CLINICAL PRACTICAL GUIDELINES FOR DIAGNOSTICS, TREATMENT AND MONITORING OF NON-ALCOHOLIC FATTY LIVER DISEASE

Riga, 2020

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Clinical practical guidelines for diagnostics, treatment and monitoring of non-alcoholic fatty liver disease

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PURPOSE OF THE RECOMMENDATIONS

The recommendations offer advice for the diagnosis, treatment and monitoring of non-alcoholic fatty liver disease. Their basis is an evidence-based medical opinion, thus improving the effectiveness of diagnosis and treatment and reducing complications and deaths.

RECOMMENDATION TASKS

- To introduce students to the essence, pathogenesis, clinic, diagnosis, treatment and observation of non-alcoholic fatty liver disease.
- To assist doctors in identifying and reducing risks of caring for patients with this disease.
- To recommend a common approach to treatment, control and evaluation of therapy effectiveness.

INTENDED USERS OF THESE RECOMMENDATIONS

Family doctors (general practitioners), internists, gastroenterologists, hepatologists, surgeons, residents of internal medicine and other specialties.

BENEFITS, ADVERSE EFFECTS AND RISKS WHICH MAY ARISE FOLLOWING THE RECOMMENDATIONS

Benefit

1. **Medical** - a rational approach to patients with non-alcoholic fatty liver disease reduces errors, complications, functional or permanent incapacity/disability and potential mortality, reducing overall both direct and indirect (faster diagnosis and treatment) medical costs.
2. **Social** - ensuring fulfilling/good quality of life for such patients.
3. **Financial** - justified, targeted and timely identification, diagnosis, treatment and dynamic follow-up of these patients reduce the number of complications and fatalities as well as the frequency of adverse reactions.

SCOPE OF THE CLINICAL RECOMMENDATIONS

Clinical recommendations for the first time in Latvia represent this type of guidance for patients with, or with clinical suspicion of non-alcoholic fatty liver disease. The authors consider that recommendations are necessary because the risk factors for the disease, which also affect the development of complications, may be predictable and partially preventable by providing the patient with recommendations of safe use of medications. These clinical recommendations are intended for the Latvian audience, but may be useful and applicable also in other countries. They are designed for medical service providers who encounter non-alcoholic fatty liver disease in their practice.

Explanatory table of standards of evidence and recommendations

Standard of evidence	Notes	Symbols
High quality	Further research is unlikely to change a view of a specific issue.	A
Medium quality	Further research may change the perception of a specific issue.	B
Low or very low quality	Further research is more likely to change the perception of a specific issue. Any conclusion is unclear and subject to change.	C

Standard of recommendations	Notes	Symbols
Strong recommendation	Good data quality, patient-relevant outcomes and rational costs are factors affecting the recommendation.	1
Weaker recommendation	Proposals and values are variable. The recommendation is less clear, may require higher costs and more resources.	2

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ABBREVIATIONS

AASLD - American Association for the Study of Liver Diseases
AP - alkaline phosphatase
BMI - body mass index
CAP - controlled attenuation parameter
EASL - European Association of Study of Liver
ELF - Enhanced Liver Fibrosis.
F - fibrosis
FIB-4 - liver fibrosis test
FLI - fatty liver index
GGT - gamma-glutamyltransferase
HbA1c - glycated haemoglobin
HCC - hepatocellular carcinoma
IH-MRS - magnetic resonance spectroscopy
IR - insulin resistance
MRI - magnetic resonance
NAFL - non-alcoholic fatty liver
NAFLD - non-alcoholic fatty liver disease
NAS - sum of NAFLD activity points
NASH - non-alcoholic steatohepatitis
NFS - NAFLD fibrosis score
SAF - steatosis, activity and fibrosis score
TMAO - trimethylamine-N-oxide
USS - ultrasonoscopy

DEFINITION

Non-alcoholic fatty liver disease (NAFLD) is characterised by excessive fat accumulation in the liver related to insulin resistance and is determined by histologic findings, where steatosis is found in >5% of hepatocytes.

CLASSIFICATION

NAFLD has 2 forms:

- **NAFL** - non-alcoholic fatty liver (steatohepatosis)
- **NASH** - non-alcoholic steatohepatitis. The aforementioned nosology has various manifestations and may gradually evolve over time in liver fibrosis, cirrhosis and as well as in hepatocellular carcinoma.

Other possible causes of liver disease, as well as alcohol consumption of more than 30 g/day for men and more than 20 g/day for women, should be excluded to detect NAFLD. Alcohol use beyond these limits tends to suggest alcohol-induced liver disease. A liver biopsy is required to confirm the diagnosis of NASH [1,2].

NAFLD spectrum and most frequent differential diagnosis (according to EASL Guidelines) [1]

<table>
<tr><th>NAFLD</th><th>Sub-classification</th><th>Differential diagnosis</th></tr>
<tr><td></td><td>NAFL:
• Only steatosis
• Steatosis and mild lobular inflammation</td><td rowspan="3">• Alcohol-related fatty liver disease
• Drug-induced hepatic impairment
• Fatty liver disease related to hepatitis C virus (genotype 3)
• Other causes of fatty liver disease:
» Hemochromatosis
» Autoimmune hepatitis
» Coeliac disease
» Wilson's disease
» Hypobetalipoproteinaemia
» Hypopituitarism, hypothyroidism
» Fasting, parenteral nutrition
» Congenital metabolic disorders</td></tr>
<tr><td></td><td>NASH:
• Early NASH
» F0-F1
• Fibrotic NASH
» Important F=>2
» Moderate F=>3
• NASH-related liver cirrhosis</td></tr>
<tr><td></td><td>Hepatocellular carcinoma</td></tr>
</table>

NAFLD classification (according to AASLD Guidelines) [3]

NAFLD	Includes the full spectrum of fatty liver disease in non-binge drinkers; spectrum from fatty liver disease to liver cirrhosis.
NAFL	Liver fat >5% but no evidence of hepatocellular damage, for example, ballooning degeneration or fibrosis. The risk of progression to liver cirrhosis and liver failure is minimal.
NASH	Liver fat >5% and has hepatocyte injury (ballooning degeneration) with/without cirrhosis. This form can progress to liver cirrhosis, liver failure and HCC.
NASH-related cirrhosis	Liver cirrhosis if you have a history of liver steatosis or NASH.
Cryptogenic cirrhosis	Liver cirrhosis without an obvious cause. These patients usually have several metabolic risk factors and metabolic syndrome.

