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CROATIAN GUIDELINES FOR THE DIAGNOSIS AND TREATMENT OF NONALCOHOLIC FATTY LIVER DISEASE

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SUMMARY – Nonalcoholic fatty liver disease (NAFLD) is a term describing excessive accumulation of fat in hepatocytes, and is associated with metabolic syndrome and insulin resistance. NAFLD prevalence is on increase and goes in parallel with the increasing prevalence of metabolic syndrome and its components. That is why Croatian guidelines have been developed, which cover the screening protocol for patients with NAFLD risk factors, and the recommended diagnostic work-up and treatment of NAFLD patients. NAFLD screening should be done in patients with type 2 diabetes mellitus, or persons with two or more risk factors as part of metabolic screening, and is carried out by noninvasive laboratory and imaging methods used to detect fibrosis. Patient work-up should exclude

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the existence of other causes of liver injury and determine the stage of fibrosis as the most important factor in disease prognosis. Patients with initial stages of fibrosis continue to be monitored at the primary healthcare level with the management of metabolic risk factors, dietary measures, and increased physical activity. Patients with advanced fibrosis should be referred to a gastroenterologist/hepatologist for further treatment, monitoring, and detection and management of complications.

Key words: *Nonalcoholic fatty liver disease (NAFLD); Nonalcoholic steatohepatitis (NASH); Metabolic syndrome; Fibrosis; Cirrhosis; Screening; Noninvasive methods; Diagnostics; Treatment; Hepatocellular carcinoma*

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease and one of the leading causes of death from liver disease in developed Western countries[1-3]. It is defined by excessive accumulation of fat in >5% of hepatocytes and can be proven histologically and/or by imaging methods in combination with laboratory indicators[1,4]. The definition of NAFLD valid until recently implied the absence of other liver diseases such as viral hepatitis, drug-induced toxic lesions, excessive alcohol consumption, autoimmune and hereditary metabolic diseases of the liver, and other causes of liver injury[1,4]. However, it has been recognized lately that fatty liver disease can exist concurrently with other causes of fatty liver (e.g., alcohol), so instead of the term nonalcoholic fatty liver disease (NAFLD), a new term for the disease has been introduced, i.e. metabolic dysfunction-associated fatty liver disease (MAFLD)[5,6]. According to the new definition, for diagnosis it is sufficient to establish the existence of hepatic steatosis determined histologically (by biopsy), by imaging methods or serology biomarkers in patients who are overweight or obese, and/or have type 2 diabetes, or other indicators of metabolic dysfunction, such as increased waist circumference, arterial hypertension, dyslipidemia, insulin resistance, or impaired glucose tolerance[6].

According to the classic definition of NAFLD, two entities can be distinguished histologically, namely, nonalcoholic fatty liver (NAFL), defined by the presence of fat in more than 5% of hepatocytes, with no signs of significant inflammation or hepatocyte damage, and nonalcoholic steatohepatitis (NASH), in which in addition to the presence of fat in more than 5% of hepatocytes there also are signs of inflammation and hepatocyte damage, with or without fibrosis[1,4,7]. The clinical significance of this distinction lies in the fact that disease progression towards cirrhosis is faster in patients with NASH[8,9]. Moreover, there is increasing evidence that NAFLD is the leading etiologic cause of cryptogenic cirrhosis, and a significant risk factor for the development of hepatocellular carcinoma (HCC)[2,3,9]. Despite its consequences on the liver, NAFLD is a multisystem disease, and the main cause of death in NAFLD patients are cardiovascular diseases and malignant tumors, while complications of advanced liver disease are the third most common cause of death in NAFLD patients[10,11].

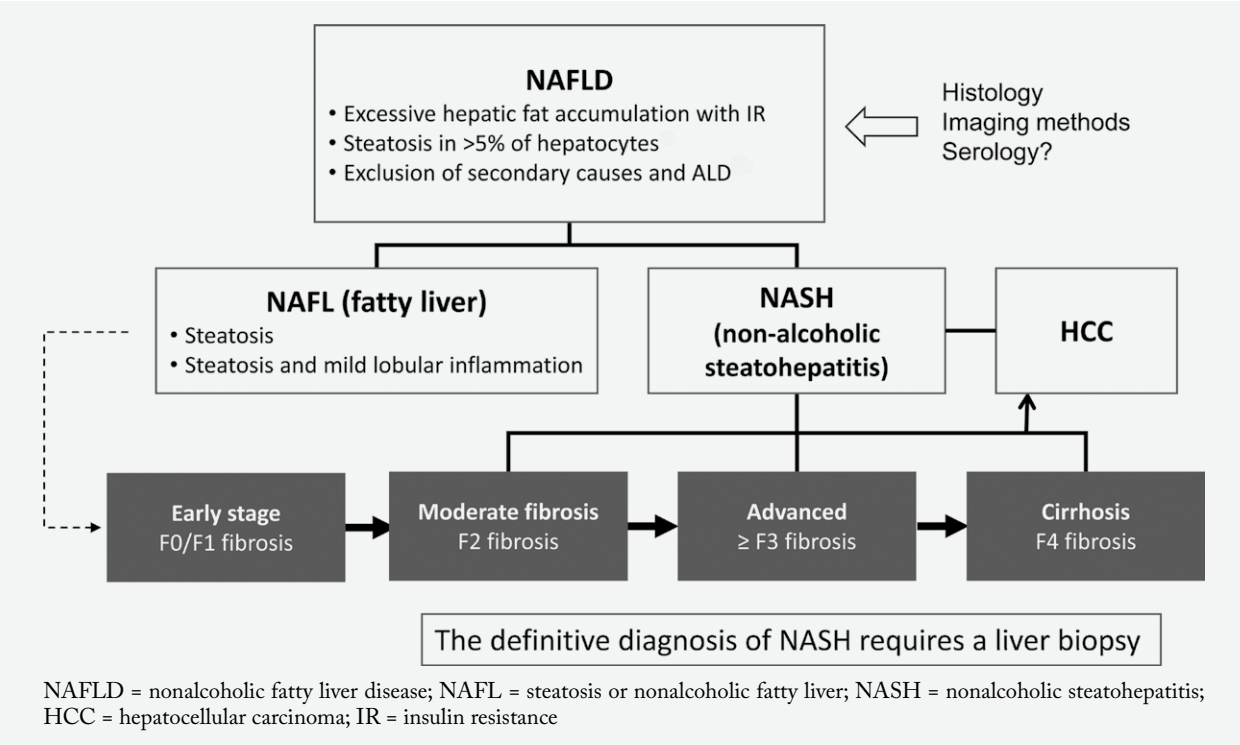
The spectrum of pathologic changes characteristic of individual entities within NAFLD is shown in Figure 1.

