The Belgian Association for Study of the Liver Guidance Document on the Management of Adult and Paediatric Non-Alcoholic Fatty Liver Disease

S. Francque[1,2], N. Lanthier[3], L. Verbeke[4], H. Reynaert[5], C. Van Steenkiste[6,7], L. Vonghia[1,2], W. J. Kwanten[1,2], J. Weyler[1,2], E. Trépo[8], D. Cassiman[5], F. Smets[9], M. Komuta[10], A. Driessen[11], E. Dirinck[2,12], E. Danse[13], B. Op de Beeck[14], E. van Craenenbroeck[15], Y. Van Nieuwenhove[16], G. Hubens[17], A. Geerts[4*], C. Moreno[8*]

(1) Department of Gastroenterology and Hepatology, Antwerp University Hospital, Antwerp ; (2) Laboratory of Experimental Medicine and Paediatrics (LEMP), Faculty of Medicine and Healthcare Sciences, University of Antwerp, Antwerp ; (3) Department of Gastroenterology and Hepatology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels ; (4) Department of Gastroenterology and Hepatology, University Hospital Leuven, Leuven ; (5) Department of Gastroenterology and Hepatology, Brussels University Hospital, Brussels ; (6) Department of Gastroenterology and Hepatology, Ghent University Hospital, Ghent ; (7) Department of Gastroenterology and Hepatology, Maria Middelares Ziekenhuis, Ghent ; (8) Department of Gastroenterology and Hepatology, ULB Erasme, Brussels ; (9) Department of Paediatrics, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, Brussels ; (10) Laboratory of Pathology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels ; (11) Department of Pathology, Antwerp University Hospital, Antwerp ; (12) Department of Diabetes, Endocrinology and Metabolic Diseases, Antwerp University Hospital, Antwerp ; (13) Department of Radiology, Cliniques Universitaires Saint-Luc, Université Catholique de Louvain, Brussels ; (14) Department of Radiology, Antwerp University Hospital, Antwerp ; (15) Department of Cardiology, Antwerp University Hospital, Antwerp ; (16) Department of Abdominal Surgery, Ghent University Hospital, Ghent ; (17) Department of Abdominal Surgery, Antwerp University Hospital, Antwerp.

Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is highly prevalent and associated with considerable liver-related and non-liver-related morbidity and mortality. There is, however, a lot of uncertainty on how to handle NAFLD in clinical practice. The current guidance document, compiled under the aegis of the Belgian Association for the Study of the Liver by a panel of experts in NAFLD, from a broad range of different specialties, covers many questions encountered in daily clinical practice regarding diagnosis, screening, therapy and follow-up in adult and paediatric patients. Guidance statements in this document are based on the available evidence whenever possible. In case of absence of evidence or inconsistency of the data, guidance statements were formulated based on consensus of the expert panel. This guidance document is intended as a help for clinicians (general practitioners and all involved specialties) to implement the most recent evidence and insights in the field of NAFLD within a Belgian perspective. (Acta gastroenterol. belg., **2018**, 81, **55-81**).

Key words : NAFLD, guidance, clinical practice, screening algorithm, treatment algorithm.

Abbreviations

AAR	AST/ALT ratio
AASLD	American Association for the Study of Liver Diseases
ALT	Alanine Aminotransferase
APRI	AST-to-Platelets Ratio Index
ARFI	Acoustic Radiation Force Impulse imaging
AST	Aspartate Aminotransferase
AUROC	Area Under ROC
BAAT	BMI-Age-ALT-Triglycerides Score
BARD	BMI-AAR-Diabetes score
BASL	Belgian Association for the Study of the Liver
BMI	Body Mass Index
CAP	Controlled Attenuation Parameter
CKD	Chronic Kidney Disease
CVD	Cardiovascular Disease
CI	Confidence Interval
CSE MRI	Chemical Shift-Encoded MRI
dB/m	Decibels per Meter
DM2	Type 2 Diabetes Mellitus
2D-SWE	2Dimensional Shear Wave Elastography
EASL	European Association for the Study of the Liver
ElastPQ	Elastography Point Quantification
ELF®	Enhanced Liver Fibrosis test
ESPGHAN	European Society of Paediatric Gastroenterology, Hepatology and Nutrition
FIB-4	Fibrosis-4
FLI	Fatty Liver Index
GGT	Gamma-GlutamylTransferase
HCC	Hepatocellular Carcinoma
kPa	KiloPascals
LR	Likelihood Ratio
MRI	Magnetic Resonance Imaging
NAFL	Non-Alcoholic Fatty Liver
NAFLD	Non-Alcoholic Fatty Liver Disease
NAS	NAFLD Activity Score
NASH	Non-Alcoholic SteatoHepatitis
NASH CRN	NASH Clinical Research Network scoring system
NFS	NAFLD fibrosis score
NPV	Negative Predictive Value
OSAS	Obstructive Sleep Apnoea Syndrome
PDFF	Proton Density Fat Fraction
PPV	Positive Predictive Value
pSWE	point Shear Wave Elastography
ROC	Receiver Operating Characteristic curves
SAF	Steatosis-Activity-Fibrosis score
ULN	Upper Limit of Normal
US	Ultrasound
VCTE	Vibration Controlled Transient Elastography

Disclaimer

The Belgian Association for the Liver (BASL) and the writing committee of the current guidance document are not responsible for the practices of physicians and provide guidance and guidance statements as indicators

of best practice only. Diagnosis and treatment remains at the discretion of physicians.

Non-Alcoholic Fatty Liver Disease (NAFLD) is highly prevalent and as such, physicians are frequently confronted with this clinical entity. There is, however, despite many research, a lot of uncertainty regarding disease pathophysiology and *a fortiori* on how to handle NAFLD in clinical practice. Therefore, besides increasing the awareness about and the knowledge of the disease (1), a guideline that addresses many questions encountered in daily clinical practice is timely and awaited by many physicians.

Some position statements, guidelines and guidance papers are available in the literature. As they are not always addressing all issues or do not always provide clear guidance, which is also applicable within the Belgian context, the Belgian Association for the Study of the Liver (BASL) took the initiative to write the current guidance paper. As high-quality clinical data are scarce or lacking on many relevant issues, it was decided to provide a guidance document with guidance statements and not a formal guideline, the latter requiring rating the quality of the evidence and the strength of the recommendations using validated systems, *e.g.* the Grading of Recommendations, Assessment Development, and Evaluation system (GRADE). A panel of experts in NAFLD, from a broad range of different specialties, assessed several relevant aspects of the clinical approach towards NAFLD and formulated guidance statements based on the available evidence whenever possible. In case of absence of evidence or inconsistency of the data, guidance statements were formulated based on consensus of the expert panel.

This guidance document is intended as a help for clinicians (general practitioners and all involved specialties) to implement the most recent evidence and insights in the field, but the care of the individual patient needs to be personalised and to integrate all knowledge of the physician on the patient's condition. The diagnostic and therapeutic management ultimately remains at the discretion of the treating physician.

Introduction

NAFLD is becoming the most important cause of chronic liver disease in the western countries. Liver steatosis is the hallmark of this condition, which regroups several potential severity stages according to the concomitant presence of liver inflammation and fibrosis.

Steatosis is defined as an abnormal amount of liver fat exceeding 5% of the total liver weight or 5% of hepatocytes containing lipid droplets (steatotic hepatocytes) on liver histology (2). This means that liver tissue and/or histology is needed for the diagnosis. However, as we will discuss further, in clinical practice, the presence of liver steatosis can also with reasonable accuracy be detected with radiological imaging modalities such as liver ultrasound, magnetic resonance

imaging (MRI) with proton density fat fraction (PDFF), controlled attenuation parameter (CAP) or computed tomography. Abnormalities in clinicobiochemical parameters are frequent findings at routine check-up or blood tests constituting potential reasons for steatosis screening.

The diagnosis of non-alcoholic steatosis also requires the exclusion of alcohol consumption as a cause for increased liver fat content. Indeed, alcohol consumption can induce steatosis and was the most common cause at the time NAFLD was first described, which also explains why this clinical entity was named non-alcoholic. The following cut-offs are usually applied for the diagnosis of NAFLD: a daily consumption equal or lower than 20 grams of ethanol per day (or 140 g per week) in women and equal or lower than 30 grams per day (or 210 g per week) in men (3). Moreover, many patients drink moderate amounts of alcohol (4), making the separation between metabolic and alcoholic liver disease sometimes troublesome. However, the effects of the metabolic components probably outweigh the attributive effects of alcohol (5,6). A standard drink with alcohol usually contains 8-10 grams of ethanol. Finally, while not included in the naming, the diagnosis of NAFLD also implies the exclusion of other secondary causes of steatosis such as medications (corticosteroids, methotrexate, amiodarone, tamoxifen, valproate), parenteral nutrition, viral disease (hepatitis B and hepatitis C, mainly genotype 3), genetic disease (alpha1-antitrypsin deficiency, a/hypobetalipoproteinaemia, Wilson's disease). The term NAFLD is hence reserved for patients with steatosis in a context of obesity, metabolic syndrome (the criteria are listed in Table 1) or high calorie intake (particularly high fat and high carbohydrate intake). Primary NAFLD and secondary steatosis may of course also coexist in some patients. Finally, NAFLD and NAFLD progression can be observed in lean patients. The risk factors for this so-called "lean NAFLD" are less well understood. Some patients have mild metabolic derangements, and (mostly unknown) specific genetic mutations in mitochondrial enzymes or other key metabolic pathways might in part be responsible for this phenotype of NAFLD (7).

As mentioned above, in some individuals, steatosis can be accompanied by necroinflammation, hepatocyte suffering and fibrosis. Liver fibrosis can lead to cirrhosis which can further evolve towards end-stage liver disease. Hepatocellular carcinoma (HCC) is a complication

Table 1. — **The criteria for the metabolic syndrome according to the International Diabetes Federation (170)**

Waist circumference	≥ 94/80 cm for men/women with ≥ 2 other criteria:
- arterial pressure	≥ 130/85 mmHg or treatment for hypertension
- fasting glucose	≥ 130/85 mmHg or treatment for hypertension
- serum triglycerides	> 150 mg/dl or treatment for dyslipidemia
- HDL cholesterol	< 40/50 mg/dl for men/women or treatment for dys-lipidemia

of cirrhosis but some cases of HCC in non-cirrhotic NAFLD have also been described or suspected (8). The term NAFLD includes all disease stages from simple steatosis without inflammation (Non-Alcoholic Fatty Liver, NAFL) to steatohepatitis (Non-Alcoholic Steatohepatitis, NASH) with or without fibrosis or cirrhosis (2).

NASH is defined by the coexistence of three components on liver histology : steatosis, inflammation within the liver lobules and hepatocyte ballooning, which is a feature of hepatocyte injury (9). This means that the diagnosis of NASH requires a liver biopsy. Further classification on disease severity (depending on the degree of necroinflammation or fibrosis) can be done by histological scoring (see below).

While NAFLD is estimated to be very common and affects an estimated 25% of the general population, NASH concerns 2.5-5% of the adult population (10). Among those patients with NASH, approximately 40% will develop progressive fibrosis (10). Fibrosis progression in patients with NAFLD without NASH at baseline also seems to exist but at a much lower rate (11,12). NASH is thus becoming a leading cause for liver transplantation (currently the second cause after hepatitis C in a recent report in the United States) and is predicted to become the first cause in the next years, due to the rising prevalence of obesity and metabolic syndrome together with the highly effective current therapies for hepatitis C (13).

Apart from those liver-related problems (development of NASH, fibrosis/cirrhosis, HCC and end-stage liver disease), NAFLD is associated with other complications affecting extra-hepatic organs. The majority of deaths in NAFLD patients are indeed related to cardiovascular disease (CVD) and, beyond the risk factors in common, NAFLD independently increases the risk of CVD (14). Other strong evidence exists for a causal link between NAFLD and type 2 diabetes mellitus (DM2) or chronic kidney disease (CKD) (15,16). Increasing evidence indeed exists supporting the fact that NAFLD itself participates in the pathogenesis of these complications, rather than being a simple marker of shared metabolic risk factors. Other diseases such as obstructive sleep apnoea syndrome (OSAS), colorectal and other cancers, osteoporosis, psoriasis and endocrinopathies such as polycystic ovary syndrome are also associated with NAFLD (17). Finally, NAFLD has been repeatedly reported to impair quality of life, both physically and mentally, compared to healthy controls or other hepatic disorders.

Due to the global obesity epidemic and the potential hepatic and extra-hepatic complications of NAFLD, economic analyses have identified enormous and growing health care costs (18). Screening and early treatment therefore seem of plausible benefit. The cost-effectiveness and long-term benefits of routine screening for NAFLD in high risk groups are, however, unknown.

