46389/rjd-2023-1273
www.rjdnmd.org

Rom J Diabetes Nutr Metab Dis
2023; volume 30, issue 3, pages 351-356

Original Article

The natural course of liver fibrosis in non-alcoholic fatty liver disease and after chronic hepatitis C cure in patients with normal body weight and obesity

Nadiia Gavryliuk [1], Ihor Hospodarskyy [1], Oksana Prokopchuk [1]*, Olha Kozak [1], Lyudmyla Mazur [1], Svitlana Danchak [1]

[1] Department of Higher Nursing Education, Patient Care and Clinical Immunology, I.Horbachevsky Ternopil National Medical University, Ternopil, Ukraine

* Correspondence to: Oksana Prokopchuk, Department of Higher Nursing Education, Patient Care and Clinical Immunology, I.Horbachevsky Ternopil National Medical University, 3A Kotsyubynskyy Street, Ternopil, 46001, Ukraine. E-mail: prokopchuk_oksana@tdmu.edu.ua

Received: 12 December 2022 / Accepted: 18 May 2023

Abstract

Liver cirrhosis and fibrosis is an increasing medical problem all over the world. Several decades ago, hepatitis C infection was the main cause of liver cirrhosis and severe liver fibrosis. After the change of antiviral treatment strategy, we have non-alcoholic fatty liver disease as the main origin factor of liver cirrhosis. After effectively eliminating hepatitis C virus, many patients still have residual post-hepatitis fibrosis. In case of accompanying non-alcoholic fatty liver disease, the natural course of liver lesion is the progress of fibrosis. The presented article highlights the main methods of post-hepatitis liver fibrosis diagnostics after curing viral hepatitis type C and in patients with non-alcoholic fatty liver disease affected by overweight and obesity. We have evaluated fibrosis development with serum markers such as FIB-4, FibroTest and transforming growth factor β1 level. Post-hepatitis liver fibrosis status after elimination of viral hepatitis type C should be observed, especially in patients with non-alcoholic fatty liver disease. FIB-4 and FibroTest correlate with each other and with biochemical liver indices and generally depend on overweight and obesity. TGF-β blood level significantly increases in case of fibrosis progression with maximum values in the group of patients with NAFLD and BMI level above 30 kg/m^2 ($p<0.05$). In the case of liver fibrosis progression, the concentration of TGF-β1 in blood increases ($r=0.78$, $p<0.05$). It confirms the role of this cytokine in the activation of hepatic stellate cells and stimulation of the synthesis of collagen and other extracellular fibrotic components and can be a marker of NAFLD progression.

Keywords: liver fibrosis; NAFLD; overweight; FibroTest; FIB-4; TGF-β1.

Abbreviations: NAFLD – non-alcoholic fatty liver disease; TGF-β1 – transforming growth factor β1; VHC – viral hepatitis type C.

Introduction

Liver cirrhosis and fibrosis are an increasing medical problem worldwide [1]. Several decades ago, hepatitis C infection was the main cause of liver cirrhosis and severe liver fibrosis [2, 3]. After the change of antiviral treatment strategy, we have non-alcoholic fatty liver disease as the main origin factor of liver cirrhosis [4, 5]. However, after effective elimination of hepatitis C virus, many patients still have residual post-hepatitis fibrosis that can regress in some patients [6]. Nevertheless, in case of accompanying non-alcoholic fatty liver disease, the natural course of liver lesion is the progress of fibrosis [6, 7].

Non-alcoholic fatty liver disease (NAFLD) is dramatically increasing worldwide but still has limited treatment options. It is one of the global problems of contemporary medicine in many countries [4–6]. According to

physiology, fibrosis is the universal repairing and therapeutic reaction to liver injury characterized by excessive extracellular matrix protein deposition after the liver injury. Under long-term necrosis or apoptosis of liver cells, their replacement by connective tissue results in the distortion of blood vessel architecture [5, 7, 8]. The end stage of fibrosis is liver cirrhosis.

