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CURRENT AND FUTURE APPROACHES TO THE DIAGNOSIS OF NON-ALCOHOLIC FATTY LIVER DISEASE

Parpibaeva D. A.,
Buvamukhamedova N. T.,
Salaeva M. S.,
Boltaboev X. Q.,
Turbanova U. V.
Tashkent Medical Academy, Tashkent, Uzbekistan

ABSTRACT
Nonalcoholic fatty liver disease (NAFLD) is one of the most common chronic liver disease, are prone to a progressive flow. NAFLD is a diagnosis of exclusion, and therefore rational diagnosis of this disease is based on a combination of clinical, laboratory and instrumental data. The article provides an overview of current and prospective methods of diagnosis of NAFLD. The role of biomarkers as a promising non-invasive diagnostic tools for assessing the severity of necroinflammatory and hepatic fibrosis in patients with NAFLD.

Keywords: non-alcoholic fatty liver disease, SFRP4, non-alcoholic steatohepatitis, steatosis, fibrosis of the liver, cirrhosis of the liver.

Introduction

Non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in the world. This nosological group combines a spectrum of pathological conditions, including fatty steatosis (hepatosis), which in most cases has a benign course, and non-alcoholic steatohepatitis (NASH), characterized by the potential for progression to cirrhosis and hepatocellular carcinoma (1, 2). The prevalence of NAFLD is already pandemic, correlating with levels of obesity in the population [3, 4]. It occurs in both adults and children and does not differ significantly by gender. Recent US studies show that NAFLD affects between 20 and 46% of asymptomatic patients [5-7]. Population-based cohort studies in Asian countries have also found a high prevalence of NAFLD in this region, ranging from 12 to 27.3% [8-11]. In the Russian Federation, objective data on the prevalence of NAFLD were not available until recently. However, an open multicentre prospective epidemiological study, DIREG_L_01903, aimed at analysing the incidence in the urban population, was completed in 2007. This study helped to obtain comprehensive information on the epidemiological structure of NAFLD in Russia: this nosology was detected in 26.1% of patients, with cirrhosis in 3% of patients, steatosis in 79.9%, and steatohepatitis in 17.1% [12]. Due to the high prevalence of NAFLD, as well as the potential for its progressive course, the issue of accessible, effective, and early diagnosis of this pathology is extremely relevant. The purpose of this review article is to cover various

modern laboratory and instrumental methods for NAFLD and NASH diagnosis used in Russia and abroad, as well as promising developments in this field of medicine. Clinical criteria In routine clinical practice, the vast majority of cases of NAFLD are diagnosed accidentally based on clinical, laboratory, and instrumental data. Only a few patients complain of weakness, discomfort, or mild right-subcostal pain (13,14). The disease is asymptomatic in 48-100% of patients (4,15). On physical examination, there is a subtle hepatomegaly, which may be difficult to detect by palpation and percussion if the patient is obese. Small hepatic signs (telangiectasia, erythema palmaris, gynaecomastia), splenomegaly, ascites are indicative of the progression to cirrhosis (Table 1). It is worth noting that during history taking in a patient with NAFLD, it is important to rule out alcohol abuse as the etiological cause of the liver damage. The non-alcoholic nature of the lesion is established if the patient does not consume alcohol at all or the amount of alcohol does not exceed 30 g per day for men and 20 g per day for women (in ethanol equivalent) (13, 16). Laboratory diagnosis Laboratory diagnosis allows differentiation of the form of NAFLD. In the absence of a cytolytic syndrome, steatosis is diagnosed, whereas cytolysis indicates inflammation and is consistent with NASH, which is defined according to the degree of elevation of liver transaminases (usually less than 2-4 units) [4, 14]. Extremely rarely, liver transaminases can reach ten times the reference interval, which requires differential diagnosis with acute hepatitis, especially of viral origin. In assessing the ratio of alanine aminotransferase (ALT) to aspartate aminotransferase (AST) levels, the predominance of ALT is more common, in contrast to alcoholic hepatitis, where, in contrast, AST levels exceed ALT levels in more cases [17]. Retrospective studies have reported an average ALT level of 83 units/l and an AST level of 63 units/l in NAFLD [18]. To differentiate histologically verified NASH from steatosis, threshold values for ALT (greater than 60 U/L) and AST (greater than 35.2 U/L) have been proposed; Table 2 [19]. As patients with NAFLD require long-term follow-up and, depending on the use of different test systems, the reference values may vary widely, it is useful to indicate, in addition to the absolute values of ALT and AST, how many times the normal value is exceeded. This helps to illustrate the dynamics of the biochemical parameters at different stages of the patient's follow-up. Elevations in alkaline phosphatase and g-glutamyl transpeptidase are usually mild and not specific for NAFLD. The levels of total bilirubin, albumin and prothrombin time do not exceed the limits of normal reference values (2). In patients with cirrhosis, the synthetic liver function (albumin, cholinesterase, prothrombin index) must be determined in order to judge the severity of the process. NAFLD is a diagnosis of exclusion, so if it is first suspected, copper (Wilson-Conovalov disease), iron (haemochromatosis), and markers of chronic viral hepatitis B and C must be excluded. It is not uncommon for NAFLD patients to have elevated ferritin (on average 50% of patients) and for the percentage of transferrin iron saturation to be increased by 10% (1). These findings are more often due to iron overload syndrome, but when transferrin saturation is 45% or higher, genetic testing should be performed to rule out hereditary haemochromatosis. It is extremely important to exclude markers of viral hepatitis C in patients, as in this case the viral lesion can be the cause of the onset and progression of NAFLD, especially when infected with genotype 3 virus [20].