These guidelines present the NAFLD screening protocol in patients with metabolic risk factors, the recommended diagnostic work-up of patients with suspected NAFLD, and their treatment.

Epidemiology

Nonalcoholic fatty liver disease is the most common liver disease in developed countries, affecting between 17% and 46% of adults, depending on the population[1,3]. Its prevalence in Europe and the United States is 25%-37%[12-14]. NAFLD prevalence is continuously growing and keeping up with increase in the prevalence of metabolic syndrome and its components, the presence of which also increases the risk of advanced liver disease, i.e. NASH and cirrhosis[1]. The diagnosis of metabolic syndrome, defined according to the International Diabetes Federation for persons of European origin, can be established if at least 3 of the following 5 components are present[1,15]:

- waist circumference ≥94 cm for men and ≥80 cm for women;
- fasting glucose ≥5.6 mmol/L (100 mg/dL) or pharmacologically treated;
- blood pressure ≥130/85 mm Hg or pharmacologically treated;



NAFLD = nonalcoholic fatty liver disease; NAFL = steatosis or nonalcoholic fatty liver; NASH = nonalcoholic steatohepatitis; HCC = hepatocellular carcinoma; IR = insulin resistance

Fig. 1. Clinical pathological spectrum of changes in nonalcoholic fatty liver disease (adapted from reference 1).

- triglycerides ≥1.7 mmol/L (150 mg/dL) or pharmacologically treated; and
- HDL cholesterol <1.0 mmol/L (40 mg/dL) for men and 1.3 mmol/L (50 mg/dL) for women.

Epidemiological studies confirm a higher prevalence of NAFLD in risk groups. Hepatic steatosis is present in 50%-60% of overweight persons[14], and in as many as 93% of obese persons who have had a gastric bypass[16], while in patients with type 2 diabetes it is about 55% globally and 68% in Europe[17-19]. In Croatia, on a cohort of 454 outpatients with type 2 diabetes, the prevalence of fatty liver established using the controlled attenuation parameter was 78%[20]. Nevertheless, NAFLD also affects approximately 7% of persons with normal weight[21]. It is more common in younger women, persons with insulin resistance and hypercholesterolemia, whose liver enzymes are frequently within the reference range[1]. In principle, NAFLD is more common in men; however, according to a recent meta-analysis, once it develops, it progresses more quickly in women, especially above the age of 50[22].

Although the prevalence of NAFLD in Croatia is believed to correspond to that in Western Europe (20%-30%), these data are not reliable due to the lack of epidemiological studies in the general population[23,24]. In a study conducted using transient elastography in 648 patients with at least one metabolic syndrome feature, steatosis determined using the controlled attenuation parameter was present in 88.3% of patients[25]. In a study on outpatients who were referred to abdominal ultrasound for any reason, 48.5% were found to have fatty liver based on ultrasound results[26]. Full insight into the scope of the problem requires additional studies to assess the prevalence of NAFLD in the general population and high-risk groups in Croatia.

Diagnosis of NAFLD

The majority of NAFLD patients have no symptoms, while few report complaints such as fatigue, weakness, or discomfort below the right costal margin[27]. As the disease is usually asymptomatic, it is most often detected by chance based on elevated liver enzymes in laboratory results, by an incidental finding of hepatic steatosis through imaging methods (or rarely during surgery), or by targeted screening of persons at risk with metabolic syndrome. Medical history of

Table 1. Recommended work-up algorithm for suspected nonalcoholic fatty liver disease patients

Level	Parameters
Initial work-up	1. Alcohol intake: <20 g/day (women), <30 g/day (men) 2. Personal and family history of T2DM, hypertension and CV disease 3. History of steatosis-associated drugs, change in body weight 4. Physical examination: BMI, waist circumference, signs of advanced liver disease 5. Liver enzymes (ALT, AST, GGT, ALP), bilirubin, CBC, PT 6. Fasting blood glucose, HbA1c, OGTT, (insulin [HOMA-IR]) 7. Total cholesterol, HDL, LDL, triglycerides, uric acid 8. HBV and HCV infection (HBsAg, anti-HBs, anti-HBc, anti-HCV) 9. Abdominal US
Extended work-up (consider)	1. Hemochromatosis: ferritin, Fe, TIBC, UIBC 2. Autoimmune and cholestatic liver disease: ANA, AMA, ANCA, AGLM, LKM-1, IgA, IgG, IgM 3. Wilson's disease (Cu in the serum and 24-hour urine, ceruloplasmin) 4. Alpha-1 antitrypsin deficiency (serum alpha-1 antitrypsin) 5. Coeliac disease (tTGA IgA), thyroid diseases (T3, T4, TSH), polycystic ovary syndrome

T2DM = type 2 diabetes mellitus; CV = cardiovascular disease; BMI = body mass index; BW = body weight; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; ALP = alkaline phosphatase; CBC = complete blood count; PT = prothrombin time; BG = blood glucose, OGTT = oral glucose tolerance test; HOMA-IR = homeostatic model of insulin resistance; HDL = high-density lipoprotein; LDL = low-density lipoprotein; HBV = hepatitis B virus; HCV = hepatitis C virus; US = ultrasound; Fe = serum iron; TIBC = total iron-binding capacity; UIBC = unsaturated iron-binding capacity; ANA = antinuclear antibodies; AMA = anti-mitochondrial antibodies; ANCA = antineutrophil cytoplasmic antibodies; SMA = smooth muscle antibodies; LKM = anti-liver-kidney microsomal antibodies; IgA = immunoglobulin A; IgM = immunoglobulin M; IgG = immunoglobulin G; Cu = copper

those affected includes comorbidities such as overweight or obesity, type 2 diabetes mellitus, arterial hypertension, dyslipidemia, or other indicators of metabolic dysfunction[1,5,28]. Common concomitant conditions are hypothyroidism and sleep apnea, and in women polycystic ovary syndrome[1]. Signs of advanced chronic liver disease may be present in a small portion of patients whose disease progresses to end-stage liver disease.