Liver fibrosis is a significant medical and economic problem in many countries, not only in developed ones, with excess incidence of overweight and obesity, as well as in the countries with high incidence of viral hepatitis [1, 6]. This leads to the spread of non-alcoholic fatty liver disease, where steatosis and steatohepatitis are involved in developing liver cirrhosis and hepatocellular carcinoma and increased mortality from liver disease [7].

Nowadays, we have significant progress in understanding precise molecular mechanisms of liver fibrosis [5, 7]. However, we still have the problem of early diagnostics of liver fibrosis, disease course prediction and effective evidence-based treatment with confirmed antifibrotic effect [8, 9]. Often, but not in all cases, NAFLD and liver fibrosis develop in overweight patients and patients with obesity [9]. Other trigger factors of NAFLD can be type 2 diabetes and polycystic ovary syndrome [9, 10], but a very special case is the NAFLD progression after HCV cure. We have two different models of fibrosis development and a natural history of progression that can be potentiated in combination with obesity [10, 11].

During the last 2 decades, we have passed the impressive way – from liver biopsy as a gold standard to non-invasive biomarkers in evaluating liver fibrosis [10]. Different types of fibrosis stage diagnostics now exist, including laboratory and instrumental ones [11–15]. They have different levels of precision and cost [10, 14, 15]. So, we have used different laboratory markers to estimate liver fibrosis in this investigation.

Our investigation aims to improve the diagnostic strategy of post-hepatitis liver fibrosis after HCV infection cure and in patients with non-alcoholic fatty liver disease and obesity by evaluating fibrosis serum markers.

Material and methods

The investigation was carried out at the Department of Gastroenterology and Hepatology in Ternopil University Hospital. We examined 115 patients with different body mass index. The first group included 56 patients with liver fibrosis without decompensated concomitant pathology and registered cure from HCV infection (2 negative HCV-PCR results after the end of antiviral treatment).

The second group included 59 patients with NAFLD. According to the classification of the WHO International Obesity Group (1997), all patients were divided into subgroups: 18.5–24.9 kg/m^2 – normal body weight; 25–29.9 kg/m^2 – overweight (pre-obesity); 30.0–34.9 kg/m^2 – obesity of the class 1.

Biochemical analysis, including biochemical liver function indices, has been performed. FIB-4 index was calculated. The patients were also examined for serum levels of alpha2-macroglobulin, haptoglobin and apolipoprotein A1 to calculate the FibroTest index for more correct fibrosis stage verification.

In order to interpret FibroTest results and data translation into the fibrosis stage, the most common scale of histological METAVIR indices was used (Table 1).

Table 1: Interpretation of the FibroTest results based on the scale of histological METAVIR indices.

FibroTest	METAVIR stage of fibrosis
0.75–1.00	F4
0.73–0.74	F3–F4
0.59–0.72	F3
0.49–0.58	F2
0.32–0.48	F1-F2
0.28–0.31	F1
0.22–0.27	F0-F1

FIB-4<1.45 value indicated the absence of significant fibrosis (F0-F2 fibrosis), and FIB-4>3.25 value indicated the presence of significant fibrosis (F3-F4 fibrosis).

Additionally, serum transforming growth factor β1 (TGF-β1) level has been evaluated as a predictor of liver fibrosis progression. For TGF-β1 quantitation in blood serum, we used the ELISA analyses on automated enzyme-immunoassay analyzer "MultiskanFC-357" and test system Human TGF β1 Platinum ELISA (BMS249/4 BMS249/4TEN; eBioscience, Austria.

Results and discussion

Group 1 included 30 men with a mean age of 45.84± 2.11 and 29 women with a mean age of 53.52±1.92 years.

https://doi.org/10.46389/rjd-2023-1273

Group 1 has been divided into 3 subgroups according to BMI level:

- 1st – BMI=18.5–24.9 kg/m²;
- 2nd – BMI=25.0–29.9 kg/m²;
- 3rd – BMI=30.0–34.9 kg/m².