Histology

As NAFLD and NASH have been defined based on liver histology, the liver biopsy remains the gold standard for an accurate diagnosis of NAFLD. Pathologists play hence an important role in NASH management. The task comprises two parts, histological diagnosis and grading of NASH and determining its prognosis by staging the fibrosis (3,19). This is particularly relevant, as steatohepatitis is considered the main driving force of disease progression and adverse outcomes, whereas fibrosis has been shown to be the strongest predictor of long term liver- and non-liver related morbidity and mortality. The diagnosis of NASH and its severity and the staging of the fibrosis are hence of major clinical importance.

Pathological diagnosis and pitfalls

In order to provide proper clinical management, accurate histological diagnosis is required. NASH is defined as a steatotic liver ($>$ 5% of hepatocytes containing fat vacuoles) associated with hepatocellular ballooning and lobular inflammation. The concomitant presence of these three criteria are mandatory to make the diagnosis of NASH. In contrast, NAFL doesn't have the complete picture, missing more specifically ballooning and/or inflammation (20).

Given that, recognizing these pathological alterations is crucial to make an accurate diagnosis. However, there are some issues to be addressed. First, small inflammatory infiltrates can be easily overlooked, which can make it difficult to distinguish between NASH and NAFL. Therefore, we usually use cluster-of-differentiation 68 (CD68) immunohistochemistry to check the presence of small clusters of macrophages, as they are usually seen in NASH but not in NAFL. Another diagnostic problem is the assessment of hepatocyte ballooning as there is no unequivocal definition of hepatocellular ballooning, including its severity. Hepatocyte ballooning results from degeneration of the cytoskeleton, making the hepatocyte losing its classical angulated shape. The pathologist should hence distinguish hepatocyte ballooning from the accumulation of lipid droplets.

Although histological aspects of NASH have been well documented, there are some issues that need to be considered at the time of diagnosis. First, NASH patients may mimic other chronic liver diseases such as viral hepatitis, drug-induced liver injury and autoimmune hepatitis. Therefore, if the histology presents severe lobular and/or portal inflammation with/without interface hepatitis, the pathologist should mention the possibility of other aetiologies to the clinician. In addition, NASH patients may combine several aetiologies of chronic liver diseases, such as the concomitant presence of chronic hepatitis C and NASH (21). NASH can be diagnosed as the only disease if the other causes are excluded. Second, the pathological changes are similar between NASH and alcoholic steatohepatitis, especially in the early phase.

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Therefore, clinical information is mandatory to come to the correct diagnosis. Finally, NASH in the cirrhotic stage often shows absence of the steatosis, although inflammation and ballooning can still be obviously present (22). A recent study showed that approximately 20% of cirrhotic patients were diagnosed as 'no NASH' in a large cohort of NAFLD-patients (21). Therefore, NASH should be considered as a differential diagnosis if the cirrhotic liver presents lobular inflammation with ballooned hepatocytes without steatosis. It should also be mentioned that in the cirrhotic stage, finally all features of NAFLD can disappear. Therefore, in patients with cryptogenic cirrhosis, especially in those with a history of metabolic risk factors, burned-out NASH is a likely cause of the established cirrhosis (22).

The role of a liver biopsy during follow -up is less well defined and the liver biopsy is not routinely repeated, except in patients at high risk for advanced liver disease.

Histological scoring system

The NASH Clinical Research Network (NASH CRN) scoring system, including the NAFLD Activity Score (NAS), and the Steatosis-Activity-Fibrosis (SAF) score are currently used for the diagnosis. The NASH CRN with the NAS is the most widely used system, especially in the United States (Table 2) (23) . This scoring system, originally developed for use in clinical trials, is nowadays also used for routine histological examination. The NAS score is the unweighted sum of steatosis, ballooning and lobular inflammation, hence ranging 0-8 (see Table 2). A definition of NASH has been based on the NAS: NAS 0-2 is not NASH, NAS 3-4 borderline NASH and NAS 5-8 definite NASH. This system does, however, not separate steatosis from necroinflammation, creating confusion about the term "activity". Furthermore, it gives a higher weight to lobular inflammation than to ballooning, although the latter is more important in defining steatohepatitis. Another problem is the grading of ballooning, based on the number of the ballooned cells, without, however, any clear definition of ballooning. This can easily cause interobserver variability in the diagnosis. The NAS score should hence not be used for diagnosis, but for the evaluation of the severity of the disease, as it was originally intended (Table 2). The SAF score, established by the Fatty Liver: Inhibition of Progression (FLIP) consortium, assesses 3 elements: steatosis (S), activity (A), and fibrosis (F) (Table 2) (20). The activity score is a combination of lobular inflammation and ballooning, both scored 0-2 and hence with an equal weight. Importantly, this

Table 2. — **Comparison between the histologic scoring of NAFLD according to NASH CRN system and SAF system. NAS : NAFLD Activity Score ; NASH CRN : Non-Alcoholic Steatohepatitis Clinical Research Network Scoring System ; SAF : Steatosis-Activity-Fibrosis.**

	Score	NASH-CRN (23)	SAF (20)	SAF Score
Steatosis	0	< 5%	< 5%	0
	1	5% - 33%	5% - 33%	1
	2	> 33% - 67 %	> 33% - 67 %	2
	3	> 67%	> 67%	3
Lobular inflammation	0	No foci	No foci	0
	1	< 2 foci/20X	< 2 foci/20X	1
	2	2 – 4 foci/20X	> 2 foci/20X	2
	3	> 4 foci/20X		
Ballooning	0	No ballooning	Normal hepatocytes	0
	1	Few ballooned cells	Clusters of rounded, pale hepatocytes	1
	2	Many ballooned cells	Many enlarged (2X normal size) hepatocytes	2
Portal inflammation	0	None	-	
	1	Mild	-	
	2	More than mild	-	
Fibrosis	0	No fibrosis	No fibrosis	0
	1	1a Mild, zone 3 perisinusoidal/pericellular fibrosis 1b Moderate, zone 3 perisinusoidal/ pericellular fibrosis 1c Portal/periportal fibrosis	1a Mild, zone 3 perisinusoidal/pericellular fibrosis 1b Moderate, zone 3 perisinusoidal/pericellular fibrosis 1c Portal/periportal fibrosis	1
	2	Perisinusoidal/pericellular and portal/ periportal fibrosis	Perisinusoidal/pericellular and portal/periportal fibrosis	2
	3	Bridging fibrosis	Bridging fibrosis	3
	4	Cirrhosis	Cirrhosis	4
Composite score for activity	0-8	NAS = NAFLD Activity Score = steatosis + ballooning + lobular inflammation	A = ballooning + lobular inflammation	0-4

score provides a clearer definition of the severity of ballooning by assessing the exact hepatocellular size: if the hepatocyte's size is twice as big as the normal size, it is considered as severe ballooning. Fibrosis staging (F) is comparable to the NASH CRN. The SAF score is an easy-to-use scoring system with well-defined criteria, and has high reproducibility. This scoring system is appropriate for routine diagnosis and clinical trials. Although the European Association for the Study of the Liver (EASL) (3) and the American Association for the Study of Liver Diseases (AASLD) (19) guidelines mention both scoring systems, based on these aspects we encourage to use the SAF score for the NASH diagnosis.

Furthermore, the definitions of significant or advanced fibrosis deserve to be mentioned, as it is of relevance for this guidance document. Significant fibrosis is defined as a fibrosis stage of 2 or more, whereas advanced fibrosis refers to F3 or 4.

Besides the 4 lesions mentioned, other lesions can be present and will be reported by the pathologist (*e.g.* the presence of Mallory-Denk bodies (which are hence not pathognomonic for alcoholic steatohepatitis), megamitochondria, eosinophilic bodies and portal inflammation (which has been linked with the severity of the disease and with a worse prognosis in terms of fibrosis progression)).

NASH in children

The histological aspect of NASH is different between adult and paediatric patients with more prominent portal inflammation, periportal macrovesicular steatosis, absence of ballooning and more periportal fibrosis in children (the so-called type 2, whereas the type 1 is the classical adult NASH phenotype) (24). In children, a liver biopsy is indicated in case of doubt on the clinical diagnosis of NAFLD, the possibility of a different or associated chronic liver disease, before starting pharmacological therapy or to evaluate the changes in liver histology after therapy (25). The Paediatric NAFLD Histological score has been developed for NASH in children. As the diagnosis is based on a calculated algorithm of its morphological features, pathologists have some doubt about the routine use of this scoring system (26,27).

Liver biopsy quality criteria

Accurate diagnosis requires a good quality biopsy, which ideally implies a length of at least 2 cm, a width of 2 mm (16 G needle) and ≥ 10 portal tracts (28). Biopsies that do not fulfil these criteria, give a higher risk of sampling variability or interobserver variability. These risks can, however, be reduced by the experience of the pathologist or by consensus diagnosis.

Guidance statement: Liver biopsy remains the gold standard for an accurate diagnosis of NAFLD (with grading its severity and staging the fibrosis) and for the exclusion of other chronic liver diseases. It is not routinely repeated except in patients at high risk of advanced liver disease during long-term follow-up. Ideally a core biopsy should be at least 2 cm in length and 2 mm width (16G needle). For the biopsy report, it is highly recommended to use, in addition to the description of the observed lesions, the SAF scoring system along with the NASH CRN.

Non-invasive assessment

Non-invasive methods rely on two different approaches: a "biological" approach based on the quantification of biomarkers in serum samples or a "physical" approach based on the measurement of liver stiffness. Isolated parameters usually do not reach diagnostic accuracy indices that are satisfactorily and consistent over different cohorts. Therefore, a combination of tests is likely to represent a diagnostic improvement for non-invasive assessment over individual tests.

Several non-invasive methods aim at diagnosing and quantifying hepatic steatosis, while others were designed to predict NASH or significant/advanced fibrosis. As one of the main issues in clinical practice is to make an adequate assessment of fibrosis in the management of patients with NAFLD, most extensive data are available for non-invasive fibrosis markers.

In this section, we will give a guidance on which tests can be useful for non-invasive assessment of either steatosis, steatohepatitis and fibrosis.

In general, a good screening test should have a high sensitivity. The most common approach to evaluate a test is the analysis of the Receiver Operating Characteristic (ROC) curves and the area under ROC (AUROC), which evaluates the probability of a test to identify a true positive against the probability to find a false positive result. When the AUROC is higher than 0.8, it suggests good accuracy. Optimal cut-offs can then be derived to be used for patient classification and clinical decision making.

Steatosis

The prediction of fatty change in the liver from general laboratory test values has been investigated and various indices have been proposed. The best-validated steatosis scores are the Fatty Liver Index (FLI) (29) (https://www.medicalalgorithms.com/fatty-liver-index-fli-of-bedogni-et-al-for-predicting-hepatic-steatosis), the NAFLD Liver Fat Score (30) and the SteatoTest® (31) (Table 3).

The SteatoTest® incorporates 12 variables in an undisclosed formula (Table 3) (31). Poynard showed a 0.79 AUROC for moderate-to-severe steatosis (steatosis > 33%), a good negative predictive value (NPV) of 93% but a low positive predictive value (PPV) of 63%. Disadvantages of this test are first that it is patented,

Table 3. — **Non-invasive test for Steatosis. ALT : alanine aminotransferase ; AUROC : area under the curve of receiver operator characteristic ; BMI : body mass index ; FLI : Fatty Liver Index ; GGT : gamma-glutamyl transpeptidase ; Sens : sensitivity ; Spec : specificity.**

Test acronym	Reference	Formula	Diagnostic performance
SteatoTEST	Poynard 2005 (31)	Undisclosed formula including : alfa2-macroglobulin, haptoglobin, apolipoprotein A1, total bilirubin, GGT, fasting glucose, triglycerides, cholesterol, ALT, age, gender and BMI	AUROC 0.79 for steatotis > 33%; 2 cutoffs: 0.3 and 0.72 ; Sens 90%, Spec 70%, PPV 63%, NPV 93%
Fatty Liver Index (FLI)	Bedogni 2006 (29)	$(e^{\,0.953*\log e\,(\text{triglycerides})\,+\,0.139*\text{BMI}\,+\,0.718*\log e\,(\text{GGT})\,+\,0.053*\text{waist circumference}\,-\,15.745}$ / $(1 + e^{\,0.953*\log e\,(\text{triglycerides})\,+\,0.139*\text{BMI}\,+\,0.718*\log e\,\,(\text{GGT})\,+\,0.053*\text{waist circumference}\,-\,15.745}) * 100$	AUROC 0.84 ; 2 cutoffs : < 30 for excluding and > 60 for ruling in ; Sens 87%, Spec 86%
NAFLD Liver Fat Score	Kotronen 2009 (30)	-2.89 + 1.18 x (metabolic syndrome yes = 1/ no = 0) + 0.15 x (fasting serum insulin, mU/L) + 0.04 x (AST, IU/L) – 0.94 X (AST/ALT)	AUROC 0.87; 2 cutoffs : -1.413 and 1.257; Sens and Spec 95%

and second that it incorporates parameters that are not routinely used.