The work-up in patients with suspected NAFLD should exclude the concomitant presence of other causes of liver disease (viral hepatitis, autoimmune, toxic and other metabolic diseases), prove the presence of steatosis by an imaging method (most commonly abdominal ultrasound and/or transient elastography), and rule out secondary causes of steatosis, alcohol being the most common one, and rarely certain medications or other causes (e.g., total parenteral nutrition, starvation, sudden weight loss)[1]. According to the European Association for the Study of the Liver (EASL) guidelines, daily alcohol consumption above 30 g for men and 20 g for women suggests the possibility of alcohol-induced liver injury[29], which also corresponds to the American Association for the Study of Liver Diseases (AASLD) recommendations[30]. Among the drugs that may cause steatosis, the most significant and best known are amiodarone, methotrexate, tamoxifen, valproic acid, some antiretroviral and chemotherapeutic agents[1,31]. It is also important to rule out the use of other drugs in medical history that could lead to toxic liver injury[32].

Nonalcoholic fatty liver disease is the most common cause of abnormal liver enzyme levels in developed countries[33-35]. Although aminotransferase levels in some patients may be within the reference range, in the majority of patients they are abnormal, which is often the principal reason for patient work-up[36,37]. Aminotransferase levels, primarily alanine aminotransferase (ALT), are usually 1.5-5 times above the reference range, while increased gamma-glutamyl transferase (GGT) is also found often[27,38]. One should keep in mind that normal ALT levels do not rule out the presence of NAFLD, NASH or significant fibrosis, and *vice versa*, that elevated ALT can be found in

50% of patients with inconspicuous histology[39]. Laboratory results often concurrently include elevated triglycerides, total and LDL cholesterol, with lower HDL, increased fasting and postprandial glucose or oral glucose tolerance test, as well as elevated glycosylated hemoglobin (HbA1c), which suggest abnormal glucose regulation or type 2 diabetes mellitus within the framework of metabolic syndrome[27]. Some patients may also have elevated uric acid, and urea and creatinine in case of renal impairment, which is often present in patients with metabolic syndrome[27,40].

As there are still no specific serologic markers for NAFLD that can confirm the diagnosis, diagnosing requires establishing the existence of metabolic syndrome, and proving or ruling out concomitant presence of other chronic liver conditions that require specific treatment. Table 1 presents the work-up algorithm for patients with elevated liver enzymes and/or suspected NAFLD.

In addition to laboratory work-up, imaging work-up should also be used to exclude other causes of elevated liver enzymes and to establish the existence of liver disease complications[1].

Diagnostic work-up in suspected NAFLD patients should establish the existence of key histological categories that define this disease, such as the existence and degree of steatosis, inflammatory activity (NASH), and stage of liver fibrosis.

Diagnostic methods for hepatic steatosis

Abdominal ultrasound (US) is a widely available, safe and affordable, and most often the first imaging method in the work-up of liver disease. The greatest shortcomings of US in diagnosing patients with fatty liver are low sensitivity in detecting initial steatosis (if the percentage of hepatic steatosis is <20%), difficulty of detection in obese patients (which are common among patients with fatty liver), and relative subjectivity of the method which depends significantly on the operator[41-47]. As negative US results do not rule out mild steatosis, in that case another, more sensitive method should be used. Computerized tomography (CT) and magnetic resonance imaging (MRI) can also be used to detect moderate to severe steatosis, at a higher price, lower availability, and in case of CT also the presence of radiation[1,5,47]. Proton magnetic resonance spectroscopy has the highest sensitivity and specificity in detecting fatty liver; however, its use is limited by high price and low availability[1,48]. Transient elastography (TE) (Fibroscan®, Echosense, Paris, France) with the possibility of concurrent quantification of liver fibrosis additionally enables detection and grading of hepatic steatosis by determining the controlled attenuation parameter (CAP)[1,47,49]. CAP has a relatively good sensitivity and specificity to detect even low-grade hepatic steatosis (<10%). The latest EASL guidelines suggest the CAP level of 275 dB/m for steatosis screening[49].

There is also intensive research under way into the possible steatosis indexes obtained by calculations using formulas that include several variables[1,47,49]. The most frequently mentioned is the fatty liver index (FLI), which consists of the body mass index (BMI), waist circumference, triglycerides and gamma-glutamyl transferase (GGT)[47,49,50]. The values <30 are used to rule out NAFLD and ≥60 to confirm steatosis, but with no possibility of distinguishing between moderate or severe steatosis[47,50]. There is also research on the hepatic steatosis index (HSI), SteatoTest and NAFL screening score[47,51-53]. However, based on the available data on their diagnostic and prognostic value, these steatosis indexes can currently be recommended only for large epidemiological studies, but not for routine clinical practice[5].