In NAFLD, low antinuclear antibody (ANA) or smooth muscle antibody (ASMA) titres are detected in 23-36% of cases, requiring differential diagnosis with autoimmune liver disease [1]. However, serum immunoglobulin G levels in patients with NAFLD are usually within normal limits. In addition to a comprehensive assessment of the cytolytic syndrome over time, a liver biopsy is required in these patients to verify the diagnosis.

Table 1.

Symptoms, physical examination and laboratory criteria for NAFLD		
Symptoms	**Physical examination data**	**Laboratory criteria**
Frequent signs		
- No symptoms (48-100% of patients)	- Moderate hepatomegaly	-A 2-4 times increase in ALT and AST levels -De Ritis coefficient (AST/ALT)<1 - Slight increase in alkaline phosphatase levels in 1/3 of patients - Normal bilirubin, albumin and prothrombin time -Elevated serum ferritin levels
Rare signs		
- Slight pain in the right side of the stomach - Unwellness, weakness	- Telangiectasia - Palmar erythema - Gynecomastia - Splenomegaly - Ascite	- Low antinuclear antibody titer

Table 2.

Specificity and sensitivity of biochemical indicators for differentiating histologically verified NASH from hepatic steatosis			
Indicator	**NASH (U/L)**	**Steatosis (U/L)**	**Threshold value for differentiation (sensitivity and specificity)**
ALT	134,9±94,1	90,5±76,4	60 (71%, 60%)
AST	75,2±41,1	46,4±23,5	35,2 (71%, 55%)

Instrumental diagnosis. It is not an exaggeration to say that abdominal ultrasound (US) is a routine instrumental method of imaging the liver parenchyma, and although NAFLD has a number of distinctive features, the diagnosis cannot be made on the basis of US findings but is the result of a comprehensive analysis of clinical, laboratory, anamnestic and instrumental data (14). The wide diagnostic application of ultrasound is due to its accessibility, widespread use and low cost (16). Ultrasound has a reasonable degree of accuracy in diagnosing NAFLD, with a sensitivity of 60-94% and specificity of 66-97% [21]. However, in morbidly obese patients (grade 2-4), the sensitivity and specificity

decline. Ultrasound criteria for NAFLD are – moderate increase in liver size; - reduced echogenicity of the parenchyma (so-called 'bright liver' effect); - impoverishment or absence of haracterizat of the vascular pattern; - 'fading' of the ultrasound beam.