RECOMMENDATIONS:

- Patients with insulin resistance and risk factors for metabolic syndrome should undergo biochemical and radiological examinations (in some cases all the way to liver punch biopsy) to exclude NAFLD (A1).
- For patients with detected hepatic steatosis other causes of hepatic impairment should be excluded and also the alcohol use should be assessed carefully (A1)

- It is necessary to bear in mind that along NAFLD other diseases can progress and they should be detected as this may result in more severe hepatic impairment (B1)

PREVALENCE UN INCIDENCE

NAFLD is the most common liver disease in Western countries, affecting 17-46% of adults (differences are determined by diagnostic methods used, age, gender, race) [4]. The prevalence of NAFLD can be assessed alongside metabolic syndrome and its components, which also increase the risk of the disease in both adults and children. NAFLD is also found in ~7% of people with a completely normal weight. More often these are young women who also have liver transaminases within normal limits. However, it is also recalled that they may also have liver disease progression, which may be related to the use of different medications or supplements, progression of metabolic syndrome, etc. [4,5]. The prevalence of NAFLD varies from country to country. This could be 20-86 cases per 1000 person-years based on increase in liver enzymes and/or USS and 34 cases per 1000 person-years based on MRI data [7].

At present, there is no evidence from literature data to support screening of the entire population. It is necessary to bear in mind also the high direct and indirect costs, the low predictive value of non-invasive tests, the risks of liver biopsies and the lack of effective treatment [8]. However, it should be remembered that there should be attempts to identify people at high risk (>50 years old, with type 2 diabetes) of an advanced form of NAFLD (for example, NASH), particularly related to pronounced hepatic fibrosis, because it is necessary to take into account the possible outcomes.

NAFLD incidence (according to AASLD Guidelines)

There is a lack of data on the total population of NAFLD.
Data from studies in Western countries:
- In a study in the UK, NAFLD incidence was 29 per 100,000 person-years, but the authors note that the number could actually be higher [8].
- A study from Israel found an incidence of 28 per 1,000 person-years [9].
- Separate meta-analysis data show that incidence in the West is 28 per 1000 person-years [10].

NAFLD prevalence (AASLD)

It should be noted that there is significantly more literature data on prevalence than on incidence.
Data from meta-analysis:
- Possible global prevalence of NAFLD could be 25.24% [3,10]
- The highest prevalence of NAFLD could be in the Middle East - 31.79% and in South America - 30.45%, but the lowest in Africa - 13.48% [3,10]

Often, the consequences of NAFLD is NASH. As aforementioned, liver biopsy is the standard for NASH diagnosis. The biopsy is carried out relatively rarely and therefore the amount of data is small and does not reflect the true number of NASH cases. However, data from separate studies indicate that:
- In NAFLD patients who underwent liver biopsy for clinical indications (e.g. for differential diagnostic purposes) NASH was found in 59.10% cases [10]
- In NAFLD patients who underwent liver biopsy without clinical indication, NASH was found in 6.67% to 29.85% cases [10].
- The prevalence of NASH in the general population is estimated to be between 1.5% and 6.45% [10]

RECOMMENDATIONS:

- All patients with hepatic steatosis should be tested for risk factors of metabolic syndrome, regardless of liver enzyme levels (A1)
- All patients with increase in liver enzymes should be examined for NAFLD as this is the most common cause of liver enzyme changes (A1)
- Patients with obesity and metabolic syndrome should have routine examinations to detect liver enzyme level and USS (A2)
- High-risk patients, for example, those over 50 years of age, with type 2 diabetes, metabolic syndrome, etc., should have examinations (biochemical, radiological, histologic) to exclude more severe forms of the disease (for example, NASH) (A2)

PATHOGENESIS

A high-calorie diet, excess fat and human consumption of carbohydrates, sugary beverages, dietary fructose are associated with weight gain and obesity, as well as NAFLD [1,2,11,12]. High dietary fructose levels increase the risk of developing NASH, speeding up the fibrotic process, although the association could be more related to a high-calorie or unhealthy and sedentary lifestyle, which also contributes to the risk of NAFLD development [12].

RECOMMENDATIONS:

- An unhealthy lifestyle (a diet high in fat and carbohydrates, sedentary lifestyle, etc.) plays an important role in the development of NAFLD (A1)
- NAFLD patient screening should evaluate the patient's diet and physical activity (A1)

ROLE OF MICROBIOME IN NAFLD DEVELOPMENT

There are several studies describing the possible relation to the microbiome with the NAFLD development. For example, patients with NAFLD more often have qualitative and quantitative changes of intestinal microbes (dysbiosis). Usually an increase of *Bacteroides* and *Ruminococcus* but lower *Prevotella* level is observed. Patients with stage 3 and 4 fibrosis have a higher *Eschirichia coli* and *Bacteroides vulgatus* level. There are also studies available showing that *E.coli* level is higher in children with obesity and proven NASH than in healthy individuals [13].

The role of various metabolites in pathogenesis should be considered when evaluating data of the possible relation, for example, patients with NASH who have increase in ethanol produced by microorganisms. Patients with NAFLD have increased TMAO and bile acid synthesis but decreased phosphatidylcholine synthesis [14,15].

It should also be noted that the microbiome and its metabolites could in the future be used as markers to help to identify and use NAFLD pathways for dynamic monitoring, as well as microbiome regulation could be one of the NAFLD treatment methods [15].

THE ROLE OF GENETIC FACTORS IN NAFLD DEVELOPMENT

Currently, several genetic factors related to NAFLD development have been identified, but only a small percentage has been statistically confirmed.

Best described is the relation to *PNPLA3* the gene identified in genome-wide association studies in multiple cohorts and various races that act as NAFLD severity modifier. Recently, the TM6SF2 gene has also been related to modifying the course of NAFLD and could be used in clinical practice to evaluate mortality due to liver disease or heart disease. PNPLA3rs738409 is also responsible for susceptibility to NAFLD development and affects the histology of NAFLD and fibrosis in obese children and adolescents. A combination of 3 polymorphisms in obese children with increase in liver enzymes that can be used to assess the risk of developing NASH has now been confirmed.

