Optimization of early diagnosis of fibrosis in patients with non-alcoholic fatty liver disease with obesity and overweight

**Objective** — to optimize of early diagnosis of fibrosis in patients with NAFLD with obesity and overweight based on ROC analysis of non-invasive laboratory and instrumental diagnostic markers.

**Materials and methods.** The study included 120 patients with NAFLD (main group) with 1—3 degrees of obesity or overweight (BMI \( \geq 25.0 — 39.9 \) kg/m\(^2\)) and 20 practically healthy volunteers (control group) 23.50 [21.35; 24.78] kg/m\(^2\). Patients in the main group were divided into two subgroups depending on BMI. The subgroup I included 85 patients with NAFLD with concomitant obesity, BMI 36.50 [32.00; 40.60] kg/m\(^2\), and the subgroup II included 35 patients with NAFLD and overweight 28.00 [27.10; 29.35] kg/m\(^2\). Determination of the degree of fibrosis according to the META VIR scale by measuring the average stiffness of the liver parenchyma, in the mode of shear wave elastography (ultrasound scanning system Sonoeus P7). The composition of the gut microbiota at the level of the main phylotypes was studied by identifying total bacterial DNA and DNA of Bacteroidetes, Firmicutes, as well as their ratio (F/B ratio), by quantitative real-time polymerase chain reaction using universal primers for the 16S rRNA gene and taxon-specific primers. Statistical processing was performed using Statistica 13.1. The data are presented in the form of Me [LQ; UQ], where Me is the median, LQ and UQ are the lower and upper quartiles, respectively.

**Results.** Determination of the characteristic curves obtained as a result of the analysis allowed us to identify the most informationally significant additional diagnostic indicators: CRP, TNF-\( \alpha \), microRNA-122, microRNA-34a (RU), the percentage of visceral fat, and F/B ratio. The analysis of ROC curves allowed us to determine the cut-off value for each of the additional diagnostic criteria: cut-off value for serum CRP concentration — 4.5 mg/l (AUC = 0.99, sensitivity 1.00, specificity 0.030; p < 0.05); for the serum concentration of TNF-\( \alpha \) — 5.5 pg/mL (AUC = 0.97, sensitivity 0.98, specificity 0.025; p < 0.05). The cut-off value for the serum level of microRNA-122 — 12.50 RU (AUC = 0.99, sensitivity 0.98, specificity 0.048; p < 0.05); for the serum level of microRNA-34a — 5.50 RU (AUC = 0.98, sensitivity 0.98, specificity 0.106; p < 0.05). The cut-off value for the proportion of VF — 8.5% (AUC = 0.99, sensitivity 1.00, specificity 0.175; p < 0.05); for F/B ratio — 1.51 (AUC = 0.95, sensitivity 0.83, specificity 0.021; p < 0.05). The analysis of the studied parameters allowed us to develop an algorithm for early comprehensive diagnosis of fibrosis in patients with NAFLD with obesity and overweight. The algorithm we propose consists of the following 4 stages. Stage I includes assessment of hepatopathy and interviewing the patient to determine complaints and anamnesis to exclude etiologic factors of secondary fatty liver. Stage II includes the determination of cytolysis markers, namely ALT and GGT. Stage III includes the determination of metabolic and proinflammatory parameters: HOMA index; TC and LDL cholesterol; CRP and TNF-\( \alpha \). Stage IV includes the assessment of genetic and biological factors determination of the main phylotypes of the gut microbiota and measurement of serum concentrations of microRNA-122 and microRNA-34a. The algorithm allows screening patients with NAFLD with obesity and overweight and identifying patients with early stages of fibrosis through a phased examination.

**Conclusions.** The generalization of traditional diagnostic algorithms with the results of our search for additional diagnostic criteria made it possible to create a 4-stage algorithm for early comprehensive diagnosis of
According to various sources, the prevalence of non-alcoholic fatty liver disease (NAFLD) reaches more than a quarter of the world's population, and it is projected to spread in parallel with other metabolic diseases [13, 51]. Such a high global prevalence and expected increase in the incidence of the disease pose a serious risk to public health and economic burden for the state, which requires interdisciplinary approaches to control the further growth of this disease [23]. First of all, this is due to the frequent mutually aggravating combination of NAFLD with obesity and type 2 diabetes mellitus, the prevalence of which is also becoming an epidemic. For example, among patients with NAFLD and non-alcoholic statin hepatitis (NASH), 51% and 81% are diagnosed worldwide with obesity, respectively [49], and among obese patients, the prevalence of NAFLD ranges from 60% to 95% [18, 40]. This close connection with metabolic disorders has led to a change in terminology. At the last EASL International Hepatology Congress (2023), it was proposed to replace the term NAFLD with «metabolic dysfunction-associated steatotic liver disease» (MASLD) and the term NASH with «metabolic dysfunction-associated steatohepatitis» (MASH).