In addition to ultrasound, computed tomography (CT) and magnetic resonance imaging (MRI) are the imaging modalities used in the diagnosis of NAFLD. Various authors have reported CT sensitivity ranging from 33% to 93%, with a positive predictive value of 62-76% (1, 2). A bolus-enhanced CT scan improves the sensitivity. In patients with NAFLD, the contrast intensity of the liver is lower than that of the spleen, so the parenchyma of the organ appears darker than the spleen. On native examination (without contrast), the density of the liver is much lower than usual, up to minus HU (normal 50-70 HU). MRI is more sensitive than abdominal ultrasound, especially in the diagnosis of moderate hepatic steatosis (20). When performed in T1-weighted mode in patients with NAFLD, a "bright liver" effect is detected. MRI has a sensitivity of 80% and specificity of 95% for detecting moderate to severe steatosis and a sensitivity of 85% and specificity of 100% for detecting mild steatosis (24). However, this study is rarely used in the clinic due to the cost and duration of the procedure (4, 11). The introduction of the proton density fat-fraction (PTFF) MRI mode, which is characteristic of steatosis, is one of the most promising areas for improving the imaging diagnosis of NAFLD [25]. Proton magnetic resonance spectroscopy is being gradually integrated into clinical practice for diagnosing NAFLD [2,3]. This non-invasive technique has great advantages over other imaging modalities in the diagnosis of hepatic steatosis. Magnetic resonance spectroscopy is based on the principle of determining proton resonance signals characteristic of triglyceride stores in hepatocytes. In verification studies, triglyceride levels greater than 55.6 mg/g have been found to indicate the presence of hepatic steatosis (6). A promising method for noninvasive diagnosis of steatosis in NAFLD patients is the ultrasound attenuation parameter (CAP, controlled attenuation parameter) [27]. This method is attractive because the degree of both steatosis and fibrosis can be measured simultaneously, as this function is integrated into the latest generation of indirect elastometry (Fibroscan FS-502). Technically, the technique consists in determining the attenuation of the ultrasound wave with a Probe-M transducer at 3.5 MHz at a depth of 25 to 65 mm. The results are expressed numerically in dB/m and correlate with the degree of steatosis: S0 – no steatosis; S1 – minimal steatosis, less than 5% of hepatocytes with steatosis; S2 – moderate steatosis, 6-32% of hepatocytes with steatosis; S3 – marked steatosis, 33-100% of hepatocytes with steatosis. At sensitivities greater than 90%, the CAP readings are 215 dB/m for S≥1, 252 dB/m for S≥2 and 296 dB/m for S3. Together with its non-invasiveness, the method has good evidence for widespread use in routine practice [8]. Another promising technique for determining hepatic steatosis in NAFLD patients is hepatoscintigraphy with radioxenon (133Ce) [7]. The labeled radiopharmaceutical actively concentrates in adipose tissues, allowing identification of the severity of hepatic steatosis (9). In a recent retrospective study, this method was shown to have 94.3% sensitivity and 87.5% specificity in the diagnosis of NAFLD [3]. However, the technique is still poorly understood in this patient population. Because NAFLD is a progressive disease associated with the formation of liver fibrosis and cirrhosis, it is an important clinical challenge to

determine the severity of fibrotic changes in patients with this pathology. Several noninvasive instrumental methods are currently available for the diagnosis of liver fibrosis, haracterizat by a number of advantages and disadvantages (Table 3).

Table 3.

Comparative haracterization of instrumental non-invasive methods of diagnosing liver fibrosis			
	Indirect elastometry (transient elastography)	**ARFI elastography**	**MRE**
Units of measure	kPa	m/s	kPa
Advantages	Getting results fast	Can be integrated into expert-class ultrasound machines	High accuracy in obese patients
Disadvantages	High risk of measurement failure in obese patients. High cost of equipment	High risk of measurement failure in obese patients	The cost of equipment is very high. Research takes a long time

Indirect elastometry (transient elastography) of the liver is a relatively new noninvasive technique that allows rapid assessment of the degree of liver fibrosis, including dynamics. The technique is based on the property of mechanical wave oscillation to propagate at different speeds in media of different density (1,2). The density of the liver parenchyma increases with the formation of fibrosis areas, which is reflected in higher elasticity values in kPa (Table 4) [3].

Table 4.

Correlation of indirect elastometry values with METAVIR liver fibrosis		
Stage of liver fibrosis according to METAVIR	**Standard values, kPa**	**Adjusted values for NAFLD/NASH, kPa**
F0	1,5–5,8	<7,0
F1	5,9–7,2	
F2	7,3–9,5	≥7,5
F3	9,6–12,5	≤10
F4	>12,5	≥14

Over 15 years, the technique has gained popularity in many regions of the world, but its use in NAFLD has been limited by the technical impossibility of measuring in morbidly obese patients (2, 4). For example, in patients with a body mass index (BMI) >30 kg/m2, the failure rate for liver fibrosis identification varies from 3% to 16% [5, 6]. Improvements

in this technique with the introduction of the new Probe-XL sensor have overcome these limitations (7). Indirect elastometry is a fairly accurate method of determining the degree of hepatic fibrosis, with an average sensitivity and specificity of 70 and 84%, respectively (8). Sensitivity is higher for moderate and severe fibrosis as well as for cirrhosis (F2-F4), exceeding 90%. Acoustic pulse-wave (ARFI) elastography and magnetic resonance elastography (MRE) can be considered as alternative noninvasive instrumental techniques for detecting hepatic fibrosis (16). The advantage of ARFI-elastography technique over indirect elastometry is its ability to be integrated into a conventional expert-class ultrasound system with B-mode [9]. The ultrasound transducer produces an acoustic pulse that generates shear waves that propagate into the liver tissue. The shear wave penetration rate increases with tissue stiffness and thus with the severity of fibrosis and is measured in m/s (10). In a study on a mixed population of patients with different chronic liver diseases, equivalent accuracy of ARFI-elastography and indirect elastometry has been demonstrated (11). In patients with NAFLD, ARFI-elastography has been shown to have high diagnostic accuracy in the diagnosis of stage III and IV fibrosis (area under the ROC curve - AUROC 0.97) [12]. MRE is also based on the principle of shear wave induction into liver tissue and subsequent recording of its reflection to form a coloured elastogram [13]. The elastogram reflects the velocity of the wave and the elasticity of the liver tissue, measured in kPa (Figure 4) [14]. A pilot study of MRE in a mixed population of patients with different chronic liver diseases has shown high sensitivity (98%) and specificity (99%) in detecting fibrosis when the elasticity index exceeds the threshold value of 2.93 kPa [15]. Separately, MRE is a highly sensitive technique for differentiating between different stages of liver fibrosis (sensitivity 85.4%, specificity 88.4%) (46).