Bedogni proposed the FLI in 2006 (29). The FLI is calculated through a formula including body mass index (BMI), waist circumference, triglycerides and gamma-glutamyltransferase (GGT). It was tested mainly in large epidemiological studies with AUROC values of 0.84 (compared to ultrasound as gold standard).

The NAFLD Liver Fat Score derived from a Finnish population (30) and includes the presence of the metabolic syndrome, the presence of DM2, fasting serum insulin, aspartate aminotransferase (AST) and AST/alanine aminotransferase (ALT) ratio (AAR). It yielded 95% specificity and 95% sensitivity.

Guidance statement : FLI and NAFLD Liver Fat score use simple indices and therefore could be useful for the diagnosis of steatosis in large-scale epidemiological studies whenever imaging tools are not available. They can also useful in routine clinical practice to diagnose steatosis (although this can also be done by ultrasound) but they are not useful for the quantitative estimation of liver fat. Formulas, cut-offs and interpretation are listed in Table 3.

Steatohepatitis

The rationale to provide non-invasive tests to identify NASH is that the diagnosis of NASH prompts a closer follow-up and indicates a risk of fibrosis progression.

Aminotransferase levels are not reliable in identifying NASH, with low AUROC of 0.6-0.7 (32). If abnormal so called "liver function tests" (although they do not reflect liver function, so the use of this wording should be discouraged) are present, this usually means mildly raised transaminases (ALT > AST) and/or GGT. However, many NAFLD patients have normal-range ALT levels (32,33). One of the problems is also the definition of the upper limit of normal (ULN), as labs usually define this ULN based on a reference "normal" population. This reference "normal" population includes patients with overweight or obesity, whom are known to have higher transaminase levels, hence a ULN based on such

a reference population are too high. Using these ULN will hence underestimate the true prevalence of liver abnormalities and erroneously classify patients as within the normal range. The American Gastroenterological Association recently proposed new cut-offs that need to be implemented in clinical practice: values above 19-25U/L for women and 29-33 should be considered abnormal (34).

Even if elevated, the ALT typically falls as fibrosis progresses to cirrhosis. ALT values do not or only poorly correlate with histological findings and are hence not helpful in both the diagnosis of NAFLD and determining disease severity.

Guidance statement : Liver tests correlate poorly with histological lesions and "normal" transaminases do not exclude significant liver disease.

The lab reference ULN for transaminases are usually too high and for ALT, values above 19-25 U/L for women and 29-33 U/L for men should be considered abnormal.

Multiple serum biomarkers have been evaluated for predicting NASH. Most biomarkers failed to demonstrate accuracy. Cytokeratin 18 fragments is the only biomarker validated in more than 1000 NAFLD patients and is the most consistent single parameter for differentiating steatosis from NASH. Plasma levels of Cytokeratin 18 fragments predict the apoptosis of hepatocytes, which is further related to the pathogenesis of NAFLD. An AUROC of 0.82 was shown in a meta-analysis with 78% sensitivity and 86% specificity (35).

Blood tests that accurately reflect NASH and that can be used in screening, diagnosis, follow-up and treatment constitute an active area of research but need to be tested and validated in different conditions and within large consortia before they can be reliably implemented in clinical practice (36).

Guidance statement: To date, non-invasive tests cannot reliably be used solely for the diagnosis of NASH.

Fibrosis serum biomarkers

Many serum biomarkers have been proposed for staging liver fibrosis, mostly validated in patients with

Table 4. — **Non-invasive serum tests for fibrosis. ALT : alanine aminotransferase ; AST : aspartate aminotransferase ; AUROC : area under the curve of receiver operator characteristic ; BMI : body mass index ; DM : diabetes mellitus ; ELF : Enhanced Liver Fibrosis ; GGT : gamma-glutamyl transpeptidase ; IFG : impaired fasting glucose ; NPV : negative predictive value ; PPV : positive predictive value ; Sens : sensitivity ; Spec : specificity ; ULN : upper limit of normal.**

Test acronym	Reference	Formula	Diagnostic performance
BAAT score	Ratziu 2000 (37)	Score calculated by the sum of 4 risk factors: age > 50 years, BMI > 28 kg/m², triglycerides > 1.7 mmL/l, ALT > 2XULN	Sens 14%, Spec 100%, PPV 100%, NPV 73%
BARD score	Harrison 2008 (40)	Formula includes 3 variables: BMI >28 kg/m² = 1 point; AST/ALT ratio > 0.8 = 2 points; diabetes = 1 point; if score > 2 odds ratio for advanced fibrosis = 17	AUROC 0.81 for advanced fibrosis; Score 2-4: PPV 43%, NPV 96%
NAFLD fibrosis score	Angulo 2007 (41)	-1.675 + 0.037 x age (year) + 0.094 x BMI (kg/m²) + 1.13 x IFG/DM (with=1, without=0) + 0.99 x AST/ALT ratio -0.013 x platelets (x109/L) – 0.66 x Alb (g/dL) (available online measurement at website www.nafld.com)	AUROC 0.84 for advanced fibrosis; cutoffs shown in Table 3
FIB-4 index	McPherson 2010 (42)	age (years) x AST (IU/L)/platelet count x ALT (IU/L)	AUROC 0.86 for advanced fibrosis; cutoff 1.3; Sens 85%, Spec 95%, NPV 95%
ELF score	Rosenberg 2004 (45)	Proprietary formula including age, hyaluronic acid, amino-terminal pro-peptide of type III collagen (ProCIII) and tissue inhibitor of metalloproteinases (TIMP-1)	AUROC 0.87 for advanced fibrosis; cutoff 0.375; Sens 89%, Spec 96%, PPV 80%, NPV 90%
FibroTest	Ratziu 2006 (46)	Undisclosed formula including age, alfa2-macro-globulin, bilirubin, GGT and apolipoprotein A1	AUROC 0.76-0.86 for significant fibrosis; 2 cutoffs: 0.3 and 0.7; Sens 77%, Spec 98%, PPV 90%, NPV 73%

chronic hepatitis C.

Simple, non-invasive fibrosis scores derived from routine clinical and biochemical indices, such as BMI-Age-ALT-Triglycerides (BAAT) score, AST-to-platelet ratio index (APRI), AAR, BMI-AAR-Diabetes (BARD) score, NAFLD fibrosis score (NFS) and Fibrosis-4 (FIB-4) index have shown promise, particularly for excluding advanced fibrosis. The tests perform best at distinguishing advanced ($\geq$ F3) versus non-advanced fibrosis (<F3).

Ratziu *et al.* proposed the BAAT score, incorporating BMI, age, ALT and triglycerides. This test has a high specificity of 100%, but a very low sensitivity of 14% (Table 4) (37).

The APRI ((AST/ULN of AST/Platelet count (10⁹)) x100), originally developed for hepatitis C patients, has also been suggested as a useful strategy for predicting significant fibrosis due to NASH (38,39).

The AAR was also shown to be useful as a simple method of identifying patients with advanced fibrosis. A cut-off of > 0.8 seems sensitive in NAFLD patients. AAR < 0.8 had a high predictive ability to exclude advanced fibrosis (AUROC of 0.83, sensitivity of 74% and sensitivity of 78%) (35).

The BARD score includes BMI, AST/ALT ratio and presence of DM2. In a large cohort of NAFLD obese patients with NAFLD, the presence of at least 2 of these factors increased the risk for advanced fibrosis by 17-fold, with high NPV (Table 4). However, in a typical NAFLD cohort, a large proportion of patients with mild disease have a score of >2 due to obesity and diabetes, which limits its utility in clinical practice (40).

The NFS (http://www/nafldscore.com) (Table 4) (41) has been confirmed to be useful in predicting the progression of fibrosis regardless of whether the ALT level is normal or abnormal, also including morbidly obese individuals and ethnically different populations with consistent results. NFS has shown to predict overall mortality, cardiovascular mortality and liver-related mortality. Advanced fibrosis can be reliably excluded (NPV 93%) using the low cut-off score (< -1.455) and diagnosed with high accuracy (PPV 90%) using the high cut-off score (> 0.676). The advantage of this score is that it contains no items that require a special test, is easy to use in routine clinical care and has been validated in many studies. However, the score is intermediate in approximately 25-30% of the patients, for whom there is further investigation needed.

The FIB-4 (Table 4) (42) was proposed as a parameter of the progression of fibrosis in patients with human immunodeficiency virus/hepatitis C co-infection. This index is advantageous because it is also based on test values that are routinely measured in health check-ups, the number of items is small, and the index is not influenced by the BMI. The usefulness for patients with normal ALT is comparable to that for patients with abnormal ALT. For stage 3-4 fibrosis, a score < 1.30 has a NPV of 90% and a score > 2.67 has an 80% PPV. The test reaches an overall AUROC of 0.86 for advanced fibrosis.

NFS and FIB-4 were principally developed and validated in patients aged between 35 and 65 years of age. A recent study showed significant fall in specificity for advanced fibrosis in older patients ($\geq$ 65 years), resulting in a high false positive rate for advanced fibrosis (43). Using new thresholds (lower cut-offs for the NFS score and FIB-4) in patients aged $\geq$ 65 years increased the specificity for advanced fibrosis using

the NFS and FIB-4 to 70%, effectively controlling the false positive rate without adversely inflating the false negative rate of the test (43). For patients ≤ 35 years there is still an unacceptable low accuracy of the NFS and FIB-4. Another problem is that these scores have been mainly developed to diagnose advanced fibrosis (F3-4 according to NASH CRN and SAF) but, as we will discuss further on, significant fibrosis (≥ F2) is currently considered clinically relevant (44), which implies that some patients with significant fibrosis will not be picked up by the NFS and FIB-4. There are currently no revised cut-offs to solve this problem, which is one of the reasons why patients at risk need a follow up even if screening is negative (see below).

Complex fibrosis panels that include markers of matrix turnover have shown also some promise for the assessment of liver fibrosis in NAFLD. The European Liver Fibrosis Study group proposed the ELF® (Enhanced Liver Fibrosis) score (Table 4) (45). This score combines age, hyaluronic acid, amino-terminal pro-peptide of type III collagen (ProCIII) and tissue inhibitor of metalloproteinases (TIMP-1). This test showed a high AUROC of 0.87 for advanced fibrosis in NASH. The FibroTest® is an undisclosed formula incorporating age, alpha2macroglobulin, bilirubin, GGT and apolipoprotein A1 (46,47). It has shown AUROC for significant fibrosis between 0.75 and 0.86 in NAFLD patients. The disadvantage of these complex scores is that they are difficult to use in clinical practice because of the inclusion of not-routine parameters. Furthermore, they are patented and not reimbursed in Belgium. Therefore, their use is limited in daily practice.

Among the different serum biomarkers studied in NAFLD, only NFS and FIB-4 have been externally validated more than once, in different NAFLD populations and with consistent results. The use of these simple non-invasive fibrosis scores such as the NFS and FIB-4 score to identify or exclude advanced fibrosis (with NPV > 90%) as part of a staged approach to diagnosis and risk stratification in patients with NAFLD is now widely adopted and recommended by most guidelines. These scores can reliably provide a useful, inexpensive first-line identification of patients with a low risk of severe fibrosis/cirrhosis for use in primary or secondary care. Several algorithms have been proposed. A combination of the NFS and the FIB-4 using their low cut-off values to rule out significant disease is proposed in Figure 1. A recently developed web application using the same scores, along with the FLI and based on a comparable algorithm, provides an easy-to-use first line screening tool based on the aforementioned data (www.antwerpnafldguide.com). Other tests like ELF® of FibroTest® can be used according to local practice and expertise, taken into account the limitations as discussed.

Guidance statement : The FIB-4 and the NFS are based on routinely available parameters and can be used to rule-in or rule-out advanced fibrosis using appropriate and age-adjusted cut-offs and with the restriction that their accuracy is unacceptably low at age ≤ 35 years. Their combined use can increase accuracy. Other tests (ELF®, FibroTest®,…) can be used according to local expertise, but are proprietary and not reimbursed.