It should be noted that each of the stages of NAFLD carries very different risks and consequences for the patient. One of the most difficult is the stage of fibrosis — in patients with NAFLD, it is considered an independent predictor of both overall and liver-related mortality [40]. Therefore, determining the stage of the disease (primarily the presence of fibrosis) in this case will be of vital importance for the patient, both for the treatment plan and for the prognosis, which emphasizes the urgent need for new diagnostic approaches to this multifactorial metabolic disorder. Having an effective diagnostic tool will provide many advantages in monitoring the course of the disease and evaluating the effectiveness of treatment.

Despite the fact that today liver biopsy is considered the gold standard for the diagnosis of NAFLD (as well as many other liver diseases), its use is becoming increasingly limited in routine practice and screening. This is due to the invasiveness of this procedure and the risk of taking biopsy material from an intact area and, accordingly, obtaining a false result [12]. In this regard, the field of non-invasive diagnosis of fibrosis in various liver diseases, including NAFLD, has been developing rapidly in recent years. Some of these methods are based on direct visualization of fibrotic processes — primarily instrumental diagnostics: ultrasound transient elastography, magnetic resonance elastography, etc. These methods are quite effective, but they also require the purchase of very expensive equipment, which also imposes certain limitations on their use, especially in terms of screening [50]. Other methods are based on the assessment of well-known serum biomarkers [42]. For example, the NAFLD Fibrosis Score allows to determine or exclude the presence of progressive fibrosis, however, the limitation of this method is its insufficiently high sensitivity [28, 41]. Another APRI index, the aspartate aminotransferase (AST) to platelet ratio, was proposed as a simple test for determining the stage of fibrosis primarily for patients with chronic hepatitis C [17] and showed good accuracy for prevalent fibrosis in this disease [24], but unfortunately, the results were much less effective in NAFLD [20], so it is not widely used in routine clinical practice [22]. Another well-known method is the Fibrosis Score 4 (FIB-4), which consists of four variables: age, AST, alanine aminotransferase (ALT), and platelet count. This index was developed as a non-invasive panel for detecting progressive fibrosis in patients with viral hepatitis and human immunodeficiency virus [43], and later its effectiveness was proven in patients with NAFLD.

We would like to emphasize that in clinical practice, for the purpose of screening a sufficiently large number of patients, the use of non-invasive serological markers is the most practical and economically feasible and, unlike biopsy, this method will cover the entire array of liver tissue [2]. This makes the search for new non-invasive diagnostic methods even more important around the world.

One of the relatively new promising diagnostic molecules is microRNA-122, which accounts for up to 70% of the total number of all human microRNAs and is the most common and studied molecule in this group [45]. In patients with NAFLD, circulating levels of microRNA-122 correlate with ALT levels and, in comparison with classical markers of liver function, such as aspartate aminotransferase (AST) and cytokeratin-18, this indicator reflects the severity of
NAFLD [16]. Thus, it has been demonstrated that the levels of microRNA-122 were higher in patients with NASH compared to patients with non-alcoholic steatosis and correlated with the degree of inflammation and fibrosis [27]. Other experimental studies have demonstrated changes in the expression of microRNA-122, which increased even in the early stages of NAFLD formation, potentially positioning microRNA-122 as a primary biomarker in the diagnosis of NAFLD [9, 10, 25]. In addition, G. Iacomino et al. studied the nature of changes in the composition of microRNAs circulating in obesity and found an increase in the concentration of microRNA-122, which decreased with weight loss [21].

Another microRNA that is the subject of current research as a marker in the diagnosis of NAFLD is miRNA-34a [44]. F. Wen et al. found changes in the expression of microRNA-34a in liver tissue associated with the formation of liver steatosis [46], and according to the experimental work of G. Yan et al., an increase in the expression of miRNA-34a correlated with the presence of fibrosis [47].

Another promising diagnostic marker in both NAFLD and obesity is the composition of the gut microbiota, in particular its main phylotypes: *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* [31, 39]. However, today, despite certain advances in the development of screening methods for detecting fibrosis using microbiological signatures in patients with NAFLD, including those with obesity comorbidity, many issues remain unresolved and need to be optimized [19, 26].

It should be noted that the use of screening methods based on clinical biomarkers in the form of mono-diagnostics often carries the risk of low sensitivity, which emphasizes the continued relevance of combining existing and new biomarkers with clinical parameters and instrumental methods to improve the efficiency of such methods.