PNPL3 148M gene variant is functional and alters intrahepatocellular triglyceride breakdown and possibly enhances hepatic triglyceride synthesis. About 40% of Europeans are carriers of at least one variant of the allele. PNPLA3 genotyping was performed in one study, which concluded that it had a low predictive value for NAFLD risk when evaluated in combination with metabolic risk factors. Genotyping could be useful as a non-invasive test for NASH. NAFLD is increasingly being identified as the cause of HCC and is therefore needed to develop methods for selecting high-risk patients from low-risk patients who would not require resource-intensive monitoring programs. For example, carriers of homozygous allele (GG) of mucus accumulation in the major airways (PMPLA) among NAFLD patients have 5 times higher risk, but 12 times higher risk in the general population than in the UK general population to develop HCC. Based on cohort and genotype frequency data, a negative predictive value of PMPLA3 for CC versus *CG/GG* genotypes is 82% in NAFLD patients and 97% in the total British population. Although the positive predictive value is low, available research suggests that PNPLA3 genotyping (in combination with other clinical factors) may help to select patients to be monitored for the development of HCC, excluding those who have low risk [16,17].

The level of information about TM6SF2 is growing rapidly. It also appears to be a contributor to NAFLD fibrosis, but further data are still needed before it can be used in clinical practice. About 15% of Europeans are carriers of the rs585422926 allele in *TM6SF2* variants. This variant of the gene interferes with the hepatic secretion of very-low-density lipoproteins (VLDL) triglyceride (TG). Like the aforementioned gene PNPLA3, it also increases the risk of developing hepatic steatosis and NAFLD fibrosis. TM6SF2 makes it possible to distinguish the cause of death from pronounced fibrosis or cardiovascular disease because individuals carrying variant rs585422926 all have a higher risk of liver-related disease and death. Given that NAFLD is associated with an increased risk of cardiovascular disease, TM6SF2 genotyping could allow to access an individual risk and detect whether liver disease will be the determining reason for death [18,19].

Gene	**Steatosis**	**NASH**	**Fibrosis**	**HCC**	**Incidence of the Caucasian race**
PNPLA3	Yes	Yes	Yes	Yes	40%
TM6SF2	Yes	Yes	Yes	No	15%

RECOMMENDATIONS:

- PNPLA3 148M and TM6SF2 E167K variants are associated with a higher fat content in hepatocytes and an increased risk of developing NASH. NAFLD related to these variants is not systematically related to insulin resistance. Genotyping could be considered in individual patients and in clinical trials for diagnosis and prognosis but is not recommended on a routine basis and is not currently widely available in clinical practice. (B2)

DIAGNOSIS

Invasive investigation

Liver biopsy

Liver biopsy is absolutely necessary for NASH diagnosis and is the only method that can accurately distinguish NAFLD from NASH, despite the possibility of technical errors, when taking the sample [20]. Detection of alcoholic steatohepatitis also requires a liver biopsy, but it is necessary to bear in mind the anamnestic factors. Changes specific to NAFLD [21]:

- Only steatosis
- Steatosis with lobular or portal inflammation but without ballooning degeneration
- Steatosis with ballooning degeneration but without inflammation

To detect the diagnosis of NASH, it is important to determine [21-23]:

- steatosis,
- ballooning degeneration,
- lobular inflammation,
- *There may be other additional histological features - portal inflammation, polymorphonuclear cell leachates, Mallory-Denk bodies, apoptotic bodies, vacuolated nuclei, microvacuolar steatosis, megamitochondria, but finding these features is optional.*

The NAFLD Activity Score (NAS) should not be used for NASH, but primarily to assess the severity of the disease once the diagnosis is already made by histopathologic methods. A good alternative is the score of steatosis, activity and fibrosis (SAF) because it is well reproducible and describes the condition more accurately. SAF uses a simplified Kleiner's classification.

NB: See NAFLD classification on pp. 10-11!

Non-invasive investigation

Steatosis

The presence of steatosis should always be documented. Its existence also predicts that in the future there could be the development of diabetes mellitus, cardiovascular events and arterial hypertension. In clinical practice, liver fat concentrations are not detected and are therefore generally not recommended.

For identification of steatosis it is recommended:

1. For radiological examinations, USS is preferred because it is more easily accessible and less expensive than the gold standard MR. However, the USS method has limited sensitivity and is unreliable in patients with steatosis affecting less than 20% of the liver and in patients with pronounced high BMI (>40 kg/m^2) [24-26]. However, although USS is a subjective method and depends on the performer (including CT and MRI), it shows credibly enough moderate to severe steatosis, moreover the biliary system can be evaluated. It should therefore be the primary method of choice.
2. In larger population studies, serum-based biomarkers are preferred because they are progressively made available and cheaper.
   Among the best are fatty liver index (FLI), SteatoTest, NAFLD liver fat score. All these indices are validated in general populations or individuals with class 3 obesity and can predict metabolic, hepatic and cardiovascular outcomes and mortality. These indices are also associated with insulin resistance and statistically reliably predict hepatic steatosis [27]. *Read more about non-invasive tests in the Fibrosis section!* There is another method, CAP, which can diagnose liver steatosis but cannot distinguish between histologic grades and it has not been compared to steatosis measured by IH-MRS. Data from studies comparing the CAP method and USS are limited. It is therefore necessary to confirm the role of this method [1].

RECOMMENDATIONS:

- USS is first-line examination for NAFLD diagnosis (A1)
- In cases where imaging techniques (for example, large-scale epidemiological studies) are not available, serum biomarkers and indices for the diagnosis of hepatic steatosis may be considered as a suitable alternative (B2).
- The only reliable method for detection of liver fat is IH-MRS, but it is necessary to bear in mind that it is expensive and is not recommended in routine clinical practice (A1)

Non-alcoholic steatohepatitis

NASH diagnosis provides important information on the prognosis of the disease and may indicate an increased risk of fibrosis, liver cirrhosis and possible development of other conditions (HCC).

Liver biopsy is used to diagnose steatohepatitis as mentioned in the section on invasive investigation techniques. Clinical, biochemical and imaging techniques cannot distinguish NASH from steatosis. [28,29] Cytokeratin 18 (CK-18) fragments resulting from cell death (M65 fragments) or apoptosis (M30 fragments) have medium importance in the diagnosis of NASH (sensitivity 66%, specificity 82%) [30,31]. CK-18 changes in parallel with histological improvement, but is generally a no more valuable marker than alanine aminotransferase (ALT). However, to date, no non-invasive tests to diagnose NASH have been confirmed. [32]

RECOMMENDATIONS:

- A diagnosis of NASH can be made histologically when steatosis, ballooning degeneration of hepatocytes and lobular inflammation are detected (A1)

Fibrosis

Fibrosis - scarring process in the liver in response to liver damage. Fibrosis presence and stage is a major prognostic factor for NAFLD and correlates with liver-related outcomes and mortality. The presence of pronounced fibrosis identifies patients who require in-depth examination, confirmatory biopsy and intensive treatment and assessment of progression of fibrosis at various time intervals. Time intervals are not specified in the literature [33].