Liver Stiffness measurement

Liver fibrosis can be staged using 1-dimensional ultrasound vibration controlled transient elastography (VCTE) (Fibroscan®, Echosens, Paris, France), which measures the velocity of a low-frequency (50 Herz) elastic shear wave propagating through the liver. The stiffer the tissue, the faster the shear wave propagates. The final result of the VCTE session can be regarded as valid if the following criteria are fulfilled: a number of valid measurements of at least 10, a success rate of valid measurements to the total number of measurements above 60%, an interquartile range (reflecting the variability of measurements) less than 30% of the median liver stiffness measurement value. The results are expressed in kilopascals (kPa), and range from 1.5 to 75 kPa, with normal values around 5 kPa. Failure to obtain any measurement has been reported in up to 20% of the cases, mostly due to obesity or limited operator experience.

VCTE is currently the most common technique used to determine the fibrosis stage of patients with NAFLD. VCTE has been investigated in NAFLD patients but in smaller number of studies than viral hepatitis. VCTE performances are better for cirrhosis than for significant fibrosis, with AUROCs ranging from 0.94 to 0.99 and from 0.79 to 0.99 respectively. However, a lot of differences in study design and populations are likely the explanation for the observed differences among proposed cut-offs for a given endpoint (ranging, for instance, from 10.3 to 22.3 kPa for cirrhosis).

Wong *et al.* (48) demonstrated the accuracy of VCTE in diagnosing NAFLD patients, considering a cut-off value of 9.6 kPa as a reliable indicator for advanced fibrosis or cirrhosis, and the specificity reached 91.6%. A meta-analysis in 2014 (49) indicated that VCTE is excellent in diagnosing ≥ F3 (85% sensitivity, 82% specificity) and F4 (92% sensitivity, 92% specificity) and has a moderate accuracy for ≥ F2 in NAFLD. The latter represents a clinical problem, which is currently unsolved, because the clinical need is also in the identification of significant fibrosis, for which hence other cut-offs are needed and the accuracy is anyhow lower due to the important overlap in stiffness values in the intermediate fibrosis categories.

The main shortcoming of VCTE is unreliable results in the presence of high BMI and/or thoracic fold thickness. Therefore, the XL probe has been developed, decreasing the failure rate in obese subjects from 35% to 6%. A caveat with the XL probe is that median measurements were 1.3 to 1.68 kPa lower as compared to the M probe, and therefore different cut-offs will be needed.

Table 5. — **Cutoff values of vibration-controlled transient elastography (VCTE)(48,51,50,39). kPa : kilopascal ; NPV : negative predictive value; Sens: sensitivity ; Spec : specificity.**

Type of probe	Stage of fibrosis	Reported Cutoffs (kPa)	Sens (%)	NPV (%)	Spec %	Proposed cutoff (kPa)
M probe	≥2	6.7-7.8	67-88	84	61-84	7.9
	≥3	>8-10.4	65-100	95	75-93	8.7
	4	>8-10.4	78-100	99	87-98	10.3
XL probe*	≥2			89		7.2
	4			98		7.9

*Use of the XL probe in case of important subcutaneous adipose thickness (the Fibroscan® device suggests the preferred probe to use) (cutoff for fibrosis stages are on average 1.3 to 1.6 kPa lower).

Table 6. — **Cutoff values of ARFI (Acoustic radiation Force Impulse) (54). Sens : sensitivity ; Spec : specificity ; AUROC : area under the curve of receiver operating characteristic.**

Stage of fibrosis	Cutoff (m/sec)	Sens % (95% CI)	Spec % (95% CI)	AUROC
≥3	1.77	100 (65.5-100)	90.9 (77.4-95.7)	0.973
4	1.90	100 (51.7-100)	95.8 (84.6-98.4)	0.976

Table 7. — **Cut-off values of the Controlled Attenuation Parameter (CAP) to detect steatotis stages (with M-probe)(73). dB/m : decibels per meter ; NPV : negative predictive value ; PPV : positive predictive value.**

Cut-off	Steatosis grade	Sensitivity	Specificity	NPV	PPV
215 dB/m	≥ S1	0.91	0.79	0.91	0.78
252 dB/m	≥ S2	0.86	0.86	0.93	0.73
296 dB/m	S3	0.67	0.97	0.93	0.80

Nevertheless, VCTE could be of interest to exclude confidently severe fibrosis and cirrhosis with high NPV (around 90%) in NAFLD patients. In Table 5 we give an overview of the proposed cut-offs for the M-probe and XL probe (48,50-52). These cut-offs are the lower cut-offs, which have a high negative predictive value (but a low positive predictive value) making them particularly suitable to rule out advanced fibrosis. Furthermore, as significant fibrosis is what needs to be identified (and not only advanced fibrosis, as outlined previously), higher cut-offs (53) imply a risk of missing patients that warrant further investigation, which further justifies this conservative approach.

Several other liver elasticity-based imaging techniques are being developed, including ultrasound (US)-based techniques. US elastography can currently be performed by different techniques based on two physical principles: strain displacement/imaging and shear wave imaging and quantification. The latter allows a better estimation of liver tissue elasticity, and includes point shear wave elastography (pSWE), also known as acoustic radiation force impulse imaging (ARFI) (Virtual touch tissue quantification, Siemens); elastography point quantification (ElastPQ) (Philips) and 2D-shear wave elastography (2D-SWE) (Aixplorer®, Supersonic Imagine, France). A major advantage of pSWE/ARFI is that it can be easily implemented on modified commercial US machines. Its failure rate (2-3%) is lower than VCTE, especially in patients with obesity. However, their quality criteria for correct interpretation are not yet well defined.

In a few studies, pSWE/ARFI has been investigated in NAFLD(3). As in viral hepatitis, pSWE/ARFI performances are better for severe fibrosis and cirrhosis than for significant fibrosis with AUROCs ranging from 0.91 to 0.98 and from 0.66 to 0.86 respectively. Bota *et al.* have summarised the studies comparing the two methods (VCTE and ARFI) in a meta-analysis, indicating comparable sensitivity (0.87 with 95% confidence interval (CI) 0.79-0.92 for ARFI vs. 0.89 with 95%CI 0.80-0.94 for VCTE) and specificity values (0.87 with 95%CI 0.81-0.91 for ARFI vs. 0.87 with 95%CI 0.82-0.91 for VCTE) of both methods in the detection of liver cirrhosis (54). Interestingly, 80% of patients with BMI between 30 and 40 kg/m² and 58% of patients with BMI > 40 kg/m² could be successfully evaluated using pSWE/ARFI. The disadvantage is still the absence of consensus on thresholds for the different SWE methods. Most data are derived with the ARFI technique in NAFLD patients and a proposal for cut-off values is given in Table 6.

Guidance statement : VCTE is the most validated US-based technique for measuring liver stiffness as a non-invasive surrogate for fibrosis assessment, although also for the other techniques, some data exist on their utility in NAFLD patients. Their exact use in screening still needs to be determined, but, taking into account their availability and current lack of specific reimbursement, they are probably to be positioned in second line in case

of non-invasive scores that show intermediate or high probability of advanced fibrosis.

Summary guidance : NAFLD fibrosis score and FIB-4 score, as well as US-based elastography, are acceptable non-invasive procedures for the identification of cases at low risk of advanced fibrosis/cirrhosis. The combination of biomarkers scores and elastography might confer additional diagnostic accuracy and might save a number of diagnostic liver biopsies.

Imaging

US

Despite its limitations, US (B Mode) is the first line imaging modality in the detection of steatosis (55). Improved objective US signs are being used to detect minor degrees of steatosis based on a quantitative measurement of the hepatorenal US gradient. Severity signs of steatosis include B mode grading and colour mode findings. When steatohepatitis and cirrhosis are considered, B mode and colour mode help to detect signs of severity and portal hypertension. US is cost-effective in this context, taking into account its limits : MRI is the imaging method of choice for steatosis quantification, for the detection of severity signs and complications (presence of HCC). Concerning steatosis detection and grading, CAP and MRI are presently the best non-invasive tests when liver fat exceeds 5 % (see below) (56,57).

B Mode changes and liver steatosis

Steatosis generates a liver overload, namely a hyper-echoic liver. The overload on the liver is evoked by an increase of the echogenicity gradient between the right liver and the kidney or spleen: the liver becomes brighter and the kidney appears more "black", with progressive attenuation of the acoustic beam in depth (58).

Since the notion of normality of this gradient is subjective, it is not surprising that the US gradation of steatosis is random. Quantification attempts based on the B-mode image have taken place: dedicated software has been used to overcome the subjective assessment of steatosis with good performance (59). This approach is promising, and competes with that derived from pulse or shear elastography. It is proposed to use post-processing of computer images via a solution developed by the National Institutes of Health (NIHimage software ImageJ, National Institutes of Health, Bethesda) (60). In clinical routine, steatosis would therefore be detectable by this free software, by calculating the ratio between the grey intensity of the liver and the renal cortex. The presence of fatty liver (> 5%) is validated if this ratio is higher than 1.2. However, the method would not be able to grade steatosis unlike the quantification allowed by CAP or MRI.

The CAP (included in the Fibroscan® system) and the MRI are the best non-invasive reference methods for the quantification/gradation of steatosis and are briefly discussed in a subsequent section (56,57,61).

With B mode ultrasound steatosis is graded in three stages (62,63) : 1) initial, minor stage: increased echogenicity of the liver compared to renal cortex and spleen ; 2) moderate stage: reduction of the sharpness of the walls of the portal and hepatic veins ; 3) severe stage: difficulty in identifying the diaphragm. Steatosis can be homogeneous, heterogeneous (in geographical mapping) or localised. The principle of overload saving sites is based on the competition between the portal flow and the systemic arterial flow, in anatomical sites where these two networks are connected. The preferential sites are the perivesicular region (anastomosis between the splanchnic venous network and the systemic cystic network, the vesicle being irrigated by an artery and a systemic vein), the hilar plate and the surface of the liver, for the same reason. We will then describe these regions as "focal fatty spare areas", *i.e.* the steatosis savings sites. The opposite situation, namely localised deposits of fat, are in the same place, but assume a particular type of lipid dysregulation. This is called "focal fatty deposit", *i.e.* localised fat deposit sites.

Colour Doppler US

Steatosis induces changes in the flow in the portal or hepatic network (64-66). NAFLD is related to a gradual decrease in velocities in the main portal vein (from 19.6 cm/sec, 17.6 cm/sec to 12.7 cm/sec) and a decrease in the resistance index (from 0.75, 0.68 to 0.64) as a function of steatosis grades from 1 to 3. In case of cirrhosis, signs of portal hypertension can be present.

Contrast-enhanced US

The impact of steatosis on the contrast acquisition mode may dictate that penetration adjustments are necessary to obtain a microbubble signal. Steatosis itself is not known to generate specific echo-contrast signs. The advantage of the contrast is the ability to help to distinguish a focal lesion in a heterogeneous steatosis, in a zone of savings or focal steatosis, because it helps to distinguish well which contributes to the perfusion disorder and to possible focal injury (67).

Liver fibrosis

Fibrosis is associated with morphological abnormalities visible in conventional sonography and changes in hepatic tissue elasticity in elastography. Portal flow disorders are easily seen in colour Doppler mode.

The gradation of fibrosis is based on US morphological criteria, which are nevertheless subjective. This is why hepatic US elastography has developed. The elementary lesions to be investigated are those that are suggestive for progressive fibrosis of the hepatic parenchyma, and at the same time for the development of portal hypertension.

Regarding steatohepatitis, no specific lesions can be seen besides steatosis. It is the progression of the fibrosis component that then leads to lesions of fibrosing steatohepatitis.

It is possible to distinguish between non-cirrhotic (< F4) and cirrhotic (F4) states by combining the following B-mode and colour Doppler features: in B-mode the surface of the liver looks irregular: to identify it early, it is necessary to look at the capsule of the liver, the contour of the hepatic veins and the vesicular fossa. It is recommended to use a high frequency probe, which gives an excellent view of the liver surface, especially to see the subtle changes in hepatic echostructure (68). The best place is the left liver, by placing the probe just below the xiphoidal appendage. The echostructure of the liver becomes coarse, even nodular (69). The micronodular or macronodular aspect of the cirrhosis liver can be distinguished on the basis of nodule size (< or > 3 mm) (69,70). Atrophy of the right lobe and hypertrophy of the caudal lobe and left lobe are to be investigated as well as signs of portal hypertension including splenomegaly and ascites.