Objective — to optimize of early diagnosis of fibrosis in patients with NAFLD with obesity and overweight based on ROC analysis of non-invasive laboratory and instrumental diagnostic markers.

**Materials and methods**

The study included 120 patients with NAFLD (main group) with 1–3 degrees of obesity or overweight (body mass index (BMI) ≥ 25.0–39.9 kg/m²) and 20 practically healthy volunteers (control group) 23.50 [21.35; 24.78] kg/m². The diagnosis of NAFLD was established by standard methods [1, 4, 6].

For the diagnosis of obesity and classification of its degree, the criteria of the World Health Organization based on the calculated BMI were used [52]. Patients in the main group (n = 120) were divided into two subgroups depending on BMI. The subgroup I included patients with NAFLD with concomitant obesity (grades I–III) — 85 patients, BMI 36.50 [32.00; 40.60] kg/m², and the subgroup II included 35 patients with NAFLD and overweight 28.00 [27.10; 29.35] kg/m².

The groups were reciprocally distributed in terms of age and gender (p > 0.05). The median and interquartile range of the age of patients with NAFLD in the main group was 48.5 [40.00, 59.00] years, and in the control group was 46.30 [35.00, 56.00] years.

The study did not include patients diagnosed with cancer or other factors of fatty liver (consumption of alcohol or other steatogenic drugs, viral infections, hereditary diseases, etc.) Other exclusion criteria were pregnancy and patient refusal at any stage of the study, use of pro/pre or symbiotic drugs within 1–2 months before and on the eve of the study, antibiotic therapy for any disease within the next 2 months, and faecal microbiota transplantation within the last year.

All patients underwent a general clinical examination (analysis of complaints, medical history, life history, objective status) and determination of anthropometric parameters, which included measuring height in meters, determining body weight, followed by BMI calculation and determination of body composition using OMRON BF 514 bio-impedancemetry. The functional state of the liver was also assessed; the concentration of glucose in venous blood samples was determined by photometric method using a general-purpose photometer «Humalyzer 2000» (Germany), the concentration of insulin in the blood serum was determined by enzyme-linked immunosorbent assay using reagent kits manufactured by DRG International Inc. (USA) according to the manufacturer’s instructions. The sensitivity of the assay is up to 1.5 μU/mL. The measurement range was from 1.76 to 100 μU/mL, followed by the calculation of the HOMA index; and the lipid spectrum of blood — total cholesterol (TC) was determined by the enzymatic method using reagent kits manufactured by Human (Germany) on a biochemical analyser Humastar 200 and a general-purpose photometer Humalyzer 2000 (Germany). Low-density lipoprotein cholesterol (LDL-C) was calculated according to the standard formula of W.T. Friedewald.

Determination of the degrees of steatosis according to NAS criterion and fibrosis according to METAVIR scale was performed by determining the real-time function of shear wave elastography/elastometry (Share Wave Elastography Imaging (SWEI)) of liver parenchymal stiffness for...
META VIR fibrosis determination (Soneus P7 ultrasound scanning system).

Determination of the content of highly sensitive C-reactive protein in the blood serum was performed by enzyme-linked immunosorbent assay using CRP-Best reagent kit (Best Diagnostics LLC, Ukraine) according to the manufacturer's instructions.

The concentration of tumour necrosis factor α (TNF-α) in the blood serum was determined by enzyme-linked immunosorbent assay using EIA-TNF-alpha reagent kit according to the manufacturer's instructions. The sensitivity of the assay is up to 1 pg/mL. The measuring range is from 5 to 250 pg/mL.

The content of microRNA-34a and microRNA-122 in blood plasma was determined by reverse transcription and real-time polymerase chain reaction using TaqMan microRNA Assay reagent kits for monitoring and analysing microRNA expression (ThermoFisher Scientific, USA). Small nuclear RNA U6 was used as a reference. Relative content of microRNAs was calculated in relative units (RU) by Delta-delta Ct method.

The composition of the gut microbiota at the level of the main phylotypes was determined by identifying total bacterial DNA and DNA of Bacteroidetes, Firmicutes, as well as their ratio (F/B ratio), by quantitative real-time polymerase chain reaction (qRT-PCR) using universal primers for 16S rRNA gene and taxon-specific primers [15, 16].

The Bioethics Committee of the L. T. Malaya National Institute of Therapy of the NAMS of Ukraine approved this study. Before enrolment, all patients were provided with written information about the purpose and nature of the study, and were informed of the possibility to leave the study at any time without further explanation of their decision. All of the interviewees voluntarily signed an informed consent to participate in this study.