The use of non-invasive markers is recommended:

- in primary care, assessing the risk of NAFLD in patients with metabolic risk factors,
- in secondary and tertiary care, assessing the prognosis of the patients, such as severe NASH,
- to monitor disease progression,
- to predict a positive outcome of therapy.

Thereby, acting according to the aforementioned could significantly reduce the need for a liver biopsy.

Examples of non-invasive markers are the Fibrosis 4 (FIB-4) score calculator, NFS, Enhanced Liver Fibrosis (ELF) and *FibroTest* that predicts overall mortality, cardiovascular and liver-related mortality. NFS is related to an increased mortality risk. The more informative figures of these indices are in situations where severe fibrosis should be distinguished from mild fibrosis, but the significance of these tests is questionable in cases when it is necessary to distinguish between mild fibrosis and non-mild fibrosis. It is important to note that negative predictive values are higher than positive predictive values.

Therefore, non-invasive tests can be safely used as a method of choice to exclude serious illness. However, predictive values depend on the prevalence and most studies have been conducted in tertiary care centres where pre-test fibrosis possibility is higher than the general population [34,35].

Non-invasive liver fibrosis and cirrhosis tests [36]

Tests	**Description**	**Accuracy**	**Repeatability**	**Practical use**	**Limitations**
Aspartate transaminase (AST): alanine transaminase (ALT) ratio	AST, ALT	F3-AUROC 0.66-0.74 (sensitivity 40%, specificity 80%)	Not tested, but it should be taken into account that transaminase levels can change rapidly	High because the required parameters are often used	Medium accuracy
AST: platelet indices	AST, platelet count	F3-AUROC 0.74 (sensitivity 65%, specificity 72%)	Not tested, but it should be taken into account that transaminase levels can change rapidly	High because the required parameters are often used	Medium accuracy
Fibrosis-4 index	Patient age, AST, ALT, platelet count	F3-AUROC 0.80 (sensitivity 65%, specificity 97%)	Not tested, but it should be taken into account that transaminase levels can change rapidly	High because the required parameters are often used	—
NAFLD fibrosis index	Age, BMI, fasting glucose, AST, ALT, platelet count, albumin level	F3-AUROC 0.75-0.82 (sensitivity 73-82%, specificity 96-98%)	Not tested, but it should be taken into account that transaminase levels can change rapidly	High because the required parameters are often used	Should be taken into account different interpretations of BMI in patients of different races

BARD score	Presence of BMI, AST, ALT, diabetes mellitus	F3-AUROC 0.69-0.81 (sensitivity 62%, specificity 66%)	Not tested, but it should be taken into account that transaminase levels can change rapidly	High because the required parameters are often used	Should be taken into account different interpretations of BMI in patients of different races

As to imaging techniques, liver elastography shows a more reliable result in cases of cirrhosis (F4) than in pronounced fibrosis (F3). Elastography has a higher probability of false positives than false negatives. Therefore, its ability to distinguish bridging fibrosis from cirrhosis is not sufficient for decision making in clinical practice. [37] The major disadvantage of elastography is its dubious results in patients with high BMI. A study in Europe found that about 20% of tests had incorrect results, mainly in obese patients. The XL probe should be used to reduce these errors (up to 35%). [37-39]

There is currently no consensus on what is best use to avoid liver biopsies. [32]

There are data showing that the combination of elastography and serum markers produces more reliable results than just one method [40].

RECOMMENDATIONS:

- Biomarkers and fibrosis indices, as well as elastography, can be used as appropriate non-invasive methods to identify cases with a low risk of severe fibrosis or liver cirrhosis. (A2) The combination of these techniques significantly improves the accuracy of the diagnosis and can prevent unnecessary liver biopsies (B2)
- Progression of fibrosis can be monitored using a combination of biomarkers, a fibrosis index and liver elastography, but these methods still need to be accepted (C2)
- Identification of pronounced fibrosis and liver cirrhosis using serum biomarkers, fibrosis indices and liver elastography is less reliable and need to be accepted using liver biopsy - histologic examination (B2)
- Individual patients with high risk for progression of liver disease should undergo liver biopsy at a 5-year interval (C2)

COMMON METABOLIC CLINICAL SITUATIONS THAT ACCOMPANY NAFLD

NAFLD is closely related to insulin resistance not only in the liver but also in muscle and fat tissue [41]. It is also related to metabolic syndrome, which is defined by 3 of the following signs related to insulin resistance:

- Increase in fasting glucose or type 2 diabetes, hypertriglyceridemia
- Low levels of high density lipoprotein
- Increased waist circumference
- High arterial blood pressure [42]

All components of the metabolic syndrome correlate with liver fat level.

If the patient has metabolic syndrome, then the risk of NAFLD should be evaluated and vice versa - for the patients with NAFLD, the presence of components of the metabolic syndrome should be evaluated.

Accumulation of triacylglycerol in the liver is related to impaired metabolism and suppression of insulin-related hepatic glucose, a very low-density lipoprotein synthesis leading to hyperglycaemia, hyperinsulinemia and hypertriglyceridemia [43,44].

For patients without diabetes mellitus, HOMA-IR can serve as a surrogate indicator of IR and it is therefore an acceptable alternative to more expensive and time-consuming dynamic testing [45]. Progression of liver disease is related to existing or advanced metabolic disorders, including HOMA-IR [45,47]. However, it must be remembered that the reliability of HOMA-IR is based on the ability of insulin to adapt to IR, which raises questions about its usefulness in patients with diabetes mellitus. Moreover, test systems for measuring insulin levels are very diverse and there is no consensus on the analytical threshold.