Signs of portal hypertension on US are the following (71,72) : the diameter of the main portal vein exceeds 13 mm and is even higher when cirrhosis is accompanied by encephalopathy; the size of the spleen (> 12 cm long axis) is larger when the varicose veins are more developed. Furthermore, the diameter of the splenic can be increased (> 8 mm). Right liver atrophy, spleen axis greater than 14 cm and splenic vein diameter greater than 9 mm are the best US signs of cirrhosis (70). Portal thrombus or splenomegaly should be systematically looked for; to do this, it is important to analyse the network first with B mode, because colour Doppler can mask a partial thrombus. Visualization and measurement of the coronary stomach vein diameter (> or < 6 mm) is an indirect sign of risk of haemorrhage on rupture of oesophageal varices.

Guidance statement : Conventional B mode ultrasound with colour Doppler ultrasound is the first line imaging modality to assess the presence of NAFLD and its severity. Careful assessment of the liver parenchyma and vasculature, along with the other abdominal organs, particularly the spleen and splenic vein, yield important information and can help to estimate the severity of the steatosis and the potential presence of advanced fibrosis. A high frequency probe examination of the liver surface is important to accurately assess cirrhosis in an early stage.

Other imaging modalities

Controlled Attenuation Parameter (CAP) estimates the amount of liver fat based on the attenuation of the ultrasound signal on the Fibroscan® device. It is expressed in decibels/m (dB/m) and can only be calculated in case of valid liver stiffness measurements. Cut-off values to

detect steatosis ≥ grade 2 vary between 215 and 300 dB/m. Proposed cut-offs with their diagnostic accuracies are listed in Table 6. CAP is increasingly recognised as a reliable tool to assess the presence of steatosis and to grade it non-invasively (56,73).

Non-invasive MRI techniques such as emerging confounder-corrected chemical shift-encoded (CSE) MR imaging and spectroscopic analysis showed excellent promise for reliable quantification of liver fat and iron content (57,74). By accounting for all known signal confounders, CSE MR imaging methods can provide accurate and precise estimates of PDFF. PDFF is a fundamental metric of tissue triglyceride concentration that is increasingly accepted as an imaging biomarker for quantifying liver fat content. Measured by using CSE MR imaging, PDFF is highly reproducible across MR systems and across imaging parameters.

In addition, CSE MR imaging methods also demonstrated excellent promise for quantifying liver iron content through estimation of R2* (R2* = 1/T2*). R2* is well known to have a linear relationship to hepatic iron concentration. It is important to further understand the associations of hepatic steatosis and liver iron overload to predict steatohepatitis and improve algorithms for diagnosis and possible intervention in patients who have the highest risk of developing cirrhosis.

To conclude, quantification of liver fat and iron over-load by using quantitative CSE MR imaging is useful for epidemiologic research. Furthermore, MR elastography has emerged as a reliable technique for assessment of fibrosis in cross-sectional analysis and is considered by some as the new gold standard, superior to liver biopsy (as it obviously allows assessing the entire liver and can map heterogeneity throughout the liver) (61,75). It requires, however, appropriate equipment and software that is not available in all MR facilities and definitely needs further validation, also in follow-up, before its exact role in the assessment and follow-up of NAFLD patients can be decided upon.

Guidance statement : CAP emerges as a reliable tool for fat quantification.
MRI techniques to quantify liver fat and to assess liver stiffness are emerging as attractive non-invasive tools to investigate patients with suspected NAFLD and can be used according to local availability and expertise, but their exact role in screening, diagnosis and follow-up of patients is currently still undetermined and hence they cannot be incorporated in a general guidance.

Body composition imaging

An accurate assessment of body composition, not only to assess visceral fat, but of whole body fat and the different compartments, has provided important information on the role of visceral fat and of sarcopenia in NAFLD pathogenesis (76,77). Measurement of visceral fat by CT correlated with disease severity, but in terms of prediction of lesions, it does not add to the accuracy of

scores based on routinely available clinicobiochemical parameters (78).

Guidance statement : Measurement of body composition by imaging can provide useful information regarding the risk of significant liver disease but its generalised use can currently not be recommended as the information provided adds little to the risk estimation in the individual patient.

Screening

Introduction

To recommend screening, several conditions need to be met. Although some data exist (79)*,* not all pre-requisites to formally recommend screening are fulfilled, reason why the most recent AASLD guidelines (19) refrain from recommending screening. Several arguments, however, plead in favour of screening in populations at risk, taking into account the increasing prevalence of the metabolic syndrome or its components, but there is to date no clear strategy who and how to screen.

The joined EASL-European Association for the Study of Diabetes (EASD)-European Association for the Study of Obesity (EASO) guidelines (3) give some hints about screening programs, primarily to identify the patients at risk for NAFLD among those with an increased metabolic risk profile and second, to identify those patients with a more severe disease stage *i.e.* NASH and/or significant fibrosis. The most recent AASLD guidelines do not suggest routine screening for NAFLD in high risk groups attending primary care, obesity or diabetic clinics but only suggest a "high index of suspicion" in diabetic patients. With the increasing incidence of NAFLD, parallel to the burden of obesity and DM2, and with the development of new drugs to treat NAFLD (of which some are in phase 3 studies), screening the populations at risk seems, however, justified. The goals of screening are on one hand to identify NAFLD in the populations at risk and on the other hand to identify patients that do have NASH and/or significant fibrosis, as this is linked to increased intra- and extrahepatic complications. Patients at risk are defined as patients with the metabolic syndrome (or its components), obesity (BMI $\geq$ 30 kg/m^2) or DM2. Patients with a history of ischemic CVD are also at risk. All these patients should be screened for the presence of NAFLD.

Guidance statement : The following populations are at high risk for NAFLD and should be screened by their general practitioner or the specialists involved: presence of the metabolic syndrome or its components, patients with obesity (BMI $\geq$ 30 kg/m^2), patients with DM2 or patients with a history of ischemic CVD.
Non-invasive risk stratification

As mentioned before, liver biopsy remains the golden standard to diagnose NAFLD and to differentiate

NAFL from NASH, but its widespread use cannot be implemented at a population level (because of invasiveness and costs). A first approach to assess the populations at risk can be based on serum markers. As outlined before, a plethora of biomarkers have been proposed in this setting. As a first test the FLI can be used to rule out (FLI < 30 ; Sensitivity = 87% ; Likelihood ratio (LR)- = 0.2) or rule in (FLI >60 ; Specificity = 86% ; LR+ = 4.3) hepatic steatosis (29). Furthermore, biomarkers have been developed to individuate the patients at risk of fibrosis. As outlined previously, the NFS and FIB-4 have the advantage of having been validated in heterogeneous NAFLD cohorts with concordant results. They perform better in discriminating advanced fibrosis (*i.e.* $\geq$ F3) *vs.* no fibrosis and have a higher NPV than the PPV, therefore they are most suitable as a tool in first-line risk stratification to exclude advanced disease. These biomarkers have also been demonstrated to predict liver related mortality as well as cardiovascular mortality (39). Furthermore, Petta *et al.* described that the combination of non-invasive tests improves the diagnostic accuracy of severe liver fibrosis in patients with NAFLD (51).

The question remains what to screen for. As fibrosis is the most important predictor of adverse outcomes and prognosis starts declining from F2 onwards, and as NASH is the driver of disease progression, the clinically most relevant question is the presence of NASH and/or presence of significant fibrosis. As already discussed, there are currently no reliable biomarkers to identify NASH and the scores for fibrosis have the highest accuracy in the diagnosis of advanced (and not significant) fibrosis. The tools therefore are mainly useful to diagnose steatosis and advanced fibrosis.

Guidance statement: Ideally patients should be screened for the presence of NASH and significant fibrosis ($\geq$ F2). Current tools are, however, more accurate to diagnose steatosis and advanced fibrosis.

We propose an algorithm based on the low and high cut-off values of the FLI, FIB-4 and the NFS combined. The combined use of non-invasive scores has shown to increase accuracy (51). If they rule out significant disease, the likelihood of finding NASH with significant fibrosis is low.

If FLI < 30 and there is no indication of liver fibrosis (NFS < -1.455; FIB-4 < 1.30) (29,80), significant liver disease is unlikely and the patient can be evaluated with an interval of 2 years. If there is an indication of important liver fibrosis (NFS > 0.67; FIB-4 > 2.67) (41) (80), however, the patient should be referred for further hepatological investigation (see Figure 1). In case of discordant values, these biomarkers should be repeated after 6 months of lifestyle modifications. If the scores are then still discordant or high at this re-evaluation, the patient should be referred for further hepatological investigation.

If the FLI > 60 and there is no indication of liver fibrosis by NFS and/or FIB-4, there is very
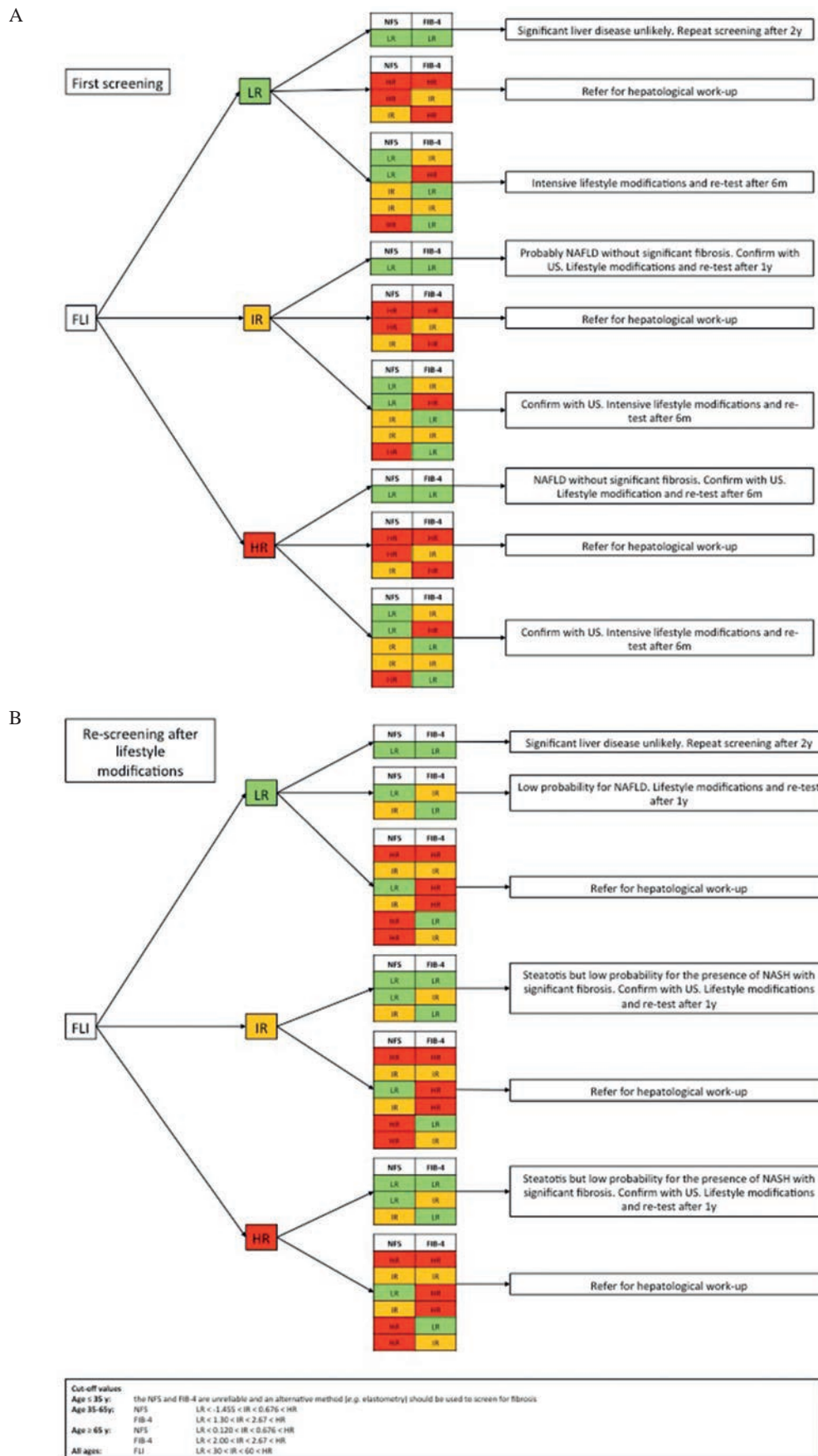
Figure 1. — Flow chart of the screening algorithm for Non-Alcoholic Fatty Liver Disease. 1A : First screening ; 1B : Re-screening after life style modifications. FIB-4 : Fibrosis 4 index ; FLI : Fatty Liver Index ; HR : High Risk ; IR : Intermediate Risk ; LR: Low Risk ; m : month ; NAFLD : Non-Alcoholic Fatty Liver Disease ; NASH : Non-Alcoholic Steatohepatitis; NFS: NAFLD Fibrosis Score ; y : year.

likely NAFLD without significant fibrosis, therefore the patient should be evaluated again after 1 year of intensive lifestyle modifications. If the NFS and FIB-4 indicate significant fibrosis, patients should be referred for further evaluation. In case of discordant values, these biomarkers should be repeated after 6 months of intensive lifestyle modifications. If the scores are then still discordant or high at this re-evaluation, the patient should be referred for hepatological investigation.