Group 2 consists of 29 men with a mean age of 48.67±2.33 and 30 women with a mean age of 51.2±1.75 years. Group 2 has also been divided into 3 subgroups according to BMI level:

- 4th – BMI=18.5–24.9 kg/m²;
- 5th – BMI=25.0–29.9 kg/m²;
- 6th – BMI=30.0–34.9 kg/m².

All groups and subgroups are representative of age and gender.

While using the FIB-4 index in patients with various BMI, we received a significant difference in index value between patients with normal body weight and pre-obesity ($p<0.01$), as well as between normal body weight and obesity class 1 ($p<0.01$), and also between pre-obesity and obesity class 1 ($p<0.01$) (Figure 1, Table 2). Correlation analyses by Spearman rank confirm liver fibrosis progression, calculated by FIB-4 index, with increasing BMI ($r=0.83$, $p<0.05$).

Whereas the average value of FIB-4 was less than 1.45 in patients with normal body weight (Figure 1, Subgroup 1), we can confirm the absence of moderate fibrosis. In patients with pre-obesity and obesity class 1, the average values of FIB-4 were less than 3.25 (Figure 1, Subgroups 2 and 3), confirming the absence of significant fibrosis.

The correlation between FIB-4 and levels of ALT ($r=0.64$, $p<0.05$), AST ($r=0.67$, $p<0.05$), LDL cholesterol ($r=0.28$, $p<0.05$) and platelets ($r=-0.55$, $p<0.05$) was also found.

FibroTest (Fibrotest) is used to evaluate liver fibrosis stages more precisely. FIB-4 and FibroTest as minimally invasive laboratory methods for liver fibrosis diagnosis show a significant difference of test values in patients with normal body weight and pre-obesity ($p<0.01$), normal body weight and obesity class 1 ($p<0.01$), pre-obesity and obesity class 1 ($p<0.01$). A comparative evaluation of FibroTest levels by subgroups is presented in Figure 2.

A significant difference between values of FibroTest in the subgroups relative to BMI and the disease type is shown in Table 3. The same difference between subgroups of the patients was observed in the analysis of