Statistical data processing was performed using the SPSS software package (version 17.0 for Windows; SPSS, Chicago, IL). The exploratory analysis of diagnostic criteria was performed by searching for informationally significant indicators using the apparatus of receiver operating characteristic (ROC) curves with the determination of the value of the diagnostic parameter separating sick and healthy people (cut-off value). The criterion for assessing the accuracy of the test for each of the indicators was the area under the ROC curve, which should be as close to 1 as possible. When finding the point of separation between healthy and sick, the sensitivity of the test was preferred despite its specificity.

**Results**

According to the data obtained, in patients with NAFLD, in both subgroups, statistically significant changes in anthropometric parameters were found compared with the control group. Thus, in patients with concomitant obesity, BMI and waist circumference (WC) were 1.55 times each (p < 0.01) higher than in the control group. A similar picture was observed in overweight patients: BMI was 1.18 times higher (p < 0.01) and WC was 1.32 times higher (p < 0.01) than in the control group. It should be noted that BMI and WC had statistically significant differences between the subgroup I and subgroup II (Fig. 1).

Among the patients in the subgroup I with grade I obesity (n = 38), 47.37% of patients had no signs of fibrosis when assessed by META VIR scale, the same number had stage 1 fibrosis, and 5.26% had...
stage 2 fibrosis. In the patients of the subgroup I with grade II obesity (n = 23), only 34.87 % had no signs of fibrosis, 30.43 % had stage 1 and 34.87 % had stage 2 of fibrosis, and no patients of the subgroup I with grade 1—II obesity had stage 3 fibrosis. In patients of the subgroup I with grade III obesity (n = 24), the results were as follows: only 20.83 % of patients had no signs of liver fibrosis, 37.50 % were diagnosed with stage 1 fibrosis and 33.33 % with stage 2 fibrosis, and stage 3 fibrosis was detected in 8.33 %.

The analysis of the functional state of the liver revealed a statistically significant increase in ALT levels in the subgroup of patients with comorbid obesity by 1.66 times (p < 0.01), AST by 1.15 times and gamma-glutamyl transpeptidase (GGT) by 1.3 times (p < 0.01), compared with the control group. The obtained results indicate the presence of cytolytic syndrome in the examined patients. A similar pattern was observed in the subgroup of overweight patients, but the rates were much lower and were mostly trending. It was noteworthy that the above indicators also differed statistically and significantly between the subgroups (Fig. 2). Further analysis of ALT, AST, and GGT revealed their statistically significant dependence on BMI (H = 36.56; p < 0.01, H = 8.57; p = 0.0138, H = 9.98; p < 0.05, respectively) (see Fig. 2).

In the analysis of lipid profile in patients of both subgroups, a statistically significant increase in LDL-C content was found compared with the control group: 1.80 times (p < 0.01) in the first and 1.65 times (p < 0.01) in the subgroup II, and when compared between subgroups, this indicator was 1.09 times higher in the subgroup I (p < 0.01). The concentration of TC was also statistically different from the control group in the subgroup of patients with comorbid obesity by 1.17 times (p < 0.01) and in the subgroup of overweight patients by 1.11 times (p < 0.01). The data obtained indicate the presence of a sufficiently pronounced dyslipidaemia in the examined patients, which is an aggravating factor.

In addition, patients in both subgroups showed signs of insulin resistance syndrome — HOMA index exceeded the control group by 2.17 times (p < 0.01) for the subgroup I and by 1.6 times (p < 0.01) for the subgroup II.

We also found a statistically significant dependence on BMI of HOMA index, TC, and LDL-C (H = 50.74; p < 0.01, H = 11.02; p = 0.004, H = 26.34; p < 0.01, respectively) (Fig. 3), which emphasizes the association of these indicators with obesity and overweight.

In the study of such a marker of systemic inflammation as CRP, it was found that its highest concentration was observed in patients of the subgroup I and amounted to 7.94 mg/L, which exceeded the control group by 4.87 times (p < 0.01). In the subgroup II, this figure was slightly lower (5.39 mg/mL), but also significantly higher than in the control group (p < 0.01). The presence of such a sufficiently active systemic inflammation will contribute to the progression of the disease and aggravate its course.

The study of microRNA levels showed that the level of microRNA-122 in subgroups I and II was almost 40 times higher than in the control group. Whereas, when comparing between subgroups, no statistically significant differences in this indicator were found (p > 0.01) (Fig. 4).