RECOMMENDATIONS:

- HOMA-IR can be used as a surrogate marker to measure insulin resistance in non-diabetic patients (A1)
- The use of HOMA-IR is limited in patients with NAFLD and metabolic risk factors. However, it may confirm altered insulin sensitivity (B2)
- In some cases, HOMA-IR may help to identify patients at higher risk of NASH and fibrosis. Improvements in HOMA-IR during weight loss may indicate metabolic changes that may have a positive effect (at least reducing progression) of NAFLD (C2)

Obesity

Obesity - excessive accumulation of fat endangering health. Body mass index and waist circumference, reflecting visceral obesity, are related to the presence of NAFLD [48] and may indicate the severity of the disease, especially in the elderly [49]. Many patients for whom the cause of liver cirrhosis is not fully understood have several metabolic risk factors that could point to NASH as the cause of most of these cirrhosis [50,51]. NAFLD is also known to be related to other obesity-related conditions such as type 2 diabetes, sleep apnoea, polycystic ovary syndrome and other endocrine disorders such as hypogonadism.

It is important to note that in patients with BMI <30 kg/m^2 (or <25 kg/m^2), but who have visceral obesity and dysfunctional adipose tissue (for example, alterations in free fatty acid composition and adipocytokine secretion, which can lead to IR and its metabolic complications) and may also be NAFLD with/without altered liver enzymes [41,42]. Patients with isolated obesity (without other manifestations of the metabolic syndrome) may have similar gene expression as patients with metabolic disorders and may have altered liver enzymes as well as health problems in a forward-looking perspective [52,53].

RECOMMENDATIONS:

- Obese patients should be carefully evaluated as an important NAFLD risk factor related to IR (A1)
- Since most NAFLD patients also have insulin resistance and altered fat accumulation in the body, although less pronounced than patients with NAFLD and obesity, they should be closely monitored for disease progression (B2).

Type 2 diabetes

Patients with type 2 diabetes also have insulin resistance. They are often overweight and have elevated liver enzyme levels and tend to accumulate fat in the liver, regardless of BMI [54-56]. The prevalence of NAFLD is higher in patients with type 2 diabetes. It is based on glycated haemoglobin of 5.7-6.4%, altered fasting glucose (5.55-6.94 mmol/L) and/or altered glucose tolerance (7.77-11.04 mmol/L).

Therefore, an oral glucose tolerance test must be carried out for these patients

Type 2 diabetes is closely related to the severity of NAFLD, its progression to NASH, severe liver fibrosis and development of HCC, regardless of liver enzyme levels. The opposite correlation can be observed: individuals with NAFLD are 2-5 times more likely to develop type 2 diabetes [57-59].

Insulin therapy increases the amount of fat in the liver but, according to the available information, it does not worsen NAFLD in diabetic patients [60-61]. Although insulin administration results in dose-dependent increases in liver fat in patients with type 2 diabetes, long-term insulin administration improves IR in adipose tissue and therefore reduces the intrusion of non-esterified fatty acids and hepatic fat [62].

RECOMMENDATIONS:

- Patients with NAFLD should be closely monitored for the development of diabetes mellitus by fasting glucose or HbA1c (A1) and, if possible, by performing an oral glucose tolerance test on high-risk patients (B1).
- Patients with type 2 diabetes should definitely be tested for NAFLD, even if liver enzyme levels are within normal limits (A2)

DIAGNOSTIC ALGORITHM

Accidentally detected steatosis should facilitate a detailed personal and family history of NAFLD related diseases and exclude other secondary causes of steatosis. All components of the metabolic syndrome should be carefully evaluated [58]. Patients with obesity or type 2 diabetes mellitus and patients with metabolic risk factors with accidentally detected elevated liver enzyme levels should undergo non-invasive screening tests as soon as possible to assess the likelihood of steatosis, NASH and fibrosis

(see table).

Table (according to EASL Guidelines)[1]

Level		**Sign**
Basic	1.	Alcohol consumption <20 g/day for women, <30 g/day for men (see Table 3 in the Annex)
	2.	Personal and family history of diabetes mellitus, arterial hypertension and other cardiovascular diseases
	3.	BMI, waist circumference, weight fluctuations (dynamics)
	4.	Hepatitis B (VHB), Hepatitis C (VHC) infections
	5.	Use of steatosis enhancing medications (see Table 2 in the Annex)
	6.	Liver values (ALT, AST, GGT)
	7.	Fasting glucose level, Haemoglobin A1c (HbA1c), oral glucose tolerance test (OGTT)
	8.	Complete blood counts
	9.	Total level and level of high-density cholesterol and triglycerides, uric acid level
	10.	USS
Additional (if suspicion remains after clinical, laboratory or radiological examination)	1.	Ferritin levels and transferrin saturation
	2.	Celiac disease, thyroid disease and polycystic ovary syndrome investigation
	3.	Rare liver disease (Wilson's disease, autoimmune diseases, alpha-1 antitrypsin deficiency) investigation

For each NAFLD patient the aforementioned fibrosis surrogate markers (NFS, FIB-4, ELF, FibroTest) should evaluate to exclude the possibility of fibrosis (at least fibrosis II). If significant fibrosis cannot be ruled out, the patient should be referred to liver elastography. If it confirms significant fibrosis, a final biopsy should be performed to reach a final diagnosis. All patients with diabetes mellitus or risk factors for diabetes should be referred to an endocrinologist.

Patients with diabetes mellitus should also be made aware of the need to change their lifestyle. If the patient is considered obese, he/she should be involved in weight loss programs and/or referred to a gastroenterologist. In all cases, cardiovascular risk factors should also be evaluated for the patients.

Patient examination algorithm [1]

There are metabolic risk factors

Ultrasonography, biomarkers of steatosis, detection of liver enzymes

There are signs of liver steatosis

There is no evidence of hepatic steatosis

Liver enzyme levels within normal limits

Altered liver enzyme levels

Liver enzyme levels within normal limits

Serum biomarkers

Low risk

Medium/high risk

Control after 2 years

The patient should be referred to a specialist

Control after 3-5 years

Detection of liver enzymes and serum biomarkers

Causes of other liver damage should be excluded. The severity of the liver disease requires in-depth evaluation. A decision on the need for a liver biopsy. A decision on monitoring and treatment tactics.

Ultrasonography. Detection of liver enzymes.

NAFLD patients with no illness progression and no metabolic risk factors should be monitored every 2-3 years. Patients with NASH and/or hepatic fibrosis should be monitored once a year, while those with NASH-related cirrhosis should be monitored every 6 months or the purpose of early detection of decompensation, HCC, etc. A liver biopsy should be planned after the evaluation of the indications in each individual case. Moreover, liver transplantation is the only lifesaving treatment of liver cirrhosis.