Diagnostic methods to establish the degree of inflammatory activity (NASH)

Nonalcoholic steatohepatitis is characterized by steatosis, hepatocyte impairment and ballooning, with or without fibrosis, which accelerate disease progression[1]. Therefore, numerous noninvasive methods are being studied and suggested to diagnose NASH, and include simple serum biomarkers, serum marker panels, and imaging[47]. In terms of serum biomarkers, the most researched are cytokeratin-18, CXCL-10, fibroblast growth factor 21, and adipocytokines: adiponectin, leptin and resistin[47]. Several biomarker panels have also been studied, such as NASHTest and NASH ClinLipMet score[47]. Given the features of NASH, it cannot be diagnosed based on routine imaging, i.e. US, CT, MRI, or transient elastography. Promising results were obtained from multiparametric MRI technology which enables quantification of steatosis, iron accumulation in the liver, and fibrosis, but requires confirmation in larger studies, and given its limited availability, it is difficult to expect its wider use in the foreseeable

Table 2. The most important biochemical indexes to assess liver fibrosis

FIBROSIS index	Age	Sex	BMI	T2DM	Plt	AST	ALT	AST/ ALT	GGT	Other parameters
FIB-4	YES				YES	YES	YES			
NFS	YES	YES		YES	YES			YES		Albumins
APRI					YES	YES				
BARD			YES	YES				YES		
ELF										PIIINP, hyaluronic acid, TIMP 1
FibroMeter NAFLD	YES				YES	YES	YES			BG, ferritin, body weight
FibroTest	YES	YES	YES						YES	A2M, ApoA1, haptoglobin, bilirubin

APRI = AST to platelet ratio index; NFS = nonalcoholic fatty liver fibrosis score; ELF = enhanced liver fibrosis; BMI = body mass index; T2DM = type 2 diabetes mellitus 2; Plt = platelets; AST = aspartate aminotransferase; ALT = alanine aminotransferase; GGT = gamma-glutamyl transferase; A2M = alpha-2 macroglobulin; APOA1 = apolipoprotein A1; BG = blood glucose; MetS = metabolic syndrome; PIIINP = procollagen amino-terminal peptide; TIMP1 = tissue inhibitor of matrix metalloproteinase 1

future[47,54]. Therefore, biopsy and histopathologic analysis of a liver sample currently remain the only method that can reliably demonstrate the existence of NASH[1].

Diagnostic methods to establish the stage of liver fibrosis

Fibrosis in NAFLD may be absent or mild (F0-1), significant (F≥2) and advanced (F≥3), while the most severe stage is cirrhosis (F4). Since numerous studies have shown the stage of fibrosis to be the most important prognostic factor for disease outcome, fibrosis staging by noninvasive methods is of great clinical significance[4,8,14,49,55-60]. To diagnose fibrosis in practice we use direct (specific) and indirect (non-specific) fibrosis indexes, and imaging techniques[47,49].

The advantage of indirect fibrosis indexes is that they are relatively affordable and widely available and therefore appropriate for wide use[47,49]. These indexes are reproducible and have a good negative predictive value to rule out significant fibrosis, but their use is limited by the low positive predictive value. On the other hand, direct fibrosis indexes imply determination of specific serum markers associated with fibrogenesis, such as type III procollagen, a C3 precursor, hyaluronic acid, and tissue inhibitor of metalloproteinase 1. These fibrosis indicators are used in proprietary (commercial) tests, have higher specificity, but also higher prices and therefore are not as available[47,49]. The most commonly used biochemical indexes to assess liver fibrosis and the parameters on which calculations are based are shown in Table 2.

Among biochemistry methods to diagnose fibrosis in NAFLD, the simplest and most researched are FIB-4 (includes information on patient age, AST, ALT, and platelet count) and NAFLD Fibrosis Score (NFS) (includes information on age, body mass index (BMI), diabetes, AST, ALT, platelet count, and albumin level)[1,47,49]. The FIB-4 and NFS biochemical indexes are equally reliable and have demonstrated good predictive value in ruling out advanced liver fibrosis, especially when combined with other methods[47,49,58,61].

Imaging techniques to assess the stage of fibrosis are based on elastography[47,49,58]. The most researched in clinical studies and most frequently used in practice is transient elastography (TE, FibroScan), but other ultrasound elastography methods are also used, such as shear wave elastography (SWE)[62]. When using elastography methods in hepatology, their limitations should also be taken in consideration. Liver elastography is based on establishing differences in the mechanical properties of tissue, such as liver parenchyma stiffness, which increases with the development and progression of fibrosis. However, other conditions such as hepatitis, cholestasis or liver congestion can also significantly increase the measured levels, and the results in such conditions cannot be deemed reliable.

The most sophisticated noninvasive method to assess liver fibrosis is magnetic elastography (MRE),