Regarding the assessment of microRNA-34a level, the results were similar, so in the control group the level of microRNA-34a was 4.08 [3.27; 5.70] RU

**Fig. 2.** Peculiarities of liver function and inflammatory markers in patients with NAFLD with obesity and overweight
and was significantly lower than in the subgroup I by 6.4 times and in the subgroup II by 6.2 times. When comparing the subgroups, no statistically significant differences were found (p > 0.01). MicroRNA-122 and 34a are known to have proinflammatory and profibrotic properties, so their significant increase may reflect the activity of the above processes in the liver (see Fig. 4).

When analysing the main phylotypes of the gut microbiome in patients with NAFLD, statistically significant changes in F/B ratio were found in both patients of the subgroups I and II (according to the Craskell-Wallis test, H = 21.17; p < 0.01) (Fig. 5).

The maximum value of F/B ratio was found in patients of subgroup I, in which this indicator was 3.67 [1.51; 9.40], which was 5.7 times higher than in the control group. A similar situation was observed in patients of subgroup II — F/B ratio 2.75 [1.45; 7.37], which exceeded the control group by 4.3 times (p < 0.01).

Further analysis showed a statistically significant correlation between F/B ratio and the duration of the disease (according to the Craskell-Wallis test: H = 12.41; p < 0.01) (Fig. 6). With increasing duration of the disease, a gradual increase in F/B ratio was observed, reaching maximum values at the disease duration of 5 to 10 years. However, with the disease duration of more than 10 years, value F/B ratio unexpectedly decreased.
It was noteworthy that the increase in the level of microRNA-122 was associated with the increase in F/B ratio in patients of both subgroups I (Spearman’s correlation coefficient \( r = 0.27; p = 0.0117 \)) and II (Spearman’s correlation coefficient \( r = 0.56; p = 0.0003 \)) (Fig. 7). This can be qualified as evidence of the role of the gut microbiota in potentiating proinflammatory and profibrotic processes.

This is confirmed by the positive correlation between the level of microRNA-122 and the serum concentration of TNF-α (Spearman’s correlation coefficient \( r = +0.23; p = 0.0353 \)) in patients of subgroup I (Fig. 8).

A cumulative analysis of all patients in the group I (\( n = 120 \)) revealed a correlation between the relative content of Firmicutes and the serum concentration of CRP (Spearman’s correlation coefficient \( r = +0.25; p = 0.002 \)) and TNF-α (Spearman’s correlation coefficient \( r = +0.17; p = 0.035 \)) (Fig. 9). This also indicates the active involvement of the gut microbiota in proinflammatory processes in patients with NAFLD and overweight or obesity.

In accordance with the aim of the study, we applied multivariate ROC analysis using the above data on diagnostic markers of fibrosis in patients with NAFLD with obesity.

Determination of the characteristic curves obtained as a result of the analysis allowed us to identify the most informationally significant additional
Fig. 10. ROC curves of diagnostic parameters for fibrosis screening in patients with NAFLD with obesity and overweight

- **Serum CRP concentration**
  - AUC = 0.99
  - Sensitivity: 1.00
  - Specificity: 0.03

- **Serum microRNA-34a**
  - AUC = 0.98
  - Sensitivity: 0.980
  - Specificity: 0.106

- **Serum microRNA-122**
  - AUC = 0.99
  - Sensitivity: 0.980
  - Specificity: 0.048

- **Visceral fat**
  - AUC = 0.99
  - Sensitivity: 1.000
  - Specificity: 0.175

- **Firmicutes/Bacteroidetes ratio**
  - AUC = 0.97
  - Sensitivity: 0.98
  - Specificity: 0.025

- **Serum CRP concentration**
  - AUC = 0.99
  - Sensitivity: 0.98
  - Specificity: 0.025

- **Firmicutes/Bacteroidetes ratio**
  - AUC = 0.95
  - Sensitivity: 0.830
  - Specificity: 0.021
The analysis of ROC curves allowed us to determine the cut-off value for each of the additional diagnostic criteria: cut-off value for serum CRP concentration — 4.5 mg/L (p < 0.05); for the serum concentration of TNF-α — 5.5 pg/mL (p < 0.05); the serum level of microRNA-122 — 12.50 RU (p < 0.05); the serum level of microRNA-34a — 5.50 RU (p < 0.05); VF — 8.5 % (p < 0.05); F/B ratio — 1.51 (p < 0.05) (Fig. 10).

The analysis of the studied parameters allowed us to develop an algorithm for early comprehensive diagnosis of fibrosis in patients with NAFLD with obesity and overweight. The algorithm we propose consists of the following 4 stages (Fig. 11).