Prospective biological markers of NAFLD. In the last few years, there has been considerable medical progress in the development of non-invasive biological markers to assess the severity of non-bloating and liver fibrosis in NAFLD patients. One of the most studied biomarkers of NAFLD is adiponectin, a hormone produced by adipose tissue. Serum levels of adiponectin are lower in obese patients with insulin resistance and type 2 diabetes than in controls (normal 9 µg/ml for women and 6 µg/ml for men) [5, 6]. Several studies in a population of patients with NAFLD have also demonstrated reduced serum adiponectin concentrations, with levels correlating with the severity of the histological picture, in particular with hepatocyte necroinflammation [3, 5]. On this basis, adiponectin is considered as a non-invasive predictor of NAFLD progression. NASH is known to be associated with the activation of apoptosis, so the determination of serum markers of this process of cell death may have promise in differentiating normal hepatic steatosis from NASH [16]. During apoptosis, activated caspases cleave various intracellular substrates, including cytokeratin 18 (CK18), a major hepatocyte intermediate filament. The cleaved CK18 fragments enter the bloodstream and can be detected by enzyme immunoassay with quantitative detection of the M30 antibody (17). The M30 antibody selectively recognises the CK18 neoepitope in the fragments cleaved by caspases. Clinical studies to date have shown that antibody M30 can identify patients with NASH with reasonable accuracy. The amount of cleaved CK18 fragments>279 units/l can differentiate NASH with a sensitivity

of 71% and specificity of 85% [18]. Another enzyme immunoassay method with quantitative detection of M65 antibodies can detect both cleaved and intact CK18 fragments. The technique has shown encouraging results in pilot studies, but needs confirmation in larger studies. A non-invasive biological marker of hepatic fibrosis is hyaluronic acid (HA), a non-sulfated glycosaminoglycan synthesised by stellate liver cells. A study in a population of children with NAFLD has demonstrated that a serum HA≥2100 ng/ml is indicative of significant liver fibrosis (F2, F3, F4) with an AUROC of 0.95 [10]. Another marker of liver fibrosis is procollagen N-terminal peptide III (PIIINP), a product of collagen cleavage. In a study of 172 patients with NAFLD, the level of PIIINP enabled a fairly accurate differentiation of fibrosis severity (AUROC 0.77-0.82 in patients with fibrosis and F0-2 and AUROC 0.82-0.84 in patients with F0-3 fibrosis) [11]. A commercially available comprehensive biochemical panel for the diagnosis of liver fibrosis, such as the Fibrotest and its analogues, is now available. In NAFLD, this panel shows moderate diagnostic accuracy (AUROC 0.75-0.86 for F2-4 fibrosis and 0.81-0.92 for F3-4 fibrosis).

Morphological changes in disease are caused by different pathological processes. In NAFLD, a number of key points ("triggers") in the development of nosology are identified: triglyceride accumulation within the hepatocyte, lipotoxicity, and a chronic systemic inflammatory response. All these processes lead to the activation of the body's regenerative systems (in particular the liver as an organ). Prolonged damage leads to hyperregeneration and development of liver fibrosis. Limited information is available about the trigger mechanism of fibrosis as a process. It is known about the existence of β-catenin-dependent Wnt-signaling pathway regulating stimulated cell apoptosis and fibrosis of organs and tissues of the body. The role of transmembrane proteins of the Wnt-pathway is performed by proteins of the Frizzled family. Tropicity to liver tissue was found in one member of the family, Secreted Frizzled Related Protein-4 (SFRP4). SFRP4, an adipokine whose expression is dependent on the intensity of adipogenesis, is an early marker of impaired carbohydrate metabolism. SFRP4 activity (and hence involvement in the inflammatory process) is largely associated with interleukin-1-β (IL-1-β) receptors. Therefore, a correlation between SFRP4 levels and the development of NAFLD is theoretically possible.

Conclusion

The increase of NAFLD share in epidemiological structure of progressive chronic liver diseases determines the urgency of the question about an effective, noninvasive and accessible method of diagnostics of this pathology. Although a number of experts are of the opinion that histological verification of NAFLD is necessary, improvements in noninvasive methods for diagnosing steatosis and liver fibrosis allow this strategy to be reassessed.

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