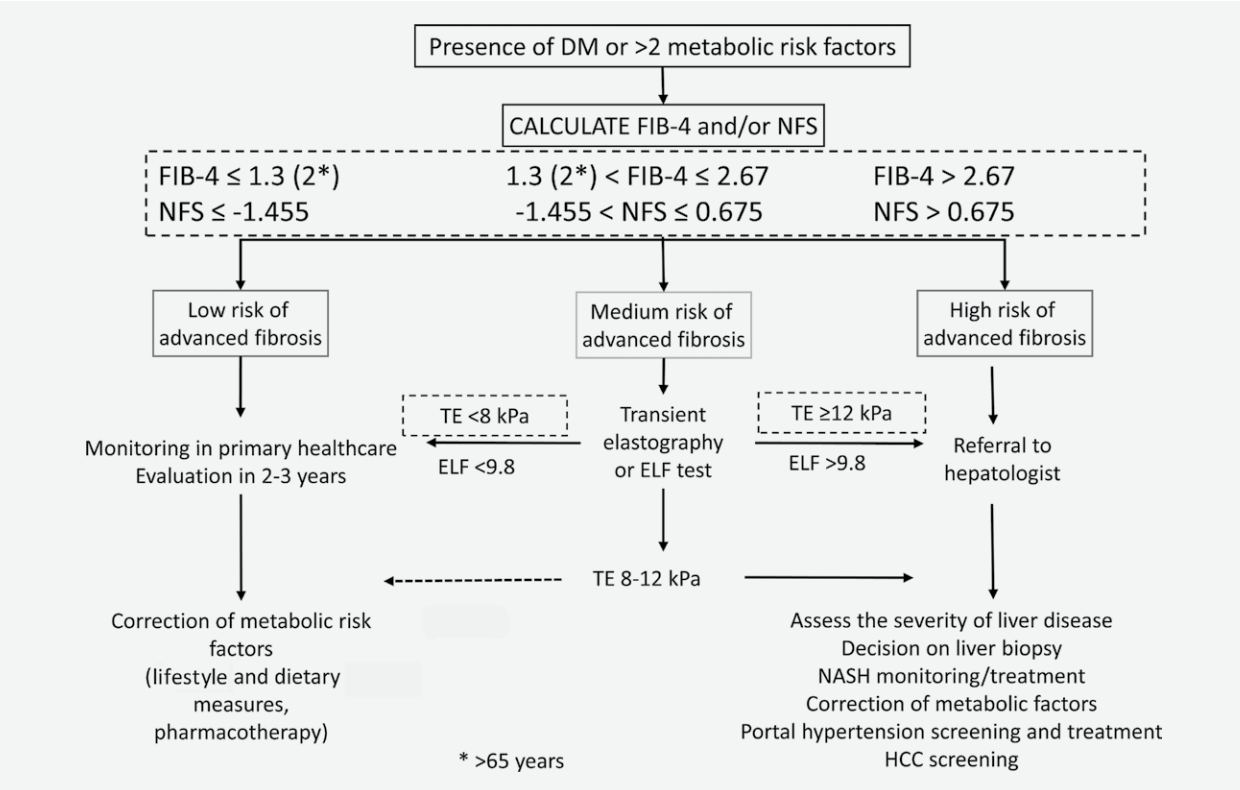


Fig. 2. Diagnostic protocol for screening for nonalcoholic fatty liver disease in patients with metabolic risk factors (adapted from references 1, 13, 66, 71–73).

which, however, due to its limited availability, duration of the examination and price, is not widely applicable to diagnosing NAFLD in routine clinical practice[1].

Patient Screening for NAFLD

Despite the relatively high prevalence in the general population, screening for NAFLD of the overall population is not recommended[1]. Namely, without additional stratification of the risk, the information on hepatic steatosis is not prognostically relevant, there is no approved efficient treatment, and the process of broad screening itself would represent a significant burden for the healthcare system[1].

Nonalcoholic fatty liver disease is mostly a slowly progressive disease, but in 20% of cases fibrosis can progress faster[57]. In the majority of patients, fibrosis progresses by 1 histological stage every 14 years in case of steatosis (NAFL), and twice as fast, i.e. every 7 years in case of NASH, while it doubles additionally in the presence of arterial hypertension[57]. In a recent study, the presence and stage of fibrosis (but not of inflammation, i.e. NASH) was demonstrated to be the most significant feature in the progression towards severe liver disease, and the risk of developing severe disease was three times higher in patients without fibrosis (stage F0) or with the initial stage (F1) of fibrosis, and as much as eight times higher in patients with high-stage fibrosis (F3) compared to the control group[55,63,64].

Therefore, in patients with risk factors, regardless of the levels of liver enzymes, it is crucial to establish the risk of fibrosis as part of NAFLD[55,65].

Subsequent to the above, as well as to the previously elaborated epidemiological data on NAFLD prevalence in certain risk groups (for instance, among the obese or diabetics) that are highly likely to have a fatty liver, it is necessary to perform testing focused on establishing the stage of liver fibrosis as the most significant prognostic factor for disease progression.

In this regard, the current joint guidelines of the European Association for the Study of the Liver (EASL), European Association for the Study of Dia-

betes (EASD), and European Association for the Study of Obesity (EASO), as well as the American Gastroenterological Association (AGA) recommend screening for NAFLD in patients with insulin resistance and metabolic syndrome, and in particular those at an increased risk of progressive NAFLD[1,13].

These are persons with type 2 diabetes, or persons with two or more risk factors as part of the metabolic syndrome, i.e. central obesity (increased waist circumference), arterial hypertension, dyslipidemia, insulin resistance or glucose dysregulation[1,7,13,66].

Testing should be carried out using simple, available, reliable and affordable methods, which can be done in family practice, as well as in other locations of frequent contact between patient groups at risk and the physician. It is recommended to use noninvasive methods to detect fibrosis in NAFLD, of which the most available are biochemical indexes that include various parameters and are formulated by mathematical models[1,4,7,11-13,47,49,58,66].

Patients with suspected advanced liver fibrosis based on biochemical indicators (e.g., FIB-4 or NFS) should be referred to a hepatologist for further work-up and monitoring[1,7,49,58,66]. NAFLD patients in whom advanced fibrosis was ruled out by noninvasive work-up, are not expected to develop complications of liver disease over a longer period (20 years), and can continue to be monitored at the primary healthcare level[7,49,58,66]. Patients who are, based on biochemical indicators, at medium risk of advanced fibrosis, require the use of an additional noninvasive diagnostic method for precise staging of fibrosis. Such patients are advised to be referred to liver fibroelastography, or patented direct biochemical test (ELF, Fibrotest or similar), depending on availability[49,58,66]. Patients with liver stiffness levels measured by transient elastography (TE) below 8 kPa or ELF test below 9.8, are at a low risk of advanced fibrosis and can continue their monitoring in primary healthcare, with modification in diet, lifestyle habits, and regulation of metabolic syndrome components[67-70]. They are recommended to repeat testing in 2-3 years. In case of higher levels on TE or ELF test, which suggest advanced fibrosis or cirrhosis (TE >12 kPa and ELF test >9.8), the patient is referred to a hepatologist for further work-up (screening for hepatocellular carcinoma and esophageal varices), potential therapy (including clinical trials), and monitoring[1,49,58,66]. In case of incongruent values on these tests, referral to hepatology and liver biopsy should be considered in order to establish reliably the histological stage of liver disease.

The recommended diagnostic protocol for NAFLD screening in patients with metabolic risk factors is shown in Figure 2. When applying this protocol, it should be taken in consideration that such screening encompasses patients at an increased risk of advanced disease, whereas the actual number of NAFLD patients with milder forms of the disease (steatosis without fibrosis or with initial fibrosis) is considerably higher, and they probably will not be detected by using these biochemical indicators.

The tests recommended in the algorithm have been chosen because they are simple and widely available, as their calculation requires easily obtained parameters (FIB-4, NFS), and they also are most validated (FIB-4, NFS, TE and ELF) in NAFLD diagnostics. However, other equally valuable biochemical tests can be used, which have adequate scientific validation for this indication, with the use of appropriate borderline values[67,70].