1. Stage I includes assessment of hepatopathy signs (hepatomegaly, changes in liver structure — increased liver echogenicity, fine-, medium- and coarse-grained liver structure, rounded and/or tuberous contour, smoothed vascular pattern) and interviewing the patient to determine complaints and anamnesis to exclude etiologic factors of secondary hepatic steatosis (alcohol consumption of more than 40 ml of ethanol per day for men and 20 ml for women over the past year, chronic viral and autoimmune hepatitis, toxic hepatitis (including drug-induced hepatitis) and anamnesis of genetically determined diseases: Konovalov-Wilson disease, idiopathic hemochromatosis, congenital α₁-antitrypsin deficiency, etc.) and a mandatory objective examination with percussion/palpation to determine the size of the liver, as well as anthropometric parameters (height, weight with BMI, WC calculations) and body composition according to bioimpedancemetry: the proportion of visceral fat.

The cut-off values for the defined diagnostic indicators are:
- BMI > 25 kg/m²;
- WC > 102 cm for men and > 80 cm for women;
- VF > 8.5 %.

2. Stage II includes the determination of cytolysis markers, namely ALT and GGT:
- ALT > 34 U/L for women and > 45 U/L for men;
- GGT > 39 U/L for women and > 61 U/L for men.

3. Stage III includes the determination of metabolic and proinflammatory parameters: HOMA index; TC and LDL cholesterol; CRP and TNF-α:
- HOMA index > 5.5 mmol/L;
- TC > 4.5 mmol/L;
- LDL cholesterol > 2.5 mmol/L;
- TNF-α > 5.5 pg/mL;
- CRP > 4.5 mg/L.

4. Stage IV includes the determination of genetic and biological factors:
- microRNA-122 > 12.5 RU;
- microRNA-34a > 5.5 RU;
- F/B > 1.51.

The algorithm for early screening of fibrosis in patients with NAFLD with obesity and overweight is as follows:

**Stage I**
- Anthropometric indicators:
  - BMI < 30 kg/m²
  - WC < 80 cm for women
  - WC < 120 cm for men
  - VF < 8.5 %

**Stage II**
- Markers of cytolysis:
  - ALT ≥ 34 U/L for women
  - ALT ≥ 45 U/L for men
  - GGT ≥ 39 U/L for women
  - GGT ≥ 61 U/L for men

**Stage III**
- Metabolic and proinflammatory parameters:
  - HOMA > 5.5
  - TC > 4.5 mmol/L
  - LDL cholesterol > 2.5 mmol/L
  - TNF-α > 5.5 pg/mL
  - CRP > 4.5 mg/L

**Stage IV**
- Genetic and biological factors:
  - microRNA-122 > 12.5 RU
  - microRNA-34a > 5.5 RU
  - F/B > 1.51

**Outcome**
- No fibrosis
- Fibrosis

**Fig. 11. Algorithm for early screening of fibrosis in patients with NAFLD with obesity and overweight**
Stage IV includes the assessment of genetic and biological factors: determination of the main phylogenies of the gut microbiota and measurement of serum concentrations of microRNA-122 and microRNA-34a:

- LDL cholesterol is > 2.5 mmol/L;
- CRP > 4.5 mg/L;
- TNF-α > 5.5 pg/mL.

4. Stage IV includes the assessment of genetic and biological factors: determination of the main phylogenies of the gut microbiota and measurement of serum concentrations of microRNA-122 and microRNA-34a:

- F/B ratio > 1.51;
- microRNA-122 > 12.50 RU;
- microRNA-34a > 5.50 RU.

The algorithm allows screening patients with NAFLD with obesity and overweight and identifying patients with early stages of fibrosis through a phased examination. If at least one of the listed diagnostic criteria is identified at the first stage, the patient is recommended to undergo stage II examinations. If at least one of the indicators is found to be increased at stage II, as well as if the disease has been present for more than 5 years, these patients are recommended to be examined in a specialized institution to determine the presence of fibrosis and its degree using high-tech instrumental methods. If the duration of the disease does not exceed 5 years or if the patient does not have the increase in the concentration of either ALT or GGT during stage II, these patients are recommended to undergo stage III examinations: metabolic and proinflammatory markers. If at least one of the stage III indicators is found to be abnormal, such patients will be recommended to clarify the stage of fibrosis in the specialized institution. Therefore, if patients do not have metabolic disorders and indicators of proinflammatory activation are within the reference values, it is necessary to determine the genetic and biological factors of stage IV NAFLD: an increase in the serum concentration of microRNA-122 > 12.50 RU, and/or microRNA-34a > 5.50 RU, and/or an increase in F/B ratio > 1.51 — also indicates a high diagnostic probability of fibrosis and justifies the referral of these patients to highly specialized institutions to clarify the stage of fibrosis.