If FLI is between 30 and 60 and there is no indication of significant fibrosis by NFS and/or FIB-4, there is probably NAFLD without significant fibrosis. Similar to a FLI > 60, the patient should be evaluated again after 1 year of intensive lifestyle modifications. If there is suspicion of significant fibrosis by NFS and FIB-4, the patient should be referred. In case of discordant values, these biomarkers should be repeated after 6 months of intensive lifestyle modifications. If the scores are still discordant at the re-evaluation, the patient should be referred for further hepatological investigation.

In the use of these biomarkers, age has to be taken into account, since the cut-offs are age-dependent. In patients ≥ 65 years adjusted lower cut-offs need to be used (NFS : < 0.12 to rule out fibrosis and > 0.675 to rule in significant fibrosis ; FIB-4 : < 2 to rule out fibrosis and > 2.67 to rule in significant fibrosis). For patients ≤ 35 years, the sensitivity drops dramatically but adjusted cut-offs could not be determined, therefore also patients with low values but with clinical suspicion of NAFLD should be referred for further evaluation (43).

Guidance statement : Populations at risk can be screened using an algorithm with the FLI, FIB-4 and NFS. Age–adjusted cut-offs must be used. In case of negative screening, patients should be followed-up and periodically retested. An easy-to-use web application based on the proposed algorithm is freely available at www.antwerpnafldguide.com.

Approach by the hepatologist

Introduction

Within the process of assessment and treatment of NAFLD, the hepatologist plays a pivotal role. Colla-boration with general practitioners and other specialists, especially with endocrinologists, obesity physicians and cardiologists, is important because of the close relationship of NAFLD with (the components of) the metabolic syndrome and as it is an established independent risk factor thereof (15).

Patients can be referred to the hepatologist via 2 distinct routes: I) patients at risk of NAFLD, namely those with the metabolic syndrome or DM2 (81,82), who screened positively for the presence of NAFLD or II) patients with an incident finding of steatosis or abnormal liver biochemistry in routine work-up for other reasons. Regardless of the reason for referral, the main goals of further assessment are the determination of the

stage of the disease, *i.e.* presence/absence of NASH and presence of fibrosis, and the evaluation of closely related co-morbidities. As it has been clearly established that prognosis declines from F2 onwards and as several drugs are in the pipeline, the main aim is to identify patients with NASH and/or ≥ F2.

Non-invasive risk stratification

In all cases most evidently an US will be performed. US can confirm the presence of steatosis, as outlined before, and can show signs of cirrhosis.

We propose an algorithm based on the low and high cut-off values of a serological score (FIB-4 or NFS) combined with a liver stiffness measurement. Fibroscan® is the most validated tool to measure liver stiffness. A cut-off of < 7.9 kPa with the M-probe (< 7.2 with the XL-probe) has a NPV of 89-95 % to rule out significant fibrosis and a cut-off of > 9.6 kPa with the M-probe (> 9.3 kPa with the XL -probe) has a PPV of 72% (50). A combined use of both (serological score and liver stiffness measurement) had been shown to increase accuracy. If both rule out significant disease, the likelihood of finding NASH with a significant fibrosis is low. In the use of these biomarkers age has to be taken into account, since the cut-offs are age-dependent. In patients ≥ 65 years adjusted cut-offs need to be used (see above). For patients ≤ 35 years, there are no cut-offs, but it has to be considered that the sensitivity in this age group drops dramatically, so that a positive liver stiffness measurement alone warrants further investigation (43). As more and more data become available on the accuracy of detecting ≥ F2, lower cut-offs might be pro-posed in the future.

Liver biopsy

Based on the non-invasive risk stratification (using at least 2 different estimates), patients with a high risk of significant fibrosis need further investigation via liver biopsy to assess NAFLD severity and the degree of fibrosis (Figure 2). Especially if the results are indicative of cirrhosis, a liver biopsy could contribute in the differentiation of the underlying cause and look specifically into signs of burned-out NASH if metabolic factors are present. If the presence of fibrosis is unlikely (*i.e.* a low risk stratification), there is no need to proceed to a liver biopsy; however, lifestyle modifications, weight loss, the treatment of metabolic traits and follow-up is warranted. A first re-evaluation of NAFLD and liver fibrosis is recommended after 3 months. Decision making in patients with an intermediate risk and/or discordant test results is less straight forward. Patients that possess a clear additional risk to have NASH or fibrosis, *i.e.* patients with diabetes mellitus or ≥ 65y, should be considered as high risk and have a liver biopsy. The decision for liver biopsy in the remaining patients has to be taken on a case-by-case manner. If one decides not to perform a liver biopsy, close follow-
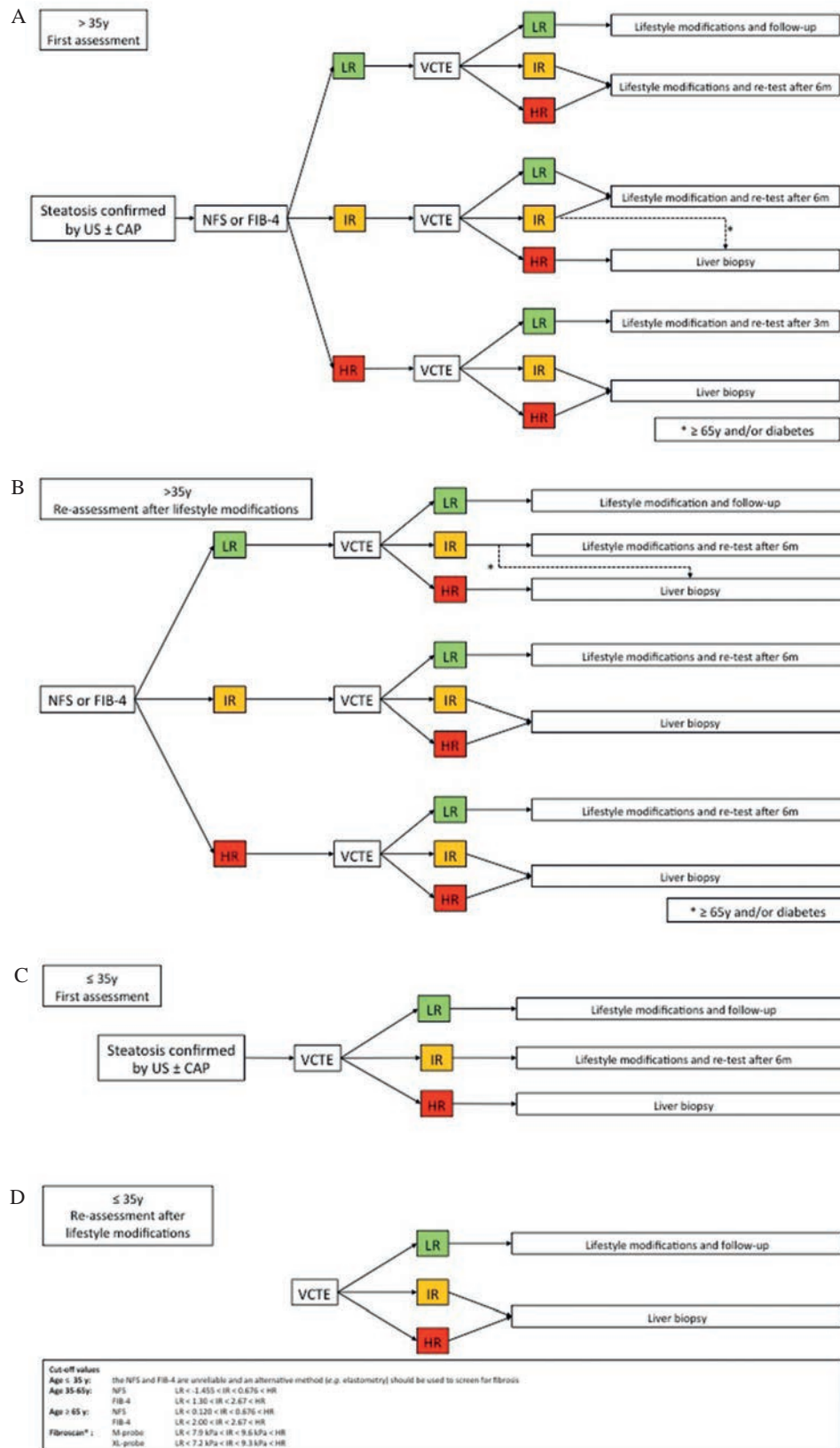
Figure 2. — Flow chart of the assessment algorithm for Non-Alcoholic Fatty Liver Disease by the hepatologist. 2A : First assessment in patients aged > 35 y ; 2B: Re-assessment after life style modifications in patients aged > 35 y ; 2C : First assessment in patients aged ≤ 35 y ; 2D : Re-assessment after life style modifications in patients aged ≤ 35 y. CAP : Controlled Attenuation Parameter ; FIB-4 : Fibrosis 4 index ; FLI : Fatty Liver Index ; HR : High Risk ; IR : Intermediate Risk ; LR : Low Risk ; m : month ; NAFLD : Non-Alcoholic Fatty Liver Disease ; NASH : Non-Alcoholic Steatohepatitis ; NFS : NAFLD Fibrosis Score ; VCTE: Vibration Controlled Transient Elastography ; US : Ultrasound ; y : year.

up with re-evaluation at 3 months is recommended for this group of patients. In the meantime, emphasis has to be put on lifestyle modifications, weight loss and the treatment of metabolic diseases. If the patients after these 3 months has the same non-invasive risk estimate, a biopsy still should be performed.

Guidance statement : Based on a combination of clinicoanamnestic parameters, US, serological score and liver stiffness measurement, the probability of the presence of clinically significant NAFLD can be estimated and based on the combined results, some guidance can be provided on the indication to perform a liver biopsy, which remains the only way to accurately diagnose the different aspects of the disease. In case there is no indication for liver biopsy according to the proposed algorithm, follow-up and periodical re-assessment is warranted (Figure 2).

Other tests

Several other biomarkers and composite scores have been proposed (*e.g.*, as discussed previously, the ELF®, the FibroTest®, Cytokeratin 18 (49)). These tests still need further validation. Furthermore, availability and pricing make these tests not useful for screening, but they can serve as an add-on to the NFS and FIB-4. More sophisticated techniques like magnetic resonance elastometry (MRE), Liver Multiscan and other imaging modalities are currently only available in specific settings and although promising, are currently insufficiently validated and/or too limited in availability to be incorporated in a general screening algorithm or diagnostic recommendation for hepatologists.

Guidance statement : Other serological tests or imaging modalities can be used upon local expertise. Some of these tests are not reimbursed and patients should be informed on this when the test is applied.

Screening for systemic complications

Diabetes

Patients with NAFLD have an increased prevalence of DM2, increasing with the severity of disease (10,83). The chance to develop new-onset DM2 within 5-10 years is increased 1.5-2-fold, which diminishes when NAFLD improves or resolves (15). Hence, all patients diagnosed with NAFLD and unknown diabetes have to be screened with an OGTT and HbA1c.

Guidance statement : All NAFLD patients not known to have diabetes should be screened for DM2 with OGTT and HbA1C.

Cardiovascular disease

Cardiovascular mortality is the main cause of death in patients with NAFLD (38% of all causes) (84) and

accumulating evidence is able to link NAFLD to a wide spectrum of CVD, ranging from subclinical carotid artery disease to overt coronary artery disease and diastolic heart failure (14,15). Even though the exact contribution of NAFLD to CVD is still to be determined, partially because of shared risk factors, the general consensus is growing that NAFLD serves as an independent CV risk factor, whereas the incidence increases with more advanced NAFLD (14,85). Therefore, even in the absence of the traditional CV risk factors, the presence of NAFLD should initiate cardiovascular screening. A cardiovascular risk score, *e.g.* SCORE or HeartSCORE (www.heartscore.org), has to be calculated in all patients. Patients at high risk, and patients with a suspicion of active cardiovascular disease based on current history and/or clinical examination, have to be referred for CV screening. Furthermore, all asymptomatic patients with proven NASH or ≥ F2 fibrosis should undergo further cardiovascular investigation that exist at least out of an electrocardiogram, echocardiography, ergo-spirometry and 1 non-invasive test for subclinical atherosclerotic disease (*e.g.* coronary artery calcium score, carotid intima media thickness, flow-mediated dilatation, pulse wave velocity). The latter can be adjusted according to the centre's experience.