One of the most used and simplest fibroelastography methods is transient elastography (FibroScan, Echosense, Paris, France); however, nowadays, different modules for elastography installed in most of better ultrasound machines are used increasingly[47,49,58]. Among elastography methods, these guidelines highlight TE because it is the one best validated in scientific studies. Preliminary results also indicate the reliability of other elastography methods in diagnosing fibrosis in NAFLD patients, however, with a currently still somewhat lower level of scientific evidence[47,49,58]. Here we recommend borderline values for liver stiffness calculated to distinguish patients with advanced (F3) fibrosis because that is precisely the stage above which the risk of the development of liver disease complications, portal hypertension and hepatocellular carcinoma increases significantly[71-73].

Liver biopsy in NAFLD diagnostics

Although not indicated for the majority of NAFLD patients, liver biopsy and histopathological analysis of the sample obtained is the gold standard in diagnosing the degree of injury and stage of liver tissue fibrosis[1]. Liver biopsy is a potentially expensive method which requires expertise in interpreting results and carries certain morbidity and a very rare risk of death. Therefore, it should be done in those NAFLD patients in which it would facilitate diagnosis, selection of treat-

ment methods, assessment of prognosis and optimal protocol for disease monitoring. In clinical practice, NAFLD diagnosis and exclusion of advanced disease stages in the majority of patients can be assumed based on medical history and status, and on the results of laboratory and imaging work-up, provided that the existence of other liver conditions has been ruled out[1,4,7,13,47,49,58,66]. However, some patients will still have an unclear diagnosis after noninvasive work-up.

Consequently, liver biopsy is most frequently indicated in cases where NAFLD diagnosis is not completely clear, i.e. there is suspicion of overlapping with other liver conditions, or to assess the degree of liver injury and fibrosis stage in patients with suspected advanced NAFLD stages (advanced fibrosis, F≥2)[1]. In addition, liver biopsy is the only currently available method to distinguish nonalcoholic fatty liver (NAFL) from nonalcoholic steatohepatitis (NASH)[1]. Identification of patients with NASH and advanced fibrosis is important for the approach to patient treatment and monitoring. NASH diagnosis provides important information on the disease prognosis by indicating an increased risk of fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Fibrosis is the most important prognostic factor in NAFLD, in direct correlation with adverse outcomes and liver-associated mortality[2,7,55,57,59,60,64,73].

In conclusion, based on the insights to date and in line with the recommendations of the European and American professional associations for the study of liver diseases, liver biopsy should be done in[1,7,58]:

- patients with suspected significant fibrosis (F≥2) based on elevated levels in noninvasive fibrosis assessment methods (e.g., FIB-4 score >1.3 and TE >8 kPa, FIB-4 score >2.67) or suspected NASH; and
- patients with an unclear etiology of liver disease or if there is a risk of another disease in addition to NAFLD.

Liver biopsy may be considered in patients with several risk factors for advanced stage NAFLD (NASH and advanced fibrosis, F≥2), e.g., >2 components of metabolic syndrome and age >45 years, or ferritin level >1.5 times the upper limit of normal.

Until the role of noninvasive methods in the monitoring of NAFLD treatment outcomes has been clarified, repeated liver biopsy may be considered within 5 years from the diagnosis of advanced stage NAFLD (NASH, F≥2)[1].

Screening for Hepatocellular Carcinoma

The risk of hepatocellular carcinoma (HCC) is well known in patients with liver cirrhosis of different etiologies. Moreover, numerous studies also show an increased risk of HCC development in obese patients and patients with type 2 diabetes mellitus[74].

Nonalcoholic fatty liver disease is considered to be one of the most common causes of HCC, which is attributed to a large number of NAFLD patients[75]. Cumulative incidence of HCC in NAFLD is 7.6% over 5 years in persons with advanced fibrosis, with an annual growth rate of 9%[2,74,76]. PNPLA3 rs738409 C>G gene polymorphism is associated with an increased risk of HCC; however, its determination is still not considered to be cost-effective in everyday clinical practice[1]. Patients with HCC in NAFLD are usually older, more frequently have extrahepatic disease, and are diagnosed in advanced stages[75]. The large number of NAFLD cases at risk of HCC make systematic monitoring to a great extent unfeasible. However, the risk of HCC development in NAFLD patients without cirrhosis is estimated to be very low given the extremely high number of NAFLD patients without cirrhosis in the general population[76].

Patients with NAFLD who, based on noninvasive work-up, are at a high risk of significant fibrosis and cirrhosis (e.g., FIB-4 >2.67 or LSM >12.0 kPa), or have a histopathological finding of advanced fibrosis or cirrhosis in liver biopsy, represent the group at the highest risk of HCC development[2,73,77]. This group should be regularly monitored by hepatologists for HCC and other potential cirrhosis complications and portal hypertension.

The monitoring program for this group implies regular examinations every 6 months with abdominal US, laboratory indicators of liver injury and function, and possibly tumor markers (most commonly alpha-fetoprotein (AFP), and based on availability also more specific markers such as AFP-L3, PIVKA-II or GALAD score)[1,73,78]. Other imaging methods such as CT and MRI, or contrast-enhanced US, in principle are not used as primary screening methods, but rather in further targeted work-up.

Treatment of Nonalcoholic Fatty Liver Disease

The basis of NAFLD treatment is the treatment of metabolic factors associated with the risk of its devel-