Discussion

As noted above, NAFLD has reached epidemic proportions worldwide, and this trend is rapidly escalating, along with the increasing prevalence of obesity and type 2 diabetes [35, 37, 48]. Considering that morbidity and mortality in patients with NAFLD are associated with the development of fibrosis, the identification of patients with early stages of fibrosis formation is crucial [5, 11]. Recent guidelines emphasize the use of non-invasive scales as the first tool for assessing fibrosis in patients with NAFLD [3, 42], which is a reasonable practical approach: they are usually not very expensive, easy to use, and have a high predictive value for excluding severe fibrosis [50]. Of particular value is the step-by-step combination of different markers, as this significantly increases the efficiency of such combined methods. Thus, in a recent large prospective cohort study using a two-stage algorithm (FIB-4 assessment in combination with ELF), 5 times more cases of fibrosis were detected than with each method alone. Accordingly, this will lead to a decrease in the number of visits for secondary care compared to standard diagnostic methods, which provides convincing new evidence of the benefits of the stepwise approach for both patients and doctors [38]. Despite this, in recent years, experts have proposed only a few algorithms and practical guidelines for the stepwise approach to stratifying patients with NAFLD [3, 5, 14]. Information on the implementation of such algorithms in real practice is very scarce and mainly concerns the use of approaches to determining the stage of fibrosis in primary care [33, 38]. Thus, the study of fibrosis biomarkers in patients with morbid obesity was considered in only 6 studies, and it should be noted that only one of them numerous blood-based tests were compared [8, 29, 30, 32, 34, 36]. An informative and promising study was conducted at the Helsinki University Hospital: researchers searched for the most effective biomarkers of progressive fibrosis and fibrosis in NASH in obese patients and studied whether BMI affects the performance of biomarkers. For this purpose, we selected, as in our study, overweight/obese patients who underwent liver biopsy and compared well-known simple combined indicators (FIB-4, NFS, APRI, BARD, HFS) with direct peptide biomarkers of fibrogenesis (PRO-C3) and hepatocellular damage (CK-18 M65/M30), as well as indicators derived from PRO-C3 (ADAPT, FIBC3) and CK-18 (MACK-3). Interestingly, the study found that BMI had a significant impact on the effectiveness of NFS, which necessitated the adjustment of new BMI values. As a result, it was found that the most effective assessments of fibrosis in obese individuals were FIB-4 and ADAPT, both of which were not affected by the degree of obesity [7]. In other words, a consistent combination of FIB-4 with ADAPT or FIBC3 scores that include PRO-C3 can significantly reduce the rate of false-positive diagnoses, potentially reducing screening-related costs and healthcare burden.

In an effort to improve the quality of diagnostic and prognostic measures, we also developed an algorithm that, in addition to standard markers (ALT, AST, GGT, HOMA index, WC, TC, LDL-CUCHASNA ГАСТРОЕНТЕРОЛОГІЯ
cholsterol), included additional most informative diagnostic markers for the presence of fibrosis in patients with NAFLD with obesity or overweight, which were identified by multivariate ROC analysis. They had sufficiently high sensitivity and specificity for liver fibrosis, which allowed us to propose their staged use with the distribution of the algorithm of actions for different levels of medical care.

The list included CRP, TNF-α, microRNA-122, microRNA-34a (RU), VF, and F/B. Using our algorithm will make it easier for first-line doctors to make decisions about further examination or referral to a specialist.

Conflicts of interest: none.

Authorship contributions: conception and design — O. Y. G.; acquisition of data, analysis and interpretation of data — N. I. C.; drafting the article, critical revision of the article — O. Y. G., N. I. C.

References


Conclusions

The generalization of traditional diagnostic algorithms with the results of our search for additional diagnostic criteria made it possible to create a 4-stage algorithm for early comprehensive diagnosis of fibrosis in patients with NAFLD with concomitant obesity or overweight. The use of the developed algorithm allows stratifying patients with NAFLD with obesity and overweight by the presence of fibrosis in the early stages, including by level of care, and timely prescribing preventive and therapeutic measures to reduce the risk of development, improve prognosis and prevent progression.
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Оптимізація ранньої діагностики фіброзу у хворих на неалкогольну жирову хворобу печінки з ожирінням та надлишковою масою тіла

Мета — оптимізувати ранню діагностику фіброзу у хворих на неалкогольну жирову хворобу печінки (НАЖХП) на тлі ожиріння та надлишкової маси тіла за допомогою ROC-аналізу неінвазивних лабораторних та інструментальних діагностичних маркерів.