Monitoring visits should include assessment of biochemical endpoints, comorbidities, should have non-invasive fibrosis tests and USS.

NATURAL COURSE OF THE DISEASE AND POSSIBLE COMPLICATIONS

Progression of the disease

For the most part, NAFLD is a slowly evolving disease. Rapid progression of fibrosis can be observed in only ~20% of patients [63]. The rate of disease progression could be one stage of fibrosis in 14 years for NAFL and one stage in 7 years for NASH patients. It is presumed that the rate in patients with arterial hypertension is doubling [63]. NASH is related to increased overall mortality when compared to the general population. Complications of liver disease are the third most common cause of death after cardiovascular disease and malignancy [64,65].

If the USS finding is the only measure of NAFLD, it is not related to an increased mortality rate, because it is believed to progress relatively rarely to NASH and fibrosis.

RECOMMENDATIONS:

- Patients with NASH with arterial hypertension should be monitored more frequently because they have a higher risk of developing NASH (B1)

Cardiovascular diseases

The prevalence and incidence of cardiovascular diseases in NAFLD patients are higher than in the general population and is related to the correlation between NAFLD and metabolic syndrome [66,67].

Cardiovascular mortality in NAFLD patients is higher than from liver damage. Patients with NAFLD have higher levels of biochemical markers of atherosclerosis (low high-density cholesterol, high triacylglycerol), higher levels of inflammatory markers (c-reactive proteins, CRPs) and higher levels of

procoagulant/prothrombotic factors than patients without steatosis [67]. Patients with NAFLD have been shown to have more pronounced pro-atherogenic lesions, such as:

- increased carotid *intima media* thickness,
- damage to coronary arteries,
- calcification of abdominal aorta and aortic valve,
- endothelial dysfunction and functional non-reactivity of the arterial walls [74].

Data show that these features correlate with the histological severity of liver damage. There are also data on other abnormalities such as electrocardiogram (ECG) and echocardiography (ECHO) changes and slower cardiac energy metabolism [68]. These factors are largely independent of traditional risk factors (see aforementioned), the duration of diabetes mellitus, glycaemic control, pharmacotherapy and the presence of components of the metabolic syndrome. In the general population, patients with USS found steatosis and associated surrogate markers (for example, FLI) have a higher long-term cardiovascular mortality and an increased risk of NASH and severe hepatic fibrosis [69,70]. The overall conclusion is that for NAFLD patients cardiovascular risk factors should be evaluated independently of traditional risk factors. Patients with high risk for cardiovascular disease should also undergo non-invasive NAFLD testing.

RECOMMENDATIONS:

- Given that cardiovascular diseases often determine the outcome of NAFLD, screening for these diseases is considered mandatory (at least with a detailed assessment of cardiovascular risk factors) (A1)

Hepatocellular carcinoma

There is a proven relation between obesity, type 2 diabetes and HCC and data about HCC in patients with NAFLD and idiopathic cirrhosis of the liver. The cumulative incidence of NAFLD-related HCC (ten times higher in patients with type 2 diabetes and obesity) varies according to the population studied: from 7.5% in 5-year patients with severe hepatic fibrosis or cirrhosis to only 0.25% in patients with a milder form of the disease [71].

At the time of diagnosis, patients with NAFLD-related HCC are older than patients with HCC related to other causes. They also have more concomitant illnesses, but less often cirrhosis (only 2/3). However, NAFLD-related HCC is often diagnosed at late stages because patients are less frequently monitored and there is no common treatment approach [72].

PNPLA3 rs738409 C>G gene polymorphism is related to a higher HCC development risk and may be important for risk stratification, but the study is not yet considered cost-effective.

RECOMMENDATIONS:

- Although NAFLD is a risk factor for HCC, HCC can develop without existing liver cirrhosis. Risk increases if PNPLA3 rs738409 C>G gene polymorphism is detected (B1)

Other extra-hepatic diseases

Among 20-50% of patients with NAFLD chronic kidney disease may also be detected, mainly in those with histologically confirmed NASH [73]. Patients with NAFLD and type 1 diabetes are 1.5-2 times more likely to develop chronic kidney disease than patients without the mentioned conditions [74].

Relation of NAFLD to colorectal cancer [75], metabolic bone diseases (vitamin D deficiency, osteoporosis), thyroid abnormalities and rare metabolic diseases such as lipodystrophy and glycogen storage disorders are also known. [57,76]

TREATMENT

Studies currently consider a histologic resolution of NASH as an indicator of the treatment efficacy.

Diet and lifestyle change

Evidence of epidemiological studies suggests a strong link between unhealthy lifestyles and NAFLD. Lifestyle change is mandatory for all patients. It should also be remembered that alcohol consumption <20 g/day for women and <30 g/day for men does not cause alcohol-related hepatic steatosis [77].

Weight loss of up to 7% can lead to histological improvement [78]. However, it should be taken into account that rapid weight loss could lead to serious complications - gallstones, liver damage and even hepatic insufficiency. A weight loss of no more than 4 kilograms per month would be recommended.

The best effect is achieved by limiting calorie intake and increasing aerobic exercise [79]. Complications of liver disease are the third most common cause of death after cardiovascular disease and malignancies. At present there is insufficient data on the long-term effects of lifestyle changes on the natural course of NAFLD.