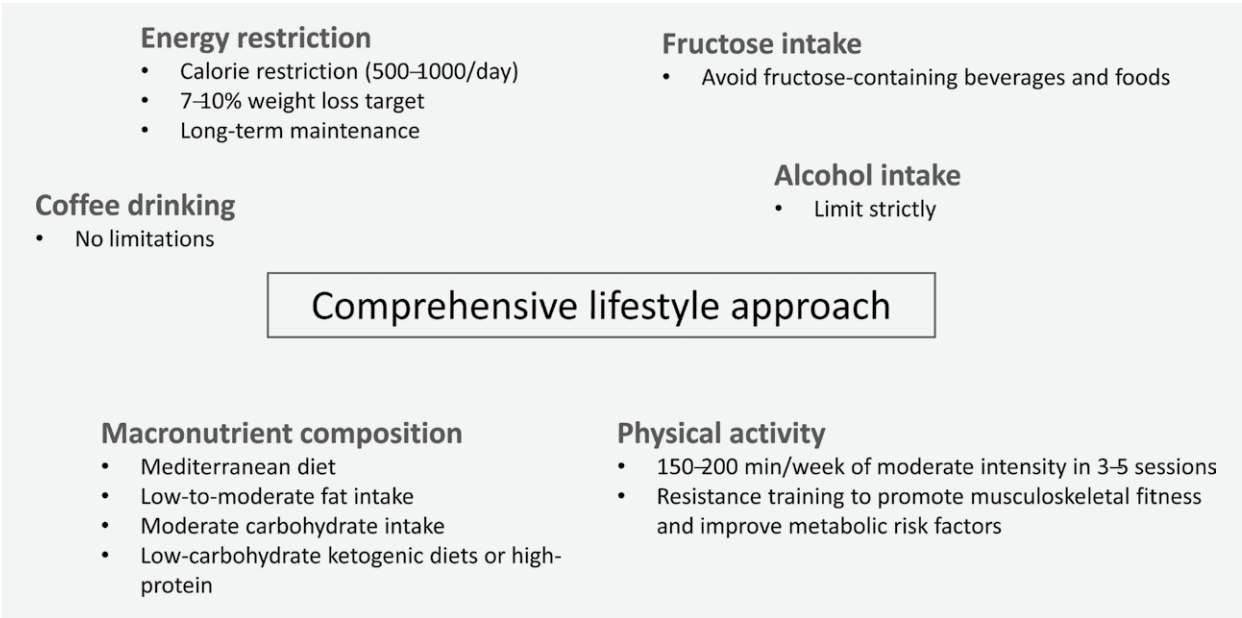


Fig. 3. The basis of treatment for patients with nonalcoholic fatty liver disease is comprehensive lifestyle modification, while pharmacotherapy and surgical methods are indicated in a small portion of patients.

opment and progression (obesity, hyperlipidemia, insulin resistance, and diabetes mellitus)[1,79-81]. According to the current European guidelines, treatment of liver disease itself is indicated in patients with biopsy-proven nonalcoholic steatohepatitis (NASH) with significant fibrosis (F≥2 according to the METAVIR scoring system), or those with risk factors relevant for development and/or progression of fibrosis[1]. Treatment options for patients with NAFLD are shown in Figure 3.

Lifestyle modification

Epidemiological studies demonstrate a significant correlation between NAFLD and unhealthy lifestyle[1-3]. That is why lifestyle changes represent the primary measure in the treatment of NAFLD[1,7,58,71,79-81]. They consist of dietary measures and increased physical activity. The goal is to reduce weight in patients with excessive body weight (BMI >25 kg/m^2) or obesity (BMI >30 kg/m^2) by a minimum of 5%-10% annually[1,7,81-86]. Study results suggest that weight loss of >5% already influences reduction in steatosis, weight loss of >7% leads to reduction in the inflammatory component in the liver and insulin resistance level, while weight loss of >10% influences fibrosis regression[81-86]. Dietary changes with an increased share of healthy food, primarily vegetables, while avoiding processed and ready-made food high in added fructose, and adequate physical activity contribute significantly to weight loss, metabolic profile improvement, fat mobilization, and cardiovascular risk reduction[1,79,80,86]. Prospective studies on the effects of certain diets with varying micronutrient compositions on NAFLD risk are quite scarce[7,86]. The Mediterranean diet high in un-

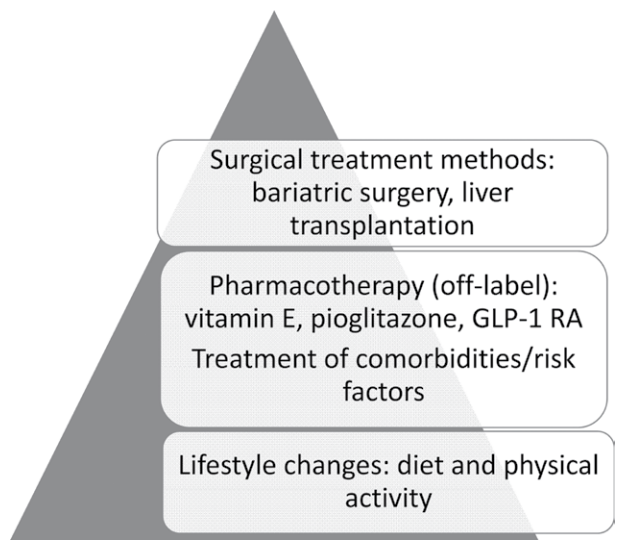


Fig. 4. Modification of habits and lifestyle are the foundation of treatment for patients with nonalcoholic fatty liver disease.

saturated fatty acids, compared to a diet high in fat and low in carbohydrates, has been associated with reduction in hepatic steatosis[1,79,80,83,86]. Nevertheless, differences in the effects of certain types of dietary regimens seem to be less significant than the overall effect of weight loss. Patients are also advised to avoid consuming considerable amounts of alcohol (more than 30 g of alcohol daily for men and more than 20 g for women) due to the potential additive effect on disease progression[7].

Although there are no controlled studies in a large number of subjects on the effects of physical activity on NAFLD, and the optimal duration, type and intensity of activity as well as long-term effects of physical activity on the course and outcome of NAFLD have not been completely defined, physical activity is a significant measure in changing an unhealthy lifestyle[1,7,80,82,87-90]. According to the results available, in patients with >150/min of physical activity weekly or an increase in total activity by >60 minutes/week, reduction in ALT was observed regardless of weight loss[1,80,89,90]. Physical activity in NAFLD patients is associated with a histological effect in terms of steatosis reduction, whereas the effect on NASH is less clear[89]. The combined effect of diet and aerobic physical activity is associated with reduction in liver enzymes and hepatic steatosis. The effect of a low calorie diet (750 kcal/day) and physical activity (walking 200 minutes/week) over one year on histological regression of NASH and fibrosis components has been demonstrated[91]. Therefore, dietary measures in combination with aerobic or resistance exercise are the basic recommendation for NAFLD patients, while the choice of diet and physical activity should be adapted individually to the person's abilities[1,80,83,86,89]. Recommended lifestyle modifications are summarized in Figure 4.