Guidance statement : All NAFLD patients should be screened for CV risk factors and CV risk stratification and appropriately referred if positive. Patients with proven significant disease (NASH and/or ≥ F2 fibrosis) should undergo further cardiovascular investigation that exist at least out of ECG, echocardiography, ergo-spirometry and 1 non-invasive test for subclinical CVD according to local expertise.

Other

Similar to CVD, the evidence that links NAFLD to CKD is increasing (15). The prevalence and incidence of CKD is about 2-fold increased in patients with NAFLD and up to 2.5-fold in NASH and persists even after correction for DM2 and other cardiorenal risk factors (86). Despite potential methodological flaws, amongst which the use of glomerular filtration rate-calculation (instead of direct measurement), and the causality and underlying mechanisms that remain to be proven, the increased incidence of CKD warrants systematic surveillance of CKD in patients with NAFLD. Pragmatically we recommend the same approach as for CVD, *i.e.* screening of patients with clinical suspicion or with steatohepatitis or ≥F2 fibrosis.

Guidance statement : Kidney function should be surveyed in patients with NAFLD. Patients with proven significant disease (NASH and or ≥F2 fibrosis) should undergo further investigation.

The second leading cause of death in patients with NAFLD is extra-hepatic malignancy (84,87) of which

the strongest association has been reported for colorectal cancer (15). The current data do, however, not allow to recommend specific colorectal screening beyond the current guidelines (88).

Finally, a full assessment pays attention to patient's concerns and the impact of the disease on daily life, especially as many patients were previously unaware of their disease. Psychosocial health not only affects severity of disease (89) or impacts on treatment outcomes (90), but is also affected itself by the diagnosis. NAFLD has been repeatedly reported to impair quality of life, both physically and mentally, compared to healthy controls or other hepatic disorders. Cirrhosis further impairs this quality of life (91–95). Therefore, quality of life is a valuable primary endpoint that has to be followed-up (96). Recently a specific tool has been developed and validated to assess QOL in patients with NAFLD (95). This tool needs, however, further external validation and translation to our native languages before it can be implemented in daily practice.

Guidance statement : Psychosocial health aspects and the quality of life should be surveyed in NAFLD patients.

Populations that deserve special attention

Surgery

Patients who are screened for bariatric surgery should be screened for the presence of NAFLD as well, since they have a high prevalence of the metabolic syndrome and NAFLD itself. NAFLD increases also the complication rate of bariatric surgery (97). They can be screened according to the same algorithm. A liver biopsy with hepatic venous pressure gradient (HVPG) measurement prior to surgery is indicated if advanced fibrosis is suspected, as it may impact on the choice of the type of procedure and also impacts on the risks related to the procedure (98,99). Other aspects of bariatric surgery are discussed in the therapy section.

Similarly, the likelihood of NAFLD in patients that need cholecystectomy is increased, mostly in those with concurrent metabolic syndrome or DM2, justifying a peroperative liver biopsy (after informed consent) in order to accurately assess NAFLD presence and severity (100,101).

Evidence on biopsy in other abdominal surgical pro- cedures is lacking.

Guidance statement : Patients considered for bariatric surgery should be screened for NAFLD applying the same algorithm as the populations at risk. Patients with DM2 or the metabolic syndrome undergoing cholecystectomy should undergo a perioperative liver biopsy.

NAFLD and hepatocellular carcinoma

HCC is one of the complications that can occur in NAFLD patients. Indeed NAFLD is one of the most common aetiologies of liver cancer, besides Hepatitis B, Hepatitis C and alcoholic liver disease, the incidences of which (% year) are respectively 2.2-3.7%, 2-8% and 2-6% (102). In cohorts of NASH-related cirrhosis patients, the cumulative incidence of HCC has been reported to be 2.4% to 12.8% (103).

In the cirrhotic population the classically recommended surveillance method is liver US every 6 months and, although not specifically studied in NASH, this guidance *mutatis mutandis* applies to NASH cirrhosis (3,19). US has a modest sensitivity of approximately 60% and a higher specificity of approximately 85-90% (104) and is an operator-dependent examination. Moreover, it has been reported to be less accurate in obese patients and in those with a nodular liver (105). A CT scan or a MRI can be suggested if the visibility at US is deemed insufficient, but there is no clear guidance on when to use these imaging modalities (except in case a lesion is detected at US for subsequent characterisation) and at what interval. There is *a fortiori* no evidence on the use of these imaging modalities for 6 monthly screening if US is deemed unreliable.

As for other aetiologies of HCC, the use of alpha- foetoprotein as a screening tool is also controversial. Although it is generally used in clinical practice, it has a limited sensitivity (lower than US) and specificity (106).

An emerging problem is the onset of HCC in NAFLD patients in the absence of liver cirrhosis. In the most recent meta-analysis of cohorts of non -cirrhotic NAFLD-patients the cumulative HCC-related mortality was reported to be 0% - 3% over a study period of up to 21 years (107). Comorbidities, such as DM2 and obesity, are major risk factors for the development of HCC. They are both associated with a 2-3-fold increase in the risk of HCC (108,109), while on the other hand some data suggest that the use of metformin could be protective (110). Also, some genetic parameters expose the patients to a higher risk of HCC. The carriage of the PNPLA3 rs738409 C >G polymorphism increases the risk of HCC in NAFLD 2.26-fold, independently of other factors such as the presence of cirrhosis (111). As to fibrosis, non-cirrhotic HCC has been reported throughout all the fibrosis stages, although with an increasing prevalence by increasing fibrosis stage. Yasui *et al.*, for example, reported a series of HCC patients in whom stage 2 fibrosis accounted for 17% of the cases, stage 3 for the 21% of the patients and liver cirrhosis accounted for the remaining 55% (112).

The surveillance program to implement in this non- cirrhotic NAFLD population is, however, not well established and cost-effectiveness is questionable. Screening policies should probably be implemented earlier in patients with multiple risk factors for HCC (113,114). Although strong epidemiological data are not yet available, it can be suggested to extend screening policies by a yearly ultrasound in NAFLD patients with ≥F2 fibrosis and metabolic co-morbidities, *i.e.* DM2 or the metabolic syndrome. Given the known onset of HCC

in the pre-cirrhotic stages and the risk of progression to cirrhosis, as well as the sampling error of the liver biopsy, patients with at least F3 should be included in the 6-month surveillance, such as for the cirrhotic patients.

Guidance statement : Patients with NASH-related cirrhosis and patients with F3 fibrosis should undergo 6-monthly surveillance with US. Patients with ≥F2 fibrosis and metabolic comorbidities, namely DM2 or metabolic syndrome, should undergo yearly surveillance with US. Other imaging modalities can at present not be recommended for regular screening, but can be used at the discretion of the treating physician in case of low quality of the US images deemed insufficient for reliably excluding the presence of HCC. Although HCC can occur on the background of non-fibrotic NAFLD, general screening of all NAFLD patients is currently not recommended.

Treatment of patients with NAFLD

Treatment of cardiometabolic co-morbid conditions

Regardless of the severity of NAFLD, all patients with NAFLD should be checked thoroughly for cardiovascular risk factors, components of the metabolic syndrome and co-morbid conditions (including OSAS) (3). These conditions should be treated according to their proper guidelines, which are beyond the scope of this guidance document. There are no contraindications to any of the drugs needed to treat these co-morbid conditions, including drugs like metformin and statins, and the risk of potential drug-induced liver injury does not seem to be substantially increased (115). Baseline elevated liver enzymes related to NAFLD are hence also not a contraindication for treatment with statins (116) or other drugs known to be potential causes of drug-induced liver injury, as long as the necessary precautions (that apply to all patients) are respected (3,115).

Guidance statement : Cardiometabolic co-morbid conditions (DM2, dyslipidaemia, arterial hypertension, overweight and obesity, OSAS) should be carefully looked for and treated according to their proper guidelines.
With the exclusion of patients with decompensated Child B and C cirrhosis, all treatments that are indicated should be applied, including metformin and lipid lowering drugs, regardless of the presence or not of elevated transaminases related to NAFLD.

Lifestyle modifications

The cornerstone of the treatment of NAFLD are lifestyle modifications. Although increased physical activity without weight loss might result in some improvement (presumably because of a shift in body composition with reduced adipose tissue and increased muscle mass) the main driver of improvement is the loss of weight. Regardless of how weight loss is achieved, a loss of ≥ 10% is needed to improve all NASH features including fibrosis. A weight loss of ≥ 5% improves steatosis and ≥ 7% steatohepatitis (117). Ideally this is achieved by a combination of reduced calorie intake and increased physical activity (118).

The type of diet seems to be less important than the actual weight loss. Mediterranean diet has shown significant benefit in well-conducted RCTs (119,120) and, because of the specific deleterious and pro-fibrogenic effects of fructose (121,122), avoiding fructose containing food and sugar-sweetened beverages are the most specific recommendations that can be made (123), along with a reduction of caloric intake of at least 500 kcal/d, but preferentially up to 750-1000 kcal/d or 30% of the baseline caloric intake, as these have shown to reduce hepatic steatosis (124). There are no high-quality data to support more specific dietary regimens and probably, if the aforementioned rules are followed, specific regimes of all kind will only marginally differ in impact on NAFLD beyond the achieved weight loss. General rules on healthy diet and food pattern should be applied and the results should be monitored. Dietary and motivational/psychological counselling should ideally be offered, but is currently in the Belgian healthcare system poorly or not reimbursed and hence at the patient's charge. As studies have shown that only 30% of patients achieve a sustained (> 1 year) 5% weight loss and < 10% a sustained 10% weight loss (117), close monitoring and counselling is advocated.

Guidance statement : Caloric restriction (30% reduction or 750-1000 Kcal/d compared to baseline) taking into account current guidelines on healthy nutrition should be implemented in order to achieve a sustained weight loss of > 7-10% of initial body weight.
Besides Mediterranean diet and reduction of fructose intake, no specific diet has proven superiority in comparison to others.

Concerning increased physical activity, recommendations vary and high-quality data are sparse. Increasing the weekly physical activity with 60 minutes or a minimum of 150 to 200 minutes per week of moderate intensity aerobic exercise is the best documented (125). It should also be regular and distributed over the week. Also for this aspect, counselling can substantially improve long term efficacy but little is reimbursed by the Belgian health insurance.

Guidance statement : Increase of physical activity with 60 minutes or to a minimum of 150-200 min per week of moderate intensity aerobic exercise is recom-mended.

Significant alcohol consumption is by definition not present in patients with a strict diagnosis of NAFLD, but many NAFLD patients consume small amounts of

alcohol, and, in case of alcohol consumption, features of the metabolic syndrome and thus NAFLD may co-exist and result in a mixed aetiology of liver disease. The negative effect of consumption of even small amounts of alcohol on the benefits of physical activity have been well documented (126). The calories present in alcohol should also be taken into account in the assessment of alimentary calorie intake. The beneficial effects of the consumption of small amounts of alcohol remains a matter of debate. Therefore, abstinence or consumption within the recently proposed limits of a maximum over 14 units/week for both men and women (although there is no safe limit) should be recommended (3). Furthermore, the impact of alcohol consumption on calorie intake and efficacy of physical training should be mentioned as well (126).

Guidance statement : There is no safe limit for alcohol consumption. Abstinence or a limit of max. 14 units/week is recommendable, and patients should be instructed on the impact of alcohol on caloric consumption and efficacy of physical training.

Pharmacological treatment

Pharmacological treatment specifically for NASH should, in our current understanding, be restricted to patients who have NASH and some degree of fibrosis. These recommendations result from the data showing that fibrosis is the strongest predictor of prognosis, with a decline in survival from F2 onwards, and the steatohepatitis being the driving force of the progression towards adverse outcomes (12,127). Generally, a steatohepatitis with a NAS of 4 (there is currently no equivalent definition based on SAF, but an A ≥ 3 could be proposed) with a fibrosis ≥ F2 is considered an indication for pharmacological treatment (128). F1 patients with a NAS ≥ 5 and/or severe (mostly) metabolic co-morbidities (persistently elevated ALT, DM2, metabolic syndrome) should, however, also be considered as they have a high risk of progression (128).