Type	Suggestion	Data from literature
Energy limitation	Calorie restriction by 500-1000 kcal so the weight loss would not be more than 500-1000 g/week	Calorie restriction drives weight loss and the reduction of liver fat, independent of the macronutrient composition of the diet
	7-10% weight loss target	A 12-month intensive lifestyle intervention with an average 8% weight loss leads to significant reduction of liver steatosis
	A long-term maintenance approach, combining diet with physical activity	Regular lifestyle changes must be followed to maximize benefits
Macronutrient composition	Low-to-moderate fat and moderate-to-high carbohydrate intake	Preference should be given to the Mediterranean diet whose effect is also confirmed by 1H-MRS
	Low-carb or high-protein ketogenic diet	
Fructose	Avoid fructose-containing beverages and foods	In the general population, an association has been reported between high fructose intake and NAFLD
Alcohol intake	Strictly keep alcohol below the risk threshold (<20 mg daily for women and <30 mg daily for men)	In epidemiological surveys, moderate alcohol (namely, wine) below the risk threshold is associated with lower prevalence of NAFLD, NASH and lower fibrosis. However, it should be remembered that total abstinence of alcohol is mandatory for patients with liver cirrhosis to reduce the HCC risk
Coffee drinking	No liver-related limitations	Protective in NAFLD and other liver diseases
Physical activity	150-200 min per week of moderate intensity aerobic activities (stationery cycling, brisk walking) in 3-5 sessions	Physical activity follows a dose-effect relationship (frequency and duration), and if possible, it should be done when possible
	Resistance or strength training that promotes musculoskeletal fitness but has no effect on metabolic risk factors	

	Avoid inactivity-promoting fatigue and daytime sleepiness	Any engagement in physical activity is however better than continuing inactivity

Table adapted from: European Association for the Study of the Liver (EASL); European Association for the Study of Diabetes (EASD); European Association for the Study of Obesity (EASO). EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease.

Resistance or strength training increases both muscle strength and mass as well as bone strength and improves metabolism. Sufficient muscle strength allows a person to perform daily activities, such as climbing stairs, getting in and out of bed and carrying loads. Strength exercises increase muscle mass, making the muscle do more work than it is used to do on daily basis.

RECOMMENDATIONS:

- Development and use of structured lifestyle change programs that focus on both diet and daily physical activity (C2) is advised
- Patients without NASH and hepatic fibrosis should only be offered lifestyle changes and no pharmacotherapy (B2)
- 7-10% weight reduction is desirable for NAFLD and obese patients, which will be visible in the test results and also morphologically (B1)
- Dietary recommendations should focus on calorie restriction and the elimination of NAFLD-promoting foods (processed foods, high-fructose foods). Preferred to follow Mediterranean diet principles (B1)
- Both aerobic exercise and resistance exercises reduce the amount of fat in the liver. An appropriate exercise pattern should be sought for each patient that is easier to maintain in the long term (B2)

Pharmacotherapy

Drug therapy is indicated in patients with advanced NASH (bridging fibrosis and liver cirrhosis), but may also be considered in patients with early-stage NASH and increased risk of fibrosis (age >50 years, diabetes mellitus, metabolic syndrome, elevated ALT) or active NASH with high necroinflammatory activity [80,81]. None of the medications have currently passed phase 3 of the study and have not been formally approved for NASH treatment. As a result, there are no approved indications for any medication and any potentially applicable medication is used “beyond the officially approved indications” [82-84]. The prerequisites for pharmacotherapy are its safety and good tolerability since many patients have a variety of comorbidities and are at high risk for medication interactions. Some of the medications described below are used to treat type 2 diabetes. Since NAFLD and type 2 diabetes also share common pathogenic characteristics, such as the insulin resistance phenomenon, these medications could also improve the situation in patients with NAFLD. However, be aware that all medications may have side effects.

Biguanides and thiazolidinediones

Metformin has little effect on hepatic fat because it is unable to restore serum adiponectin levels in the short term [84]. Some preclinical data indicate the antitumor activity of metformin in liver cancer [85], although the finding is based on retrospective data and is not sufficient to provide evidence-based recommendations [86].

Thiazolidinediones are peroxisome proliferator-activated receptor (PPAR) agonists with an insulin-sensitizing effect. For patients using pioglitazone, histologic parameters such as ballooned degeneration, lobular inflammation, etc. (except for fibrosis) may improve and signs of NASH disappeared more rapidly than in the placebo group [87]. Histological improvement could be observed in parallel with the normalization of transaminase levels and partial adjustment of insulin resistance. Side effects of glitazone should be taken into account: weight gain, increased risk of bone fracture in women and rarely congestive heart failure. However, based on safety and tolerability data, pioglitazone may be recommended for individual patients with NASH, particularly in those who already have type 2 diabetes [88].

GLP-1 agonists

Incretin mimetics acting on the glucose-insulin interaction have shown promising results in the normalization of liver enzymes in premarketing studies [89]. Daily liraglutide injections did not increase hepatic fibrosis and could improve the histologic picture in NASH [90].

Essential phospholipids

Essential phospholipids have antioxidative and anti-fibrotic effects and are therefore have pathogenetically justified use in patients with NAFL and NASH. [91]

There is data showing that patients using the essential phospholipid agents have no further complaints such as pain on the right side, dyspeptic effects.

Data shows that bilirubin, transaminases, AP and GGT level, as well as cholesterol levels, are reduced with the use of essential phospholipid agents. There is data that shows possible improvement in histological parameters (steatosis, ballooned degeneration, inflammatory response). [91]

However, there is currently no consensus on the duration of therapy as well as the optimal doses.

Available data from the studies suggest that treatment may last from 1 month to 2 years, with doses ranging from 1 g per day to 1.8 g per day. However, in most studies, the duration of therapy is 3-6 months and the dose is 1.8 g per day to which the authors of these recommendations agree.

In recent year studies of patients receiving metformin and essential phospholipid agents show positive results. For example, AST, GGT levels, as well as lipid levels in the blood are reduced and ultrasound sonography (USG) show positive diagnosis. Data show that fibrinogenesis and steatosis are reduced by the use of essential lipid agents. [91]

It is important to note that these agents have no significant side effects.

Silymarin

One of the treatment options is the use of agents of the silymarin group. Silymarin is silybum marianum extract.

Silymarin has anti-inflammatory effects as it lowers TNF-α, IL-1β and IL-6 and has antioxidative effects. It also reduces insulin resistance [92,93].

From the available literature data it can be concluded that AST and ALT levels can be reduced and normalized with silymarin. Overall, there is a tendency for the disease to progress more slowly, with a lower risk of developing liver cirrhosis and HCC. [92,93]

Also for this group of agents there is no clear dose and duration recommendation. The studies are mainly based on 70-140-280 mg doses per day and treatment duration up to 12-24 weeks. There is also data on the use of high doses (600 mg daily), but usually in complicated conditions such as patients with liver cirrhosis. [94]

There is also information on the use of silymarin in combination with statin agents, where positive results were also observed and silymarin also reduced transaminase levels, which may be a side effect of statin use. [94]

Silymarin is safe to use and no significant side effects are observed even at high doses.