Pharmacotherapy

The effects of pharmacotherapy on NAFLD are limited, therefore, the majority of guidelines in this indication are focused on patients with proven NASH and significant liver fibrosis (stage ≥2), or the presence of risk factors for fibrosis development/progression (age >50, diabetes mellitus, metabolic syndrome, elevated ALT, histologically pronounced inflammatory component), for whom dietary measures and physical activity did not yield the desired results[1,92,93].

Among the most frequently studied drugs are those that increase insulin sensitivity. The use of metformin in the treatment of diabetes, in addition to glycemic regulation, is associated with reduction in insulin resistance and liver enzyme levels, but unfortunately, did not result in histological changes in NAFLD patients and is therefore not indicated for the treatment of liver changes in NAFLD[1,7,94].

Thiazolidinediones are ligands of the nuclear transcription factor peroxisome proliferator-activated receptor gamma (PPAR-Ɣ) with a broad effect on the glucose and lipid metabolism, vascular biology, and inflammation. In the treatment of NAFLD, they have been studied because of their beneficial effect on insulin resistance and fat metabolism. In patients with type 2 diabetes mellitus, the use of pioglitazone is associated with improvement in insulin resistance, reduction in liver enzymes, level of steatosis and all histological features of NASH except for fibrosis[95]. In patients without diabetes, pioglitazone had a significant effect on the improvement of histological features of NASH, as well as fibrosis[96]. The use of pioglitazone is, however, associated with a risk of weight gain, carcinoma of the pancreas and urinary bladder, congestive heart failure, and osteoporosis. Until the results of new studies become available, the use of pioglitazone is recommended only in patients with biopsy-proven NASH, taking into account the risk-benefit ratio of complications for the patient[1,7,97]. It is also important to consider that the use of pioglitazone in NASH patients without type 2 diabetes is not in line with the approved indications.

Treatment with liraglutide, a glucagon-like peptide-1 (GLP-1) agonist, is associated with a favorable effect on the regression of steatosis and reduced progression of fibrosis[98]. Its use is associated with weight loss, but also with gastrointestinal reactions. Given the small number of studies, the routine use of liraglutide in the treatment of NAFLD cannot be recommended as of yet.

Although metformin is in the first line of treatment for type 2 diabetes mellitus, owing to the beneficial effect of other mentioned drugs that affect insulin resistance (pioglitazone, liraglutide) on histological changes in the liver of NAFLD patients, they should be considered when choosing therapy for patients with NASH[7,92,93].

As oxidative stress is one of the key mechanisms in hepatocyte injury and disease progression in NASH, there are quite a few studies on the effect of vitamin E as an important antioxidant in the treatment of NASH.

The use of vitamin E (800 IU/day) in patients without diabetes results in reduced levels of liver enzymes with improvement in histological features of NASH[1,7,99]. Nevertheless, favorable results have not been proven in all studies, and the treatment has not been extensively investigated in patients with diabetes and/or liver cirrhosis[100]. Caution is as necessary with regard to other potential adverse reactions associated with the use of high doses of vitamin E (>400 IU/day), which include an increased risk of prostate cancer, hemorrhagic stroke, and fatal outcome. Therefore, treatment decisions should be made on a case-by-case basis bearing in mind the risk-benefit ratio for the patient[1]. Treatment duration has not been fully elucidated either. In patients with elevated ALT, it is believed that vitamin E or pioglitazone may be discontinued if ALT has not normalized after 6 months, whereas in patients with currently normal ALT it is not possible to provide clear recommendations on treatment duration.

The use of other agents that have been investigated for the treatment of NAFLD/NASH, such as ursodeoxycholic acid (UDCA), obeticholic acid (OCA), elafibranor, probiotics and omega-3 fatty acids, despite some beneficial effects, currently is not recommended in the treatment of NAFLD[1,7,92,93,101].

Regardless of the treatment of liver disease, NAFLD risk factors should also be managed, i.e. type 2 diabetes mellitus, arterial hypertension, and dyslipidemia. Therefore, optimization of blood glucose management, normalization of arterial pressure, and use of hypolipidemic agents are an integral part of the treatment of NAFLD patients. Although beneficial effects of the use of statins for the treatment of NAFLD/NASH have not been proven, the use of statins in patients with dyslipidemia and NAFLD is considered to be safe in patients without fibrosis and with compensated liver cirrhosis[1].

Bariatric surgery

Achievement and permanent maintenance of reduced weight by non-surgical treatment methods is difficult in some patients, therefore in patients with NAFLD who are overweight, the use of surgical methods should be considered[1,7]. Based on the data available, bariatric surgery is intended for the treatment of patients with obesity and NAFLD. Bariatric surgery methods result in reducing the risk of cardiovascular and tumor comorbidity and fatal outcome in the majority of patients with obesity and NAFLD, and furthermore, the effect on the reduction of steatosis, inflammation, hepatocyte ballooning and fibrosis in NASH patients has been demonstrated following the procedure[102,103]. In addition to efficiency, the safety of the procedure has not yet been sufficiently defined, and mortality is higher in patients with cirrhosis, primarily decompensated.

Liver transplantation

Nonalcoholic fatty liver disease complications in terms of cirrhosis and hepatocellular cancer development in the Western countries are the fastest growing indication for liver transplantation[2]. Due to significant comorbidities, this patient group is at an increased risk of surgical and post-transplantation treatment complications. With careful selection of transplantation candidates, recipient and graft survival following liver transplantation does not differ significantly from that in other indications[104,105]. Although recurrence of NAFLD features is common following liver transplantation, the risk of a progressive course of the disease is minimal. Treatment recommendations for liver recipients with NAFLD do not differ from those for the general population. The main reason is related to the fact that the number of controlled studies in this population is small and there is no knowledge of the long-term effects of therapy on the post-transplantation course of NAFLD and the associated comorbidity.

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- Croatian Family Medicine Association

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