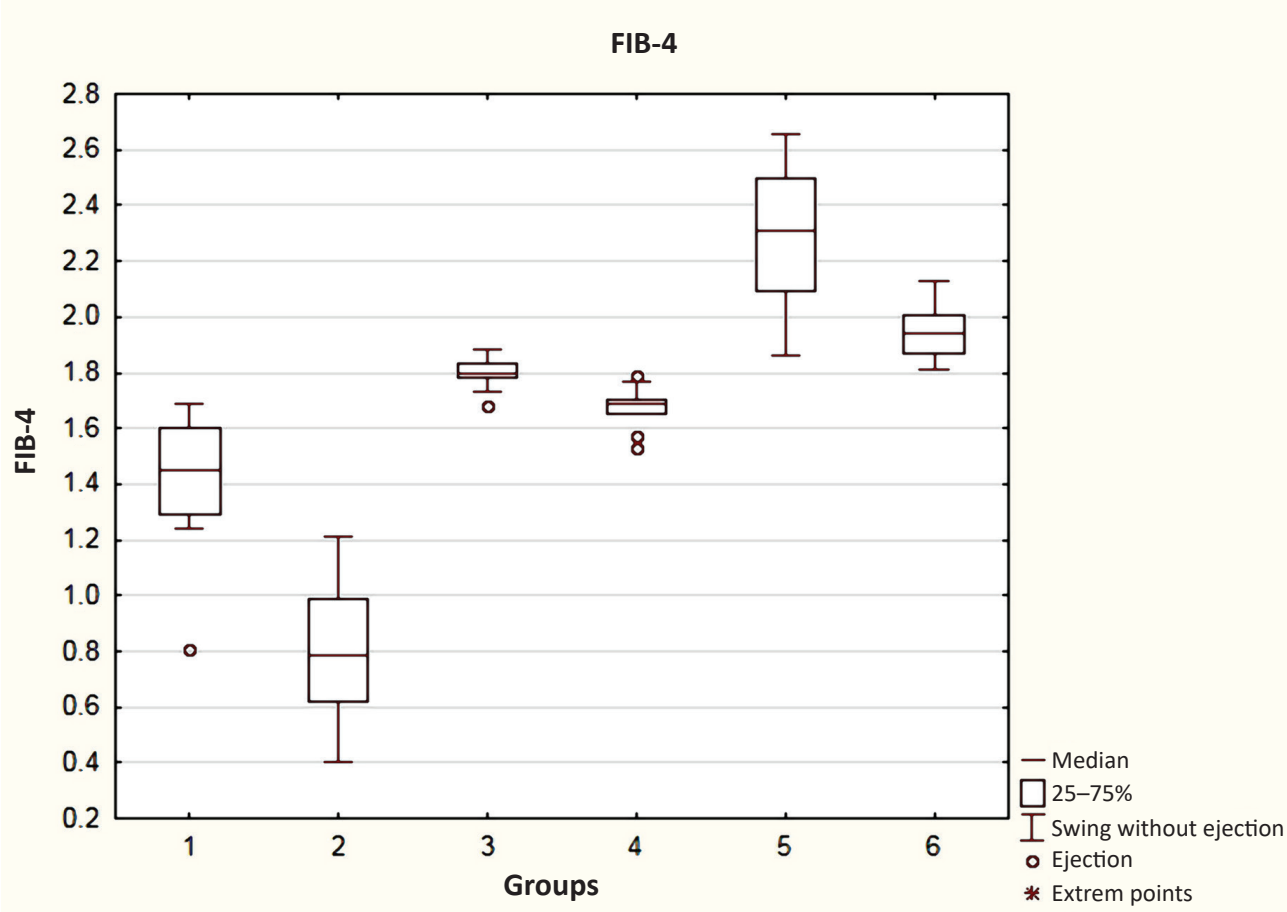

Figure 1: FIB-4 level in subgroups of patients.

Table 2: FIB-4 index in subgroups of patients (M±m).

BMI level	FIB-4		
	Post-hepatic fibrosis	NAFLD	p
18.5–24.9 kg/m²	1.45±0.05	0.81±0.05	<0.01
25.0–29.9 kg/m²	1.82±0.01	1.69±0.01	<0.01
30.0–34.9 kg/m²	2.27±0.05	1.97±0.02	<0.01

Note: Significant difference was calculated by the Kraskel-Wallis's criterion.

Table 3: Fibrotest level in patient subgroups of patients (M±m).

BMI level	FibroTest		
	Post-hepatic fibrosis	NAFLD	p
18.5–24.9 kg/m²	0.31±0.01	0.14±0.01	<0.01
25.0–29.9 kg/m²	0.43±0.03	0.33±0.01	<0.02
30.0–34.9 kg/m²	0.52±0.03	0.49±0.02	>0.05

Note: Significant difference was calculated by the Kraskel-Wallis criterion.