Antioxidants, cytoprotective and lipid level- lowering agents

Vitamin E (400-800 IU/day) induced an improvement in NASH and could improve steatosis, inflammation and ballooned necrosis [95]. Normalization of ALT correlated with histological improvement. Vitamin E could be used for NASH patients without diabetes mellitus and liver cirrhosis, but further studies are needed [96]. It should be noted, however, that high doses of vitamin E can be even dangerous, and their long-term use is debatable as they can be related to various side effects.

Several studies have been conducted on the use of ursodeoxycholic acid for NASH patients at different doses and for different duration of treatment (up to 2 years), but there are only modest improvements in biochemical parameters without histological improvement. The use of this medication could be particularly useful for NAFLD and NASH patients with cholestasis [87,97,98].

Data show that the administration of a synthetic farnesoid X receptor agonist obeticholic acid improves insulin resistance in patients with type 2 diabetes [99]. It was concluded that using obeticholic acid improves the histological picture and also reduces fibrosis. However, it should be remembered that low-density cholesterol may increase and itching of the skin may occur for the patients.

Available data on the use of pentoxifylline and orlistat are limited and currently do not provide a clear clinical justification for the use of these drugs.

Data on the use of lipid-lowering drugs suggest that they reduce cardiovascular risks without affecting NAFLD. Their use for NAFLD patients is safe and there is no increased risk of hepatotoxicity.

Data on ezetimibe use in NASH were negative [90,100]. They could even significantly reduce transaminase levels [101].

Studies of various new medications are also underway, but they are still in different stages of research. For example, medications with anti-inflammatory, anti-fibrotic and insulin-sensitizing characteristics (dual PPARα/d agonists, dual chemokine receptor (CCR) 2/CCR5) agonists and fatty acid/conjugated bile acids) and anti-fibrotic medications (similar to anti-lysyl-oxidase (anti-LOXL2) monoclonal antibodies).

RECOMMENDATIONS:

- Pharmacotherapy should be considered for patients with NASH, especially those with severe hepatic fibrosis (F2 and above), as well as those with less severe disease but at high risk of disease progression (diabetes mellitus, metabolic syndrome, persistently elevated ALT, high necroinflammatory activity) (B1)
- Although there are no strong recommendations, the use of pioglitazone in patients with NASH should be considered. Vitamin E should be administered with extreme caution, taking into account potential adverse reactions (B2)
- No data about optimal duration of treatment is available. Therapy should be discontinued in patients with initially elevated ALT if the reduction of transaminase is not achieved within 6 months. Duration of therapy in patients with pre-existing normal ALT is not known (C2)
- Use of statins is safe to lower low-density cholesterol and reduce cardiovascular risk, without benefit or loss for liver disease (B1)
- The use of hepatoprotectors (essential phospholipids and silymarin-containing compounds) could improve the biochemical parameters of the liver. They have no significant side effects. However, there is currently no consensus on the duration of therapy as well as the optimal doses.

Reducing the level of iron

The accumulation of iron in the liver is related to insulin resistance and lowering it decreases insulin resistance. Elevated levels of ferritin (>600) can often be observed in patients with NAFLD, although transferrin saturation may be variable and is not dependent on gene polymorphism as in hereditary hemochromatosis. In these patients, reaching a toxic level of ferritin may be helpful for lowering blood volume, thereby lowering ferritin levels and not worsening liver fibrosis [102]. However, there is limited data on this type of treatment in patients with NAFLD.

Bariatric surgery

Bariatric surgery to reduce weight and metabolic complications may be considered for patients who are not consistent with lifestyle change and medication therapy [103]. Surrogate markers indicate that bariatric surgery is effective in preventing NAFLD-related liver damage. There is also initial data that this therapy can improve necroinflammation and reduce fibrosis [104]. A recent study found that weight loss in 85% of patients within one year after bariatric surgery reduced the symptoms of NASH until its disappearance and reduced fibrosis by 34%. Although the potential benefit against the risk of peri-/postoperative complications should be evaluated [105]. At present, there is insufficient data on the effect of various bariatric procedures on liver fat content.

RECOMMENDATIONS:

- Because bariatric procedures reduce obesity and the risk of diabetes, they also reduce liver fat and are highly likely to slow the progression of NASH. There is evidence that they result in improved hepatic histology, incl. fibrosis (B1)

Liver transplantation

NAFLD-related liver cirrhosis is one of the three most common causes of liver transplantation. The life expectancy of 3 and 5 years of these patients is similar to as in case of transplantation for other reasons, although NAFLD is related to a higher risk of death from cardiovascular complications and sepsis, but a lower risk of transplant rejection [106,107]. The overall mortality risk is related to BMI and diabetes, because 50% of patients with BMI> 35 kg/m^{2}die within one year after transplantation [108].

RECOMMENDATIONS:

- Liver transplantation is suitable for NASH patients with end-stage liver disease. Life expectancy is similar to other transplant causes, although with higher cardiovascular mortality (A1)

ANNEX

Table 1. NAFLD incidence in Asian countries

Country	**Number of participants**	**Incidence**
South Korea	11448	12%
Japan	635	19.9 per 1000 person-years
China	565	13.5% (34 per 1000 person-years)

Table 2. Medicines that increase the risk of NAFLD development

Medicines and toxic agents	**Macrovesicular steatosis**	**Microvesicular steatosis**	**Steatohepatitis**
Amiodarone and other antiarrhythmics	+		
Chemotherapy preparations: • 5-FU • Irinotecan • Oxaliplatin		+	+ (irinotecan)
Tetracyclines		+	
Valproic acid		+	
Nucleoside reverse transcriptase inhibitors (NRTIs)		+	
Methotrexate	+		+
Tamoxifen	+		+
Aspirin (ASA)		+	
Steroid hormone medication	+	+	
Cocaine		+	
Alcohol	+		
Total parenteral nutrition	+		

Table 3. Alcohol units in different alcoholic beverages

Type of beverage	Alcohol units
Strong alcohol (vodka, gin, rum, whiskey, tequila) (40%) - low dose (25 ml)	1
Strong alcohol (vodka, gin, rum, whiskey, tequila) (40%) - high dose (35 ml)	1.4
Wine (white, pink and red) (12%) - a small glass (125 ml)	1.5
Wine (white, pink and red) (12%) - normal glass (175 ml)	2.1
Wine (white, pink and red) (12%) - large glass (250 ml)	3
Beer or cider (5%) - bottle (330 ml)	1.7
Beer or cider (5.5%) - bottle (440 ml)	2

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