Матеріали та методи. У дослідження було залучено 120 хворих на НАЖХП на тлі ожиріння 1—3-го ступеня або з надлишковою масою тіла (ІМТ — 25,0 — 39,9 кг/м²) (основна група) та 20 практично здорових добровольців (ІМТ — 23,50 [21,35; 24,78] кг/м²) (контрольна група). Пацієнтів основної групи розподілили на дві підгрупи залежно від величини ІМТ: підгрупа І — 36,50 [32,00; 40,60] кг/м² (n = 85), підгрупа ІІ — 28,00 [27,10; 29,35] кг/м² (n = 35). Визначення ступеня фіброзу за шкалою META VIR проводили за середньою величиною жорсткості паренхіми печінки в режимі зсувнохвильової еластографії (ультразвукова сканувальна система Soneus P7). Склад кишкової мікробіоти на рівні основних філотипів вивчали за допомогою ідентифікації загальної бактеріальної ДНК і ДНК Bacteroidetes та Firmicutes методом кількісної полімеразної ланцюгової реакції в реальному часі з використанням універсальних праймерів для гена 16S рРНК і таксон-специфічних праймерів, а також їхнє співвідношення.

Статистичну обробку проводили за допомогою пакета статистичних програм Statistica 13.1. Дані наведено у вигляді Me [LQ; UQ], де Me — медіана, LQ та UQ — нижній і верхній квартилі відповідно.

Результати. Визначення характеристичних кривих, отриманих внаслідок аналізу, дало змогу виділити найбільш інформаційно значущі додаткові діагностичні показники: вчСРП, чинник некрозу пухлини α (ФНП-α), мікроРНК-122, мікроРНК-34а (відн. од.), відносний вміст вісцерального жиру (ВЖ), співвідношення основних філотипів кишкової мікробіоти Firmicutes/Bacteroidetes (F/B). За допомогою аналізу ROC-кривих визначено cut-off value для кожного з додаткових діагностичних критеріїв: для сироваткової концентрації вчСРП — 4,5 мг/л (AUC = 0,99, чутливість — 1,00, специфічність — 0,030; p < 0,05), ФНП-α — 5,5 пг/мл (AUC = 0,97, чутливість — 0,98, специфічність — 0,025; p < 0,05), мікроРНК-122 — 12,50 відн. од. (AUC = 0,99, чутливість — 0,98, специфічність — 0,048; p < 0,05), мікроРНК-34а — 5,50 відн. од. (AUC = 0,98, чутливість — 0,98, специфічність — 0,106; p < 0,05), ВЖ — 8,5 % (AUC = 0,99, чутливість — 1,00, специфічність — 0,175; p < 0,05), F/B — 1,51 (AUC = 0,95, чутливість — 0,83, специфічність — 0,021; p < 0,05). Розроблено алгоритм ранньої комплексної діагностики фіброзу у хворих на НАЖХП на тлі ожиріння та надлишкової маси тіла із чотирьох етапів. Етап І — оцінка ознак гепатопатії та інтерв'ювання пацієнта з метою визначення скарг і анамнестичних даних для заперечення етіологічних чинників вторинної жирової дистрофії печінки. Етап ІІ — визначення маркерів цитолізу (аланінамінотрансфераза і γ-глутамілтранспептидаза). Етап ІІІ — визначення метаболічних та прозапальних параметрів (індекс НОМА, загальний холестерин, холестерин ліпопротеїнів низької густини, вчСРП, ФНП-α). Етап ІV — оцінювання генетичних і біологічних чинників: визначення основних філотипів кишкової мікробіоти, вимірювання сироваткової концентрації мікроРНК-122 та мікроРНК-34а. Використання алгоритму дає змогу провести скринінг хворих на НАЖХП на тлі ожиріння та надлишкової маси тіла й виявити хворих із ранніми стадіями фіброзу шляхом поетапного обстеження.

Висновки. Узагальнення традиційних діагностичних алгоритмів з визначеннями нами додатковими діагностичними критеріями сприяло розробці 4-етапного алгоритму ранньої комплексної діагностики фіброзу у хворих на НАЖХП при супутньому ожирінні або надлишковій масі тіла. Використання алгоритму дає змогу провести скринінг хворих на НАЖХП на тлі ожиріння та надлишкової маси тіла й виявити хворих із ранніми стадіями фіброзу шляхом поетапного обстеження.

Ключові слова: неалкогольна жирова хвороба печінки, фіброз, кишкова мікробіота, алгоритм.

ДЛЯ ЦИТУВАННЯ