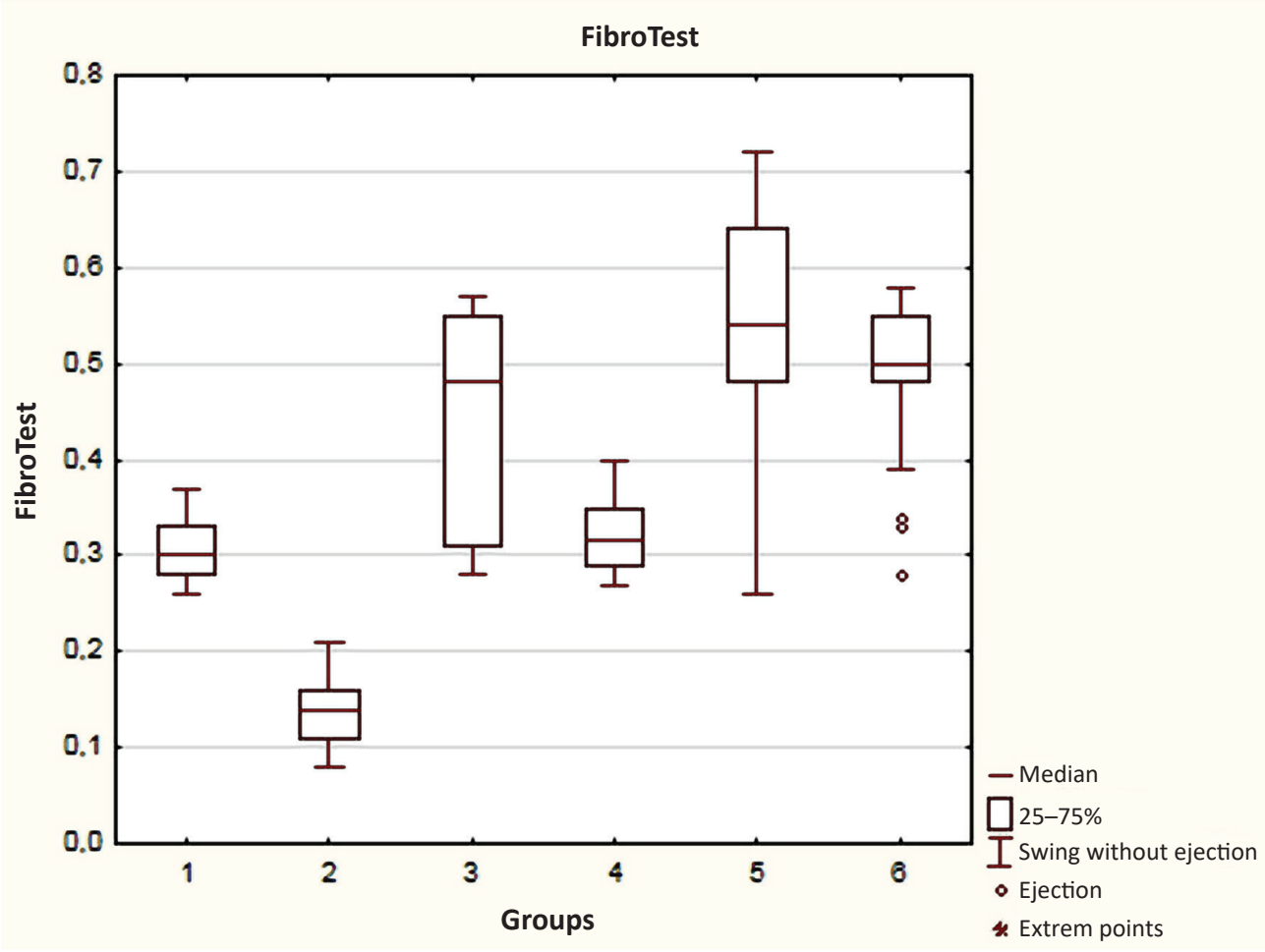

Figure 2: FibroTest level in subgroups of patients.

https://doi.org/10.46389/rjd-2023-1273

Table 4: Comparative characteristics of TGF-β1 (M±m).

Group	TGF-β1, pg/ml		
	Post-hepatic fibrosis	NAFLD	p
BMI 18.5–24.9 kg/m²	12020.76±973.9	8023.33±945.5	0.016*
BMI 25–29.9 kg/m²	14910.70±600.1	12492.83±376.9	0.006*
BMI 30–34.9 kg/m²	20529.60±948.7	18794.40±438.8	0.130

Note: significant difference was calculated by the Kraskel-Wallis's criterion: * – p<0.01.

5 laboratory indices that usually have been used to calculate the FibroTest result, such as alpha-2-macroglobulin, haptoglobin, apolipoprotein A1, bilirubin total and GGTP.

There was not any correlation between the total bilirubin level and body weight. A significant difference was found between patients with normal body weight and obesity class 1 for GGTP blood level (p<0.01). Correlation analysis showed a significant reflection between FibroTest and FIB-4 index (r=0.86, p<0.05). So, performing both methods for gradually analyzing the liver fibrosis stage is recommended.

The FIB-4 index could be used as a cheap and fast evaluation method in the 1st stage of screening to eliminate the patients without significant fibrosis (FIB-4<1.45). The next step of laboratory screening can be FibroTest, a more precise but expensive laboratory method in patients with FIB-4>1.45 and/or concomitant risk factors such as type 2 diabetes, obesity, and family cirrhosis.

The level of TGF-β1 in the blood significantly increased simultaneously with increasing fibrosis stage as well as BMI level. The maximal values of TGF-β1 were in the subgroup of patients 6 – with NAFLD and BMI 30–34.9 kg/m² (p<0.05). With liver fibrosis progression, the concentration of TGF-β1 in blood increases (r=0.78, p<0.05), confirming the role of TGF-β1 in activating hepatic stellate cells and stimulating the synthesis of collagen and other BMA components and is the probable marker of NAFLD progression (Table 4).

The anthropometric data analysis determined direct correlations between BMI and TFG – β1 (r=0.74, p<0.05). A significant relationship was revealed between the level of direct (TFG-β1) and indirect (ALT and AST) markers of fibrosis. Respectively, TFG – β1 with the level of ALT (r=0.59, p<0.05) and with the level of AST (r=0.62 p<0.05).

We found direct correlations of TFG-β with liver fibrosis stage according to the fibrotest (r=0.86, p<0.05) with FIB-4 index (r=0.77, p<0.05). In detailed correlation analysis with FibroTest parameters, a direct relationship between TFG-β and alpha-2-macroglobulin (r=0.66, p<0.05) and apolipoprotein A1 (r=0.37, p<0.05), and feedback with haptoglobulin (r=-0.57, p<0.05) were obtained.

The presence of direct correlations between liver density, TFG – β in patients with posthepatic fibrosis and patients with NAFLD in combination with increased body mass index indicates a mutually precipitating effect of these diseases, which generally contributes to the activation of fibrosis in the liver and is confirmed by literature data [10].

Conclusion

Thus, while verifying liver fibrosis with FIB-4, Fibrotest found that overweight and obesity significantly affect the fibrosis stage regardless of its etiology (p<0.01). However, patients with posthepatic fibrosis indices after HCV-infection elimination were significantly higher than those with NAFLD and BMI 18.5–24.9 kg/m² and BMI=25–29.9 (p<0.01). Analyzing the indices of serum markers for fibrosis diagnostics, such as FIB-4 and FibroTest (Fibrotest), we can state that they correlate with each other and with biochemical parameters of blood and generally complement the dependence of overweight and obesity on the liver fibrosis degree and are confirmed by many authors.

The content of TGF-β in blood significantly increases with the growth of fibrosis stage and BMI with maximum values in the group of patients with NAFLD and BMI 30–34.9 kg/m² (p<0.05). With the liver fibrosis progression, the concentration of TGF-β1 in blood increases (r=0.78, p<0.05), confirming the role of TGF-β1 in the activation of hepatic stellate cells and stimulation of the synthesis of collagen and other extracellular matrix components and is a probable marker of NAFLD progression.

Acknowledgments

The research was carried out within the scientific research work plan of the Department of Higher Nursing Education, Patient Care and Clinical Immunology on the topic – "Improvement of methods of liver fibrosis diagnosis and treatment of digestive diseases of various etiology" (State registration number – 0121U100066).

Conflict of interest

The authors declare no conflict of interest.

Ethics approval

The approval for this study was obtained from the Ethics Committee of the I. Horbachevsky Ternopil National Medical University (approval ID: No.60, September 1, 2020).

Consent to participate

Written informed consent was obtained from the participants.

References

1. Schuppan D, Afdhal NH. Liver cirrhosis. Lancet. 2008; 371: 838–51.
2. Bruguera M, Saiz J, Franco S *et al.* Outbreak of Nosocomial Hepatitis C Virus Infection Resolved by Genetic Analysis of HCV RNA. J Clin Microbiol. 2002 Nov; 40(11): 4363–4366.
3. Andrejczyn, M, Wasyliewa, N, Gospodarski, I. Some aspects of epidemiology of HCV infections in Ukraine. Przegląd epidemiologiczny, 2005, 59(2), 499–501.
4. Asrani SK, Devarbhavi H, Eaton J, Kamath PS. Burden of liver diseases in the world. J Hepatol. 2019; 70: 151– 71.
5. Koyama Y., Brenner D. A. (2017). Liver inflammation and fibrosis. J Clin Invest, 127, 1, 55–64.
6. Rockey DC, Friedman SL Fibrosis regression after eradication of hepatitis C virus - from bench to bedside. Gastroenterology. 2021 Apr; 160(5): 1502–1520.
7. Rockey DC, Bell PD, Hill JA. Fibrosis--a common pathway to organ injury and failure. N Engl J Med 2015;372:1138–49.
8. Rockey DC. Translating an understanding of the pathogenesis of hepatic fibrosis to novel therapies. Clinical gastroenterology and hepatology 2013;11:224–31 e1–5.
9. Harman DJ, Ryder SD, James MW, Wilkes EA, Card TR, Aithal GP, *et al.* Obesity and type 2 diabetes are important risk factors underlying previously undiagnosed cirrhosis in general practice: a cross-sectional study using transient elastography. Aliment Pharmacol Ther. 2018; 47: 504– 15.
10. Heyens, L.J.M.; Busschots, D.; Koek, G.H.; Robaeys, G.; Francque, S. Liver Fibrosis in Non-alcoholic Fatty Liver Disease: From Liver Biopsy to Non-invasive Biomarkers in Diagnosis and Treatment. Front. Med. 2021, 8, 615978.
11. Prokopchuk O, Hospodarskyy I, Kozak O, Gavryliuk N, Danchak S. Possibility of non-invasive diagnostics of liver fibrosis in patients after chemotherapy with normal weight and overweight. Rom J of Diabetes Nutrition and Metabolic Diseases Vol. 29, No 4 (2022):451-456.
12. Serra-Burriel M, Graupera I, Torán P, Thiele M, Roulot D, Wai-Sun Wong V, *et al.* Transient elastography for screening of liver fibrosis: cost-effectiveness analysis from six prospective cohorts in Europe and Asia. J Hepatol. 2019; 71: 1141– 51.
13. EASL-ALEH Clinical Practice Guidelines. Non-invasive tests for evaluation of liver disease severity and prognosis. J Hepatol. 2015; 63: 237– 64.
14. Majundar A, Campos S, Gurusamy K, Pinzani M, Tsochatzis ET. Defining the minimum acceptable diagnostic accuracy of non-invasive fibrosis testing in cirrhosis: a decision analytic modeling study. J Hepatol. 2020; 71: 627– 42.
15. Karsdal MA, Krarup H, Sand JMB, Christensen PB, Gerstoft J, Leeming DJ, *et al.* Review article; the efficacy of biomarkers in chronic fibroproliferative diseases — early diagnosis and prognosis with liver fibrosis as an exemplar. Aliment Pharmacol Ther. 2014; 40: 233– 49.

 https://doi.org/10.46389/rjd-2023-1273