Several drugs, not specifically licensed for the treatment of NASH but with a potential benefit based on their mode of action, have been tested. Metformin improves insulin resistance, a key pathophysiological mechanism in NASH, but failed to show histological benefit(129), hence, despite data suggest that it improves the risk of cancer (including HCC) (130), it should not be used with the intent to treat NASH but only if there is an approved indication. Ursodeoxycholic acid (UDCA) improved liver tests and some histological features, mainly inflammation, but failed to show histological benefit in 2 long term trials (129) and hence is not recommended. Fibrates were only tested in small trials, showing no benefit, and statins, although they might have pleiotropic beneficial effects, have not been tested properly (129). As highlighted above, these drugs should be used to appropriately treat dyslipidaemias according to their proper guidelines, but should not be prescribed for the sole indication of treating NAFLD until more data become available. Omega-3 fatty acids showed some promise in initial trials, but more recent trials did not show a clear benefit. Although probably not of harm, they should currently not be prescribed as a NASH treatment.

Glucagon-like-peptide (GLP)-1 analogues or incretin mimetics improve glycaemic control and reduce weight. They have been approved for the treatment of DM2 and several molecules are available for that indication on the Belgian market, according to specific reimbursement rules. In 2015 liraglutide in a dose up to 3 mg QD and commercialised under the name of Saxenda®, has also been approved for the treatment of obesity and is available on the Belgian market, but is not reimbursed (131). Saxenda®, used on top of a hypocaloric diet and increased physical activity, has shown to reduce body weight with > 5% in 63% of treated individuals after 1 year (132). Liraglutide (at a dose of 1.8 mg QD) has been reported to beneficially affect liver histology in a small RCT (133). Likely the induced weight loss is the main driver of the histological benefit, although direct intrahepatic effects cannot be excluded. Side effects include nausea and diarrhoea. Until further data become available (several other GLP-1 analogues are currently being studied) its use should be restricted to the classical indications (BMI > 30 kg/m² or > 27 kg/m² and comorbidities (diabetes, arterial hypertension, dyslipidaemia, OSAS)), with the only extension that we propose to add NASH to this list of co-morbidities of obesity that justify pharmacological treatment of obesity.

Thiazolidinediones or glitazones, used for the treatment of diabetes, are effective in improving histological lesions of NASH. The safety profile is not alike for all molecules and some harbour safety concerns. Pioglitazone, commercialised in Belgium under the name of Aktos®, clearly improved cardiovascular outcomes in diabetic patients and has a more favourable safety profile (134), but its reimbursement is restricted to patients with DM2 that is uncontrolled despite metformin or sulfamide treatment in patients with a BMI > 27 kg/m², and it is not frequently used. There are some concerns regarding the possibility of eliciting heart failure in predisposed individuals, although as mentioned, an overall significant cardiovascular benefit has been recently demonstrated for pioglitazone (134,135). We recommend that its prescription should be left at the discretion of the treating diabetologist and, if considered within its context in patients with a suspicion of NASH, NASH should be histologically proven.

Vitamin E has also shown beneficial effects on liver histology in non-diabetic and non-cirrhotic NASH patients, so its use should be restricted to this patient category (136). Vitamin E is hence not to recommend in patients in whom NASH was not histologically documented, in diabetic or cirrhotic patients. Although a

recent large meta-analysis did not confirm earlier safety issues (137), the potential of increased prostate cancer in man is an unresolved issue of concern. The dose that proved efficacy in the PIVENS trial is 800 IU/d and can be prescribed as Optovit E® (capsules of 200 IU) and is without reimbursement.

For all these treatments, evidence on efficacy has been demonstrated by histology. There is, however, no clear guidance on how to assess efficacy of treatment in routine clinical practice. Improvement in liver enzymes has been shown to be in line with histological improvement in several trials (138,139), regardless of baseline values, but there are no clear rules to interpret liver enzyme changes in individual patients.

There is hence currently no pharmacological treatment that has NASH in its label. There is, however, a large pipeline of drugs that are currently being tested, some of them already in phase 3. The first to come on the market, if the registration trials are positive, will presumably by 2020. Meanwhile, patients with significant disease eligible for pharmacological treatment as defined above, can be offered treatment in the context of a clinical trial. Given the potential benefit for the patients, the possibility of participating in a clinical trial should systematically be considered and offered to the patient, reinforcing also the recommendation to screen and adequately diagnose patients at risk.

Guidance statement : Few drugs have shown efficacy in the treatment of NASH and no drug currently has NASH in its label.

Pharmacological treatment is only warranted in patients with histologically proven NASH with a NAS ≥ 4 and F ≥ 2 (or F1 with NAS ≥ 5 or co-morbidities : persistently elevated ALT, DM2, metabolic syndrome). Conversely, if suspected, a liver biopsy should be performed.

Vitamin E can be used in non-cirrhotic non-diabetic patients with histologically proven NASH. Due to potential long term safety issues, it is not clear for how long this treatment can be safely continued. In case of absence of improvement on follow-up liver biopsy (see below), treatment should be discontinued.

Liraglutide can be used in obese patients with BMI > 30 kg/m² or > 27 kg/m² and comorbidities (diabetes, arterial hypertension, dyslipidaemia). ioglitazone can also be used in patients with DM2 that is uncontrolled despite metformin or sulfamide treatment and with a BMI > 27 kg/m² but its prescription is preferentially confined to the diabetologist.

There is no clear guidance on how to assess efficacy of treatment in routine clinical practice.

Patients meeting the aforementioned criteria should be offered the possibility to participate in a clinical trial and should hence be referred for this purpose.

Bariatric surgery is now widely used to treat obesity and increasing evidence also supports its use as a treatment for metabolic disturbances (so called "metabolic surgery"). Current reimbursement criteria restrict its use to patients > 18 years of age with obesity with a BMI ≥ 40 kg/m² or BMI ≥ 35 kg/m² with associated co-morbidities, with a list restricted to diabetes mellitus, OSAS and uncontrolled arterial hypertension despite triple therapy. NASH is hence not in this list of co-morbidities putting forward the indication for bariatric surgery.

Bariatric surgery techniques have evolved over the last decades. Jejuno-ileal bypass, biliopancreatic diversion and early gastric bypass techniques have been associated with both short term and long term severe hepatic complications (140). The currently applied techniques of Roux-en-Y gastric bypass, sleeve gastrectomy and adjustable gastric banding are, however, safe in this respect. Large series of patients with serial biopsies after bariatric surgery consistently show a marked improvement in liver histology, including regression of fibrosis (141). Therefore, it could be recommended to consider bariatric surgery in patients with clinically significant NASH that otherwise correspond to the current criteria (142).

As most of the patients are asked to adhere to a low-calorie diet prior to surgery, resulting in a marked reduction of liver fat content and volume (143), as studies with systematic liver biopsies have shown overall low prevalence of advanced disease in terms of fibrosis in this population (32), and as bariatric surgery results in marked improvement, routine systematic biopsies during surgery or in follow-up are no longer recommended. It is, however, important to diagnose cirrhosis before going to surgery. Therefore, the aforementioned algorithm should be used in every patient considered for bariatric surgery, and if suspected, cirrhosis should be further assessed, including HVPG measurement and biopsy. Patients with cirrhosis have an increased risk of peri- and postoperative complications (98,99). In case of cirrhosis without clinically significant portal hypertension (HVPG < 10 mm Hg), there is no formal contraindication (144) and, although sleeve gastrectomy is preferred, gastric bypass is probably also feasible without significantly increased risk.

In cirrhosis with clinically significant portal hypertension, pro- and cons should be carefully balanced by a multidisciplinary team, and sleeve gastrectomy is the technique of choice (19,99). In case of decompensated cirrhosis, liver transplantation should be considered, and careful attention should be paid to the patient's nutritional status. These cases should hence be referred to a transplant centre. The feasibility of bariatric surgery and the timing and sequence of the interventions, especially in relation to the liver transplantation, should be discussed on a case-by-case basis in a broad multidisciplinary discussion including all relevant parties involved. Simultaneous liver transplantation with bariatric surgery has been reported with good results, but relies on local expertise (145).

An even more challenging clinical situation is a decompensated cirrhosis as a result of prior bariatric

Table 8. — **Waist circumference percentiles in girls and boys between 5 and 16.9 years (adapted from (171))**.

Age	Girls (cm) 90th percentile	Girls (cm) 95th percentile	Boys (cm) 90th percentile	Boys (cm) 95th percentile
5y	55.4	57.2	55.6	57.0
6y	57.0	58.9	57.1	58.7
7y	58.7	60.8	58.8	60.7
8y	60.4	62.7	60.9	62.9
9y	62.0	64.5	63.2	65.4
10y	63.6	66.2	65.6	67.9
11y	65.4	68.1	67.9	70.4
12y	67.3	70.5	70.4	72.9
13y	69.1	71.8	73.1	75.7
14y	70.6	73.2	76.1	78.9
15y	71.7	74.3	79.0	82.0
16y	72.6	75.1	81.8	85.2

surgery, mostly of the Scopinaro type. Several cases have been reported, also in Belgium (146). Treatment is usually complicated by malnutrition (often despite persisting overweight or obesity) and aggressive nutritional support, which can include total parenteral nutrition, is warranted and might already significantly improve the patient's condition. Surgical conversion to a gastric-bypass-like anatomy can also result in a significant improvement, but its feasibility will depend on the severity of liver decompensation and the effects of nutritional correction. Liver transplantation should also be considered. These cases should hence be referred to transplant centre were a broad multidisciplinary team will have to decide on the optimal treatment strategy on a case-by-case basis.

Guidance statement : The presence of clinically significant NASH can be an argument in favour of bariatric surgery in patients that are otherwise eligible for this procedure. Bariatric surgery improves NASH.
Biliopancreatic diversion is prone to severe hepatic complications and should not be performed, but Roux-en-Y gastric bypass, sleeve gastrectomy and gastric banding are deemed safe.
Candidates for bariatric surgery should be screened for NASH and if advanced fibrosis or cirrhosis is suspected, a further assessment and accurate staging of the disease should be performed prior to surgery.
In case of cirrhosis with clinically significant portal hypertension, the pro and cons of surgery should be carefully weighed and sleeve gastrectomy is the technique of choice.
Patients with a decompensated NASH cirrhosis, and patients with a post-Scopinaro advanced liver disease should be referred to a transplant centre and the optimal treatment strategy decided on a case-by-case basis.
Routine liver biopsy in all patients undergoing bariatric surgery is no longer recommended.

Liver transplantation

The indications for liver transplantation in NASH-related cirrhosis are the same as for other chronic liver diseases (147). NASH-related cirrhosis patients are usually older at the time of development of a decompensated liver disease compared to other chronic liver diseases and might present cardiometabolic co-morbidities that preclude liver transplantation (147). Although in essence the pre-transplant evaluation is the same as for other patients with underlying chronic liver disease, these aspects should be carefully considered and timely referral of potential transplant candidates is warranted. The same holds true for the peri-and post-transplant follow-up: although in essence the same as for every liver transplant patient (cardiometabolic diseases are a major cause of post-transplant morbidity or mortality), these patients should be carefully monitored (147). Post-transplant recurrence of NAFLD is frequent (148).

Guidance statement : Although indications, pre-and post-transplant assessment and monitoring are in essence comparable to other chronic liver diseases, NASH patients tend to be older and frequently suffer from cardiometabolic co-morbidities that warrant in-depth assessment and close monitoring. Timely referral of potential transplant candidates to a liver transplant centre is warranted.

Follow-up

Once an adequate diagnosis has been established and an appropriate treatment started, the patient needs to be carefully followed to monitor the results of the treatment and the evolution of the liver disease. Some guidance on re-screening in case of initially negative cases is provided in the flow charts (Figure 1 & 2). In milder disease cases, a yearly follow-up can be proposed. The value of non-invasive tests for steatosis or fibrosis in follow-up has been poorly studied. Re-assessment for clinically relevant disease and especially fibrosis in order to identify progressive disease seems justified on a 2-yearly basis. Given the diagnostic inaccuracy of the non-invasive tests, a repeat biopsy after 5 years is to be considered, especially if the metabolic risk factors have

not significantly improved (3). In case of significant NASH and/or fibrosis, 6-monthly clinicobiochemical evaluation, yearly US and fibrosis assessment and 5-yearly liver biopsy can be recommended. Patients with cirrhosis should be followed on a 6-monthly basis according to the guidelines applicable to all cirrhotic patients irrespective of the underlying aetiology (3,19).

Guidance statement : After diagnosis and initial treatment, the patient should enter a follow-up program, tailored to the severity of the initial diagnosis and the efficacy of the treatment, especially with regard to the concomitant cardiometabolic comorbidities.

Paediatric NAFLD and NASH

Prevalence

Paediatric NAFLD was first described in 1983 (149). Since then, the prevalence seems to increase, although showing large variations according to populations, definitions and methodology. Worldwide estimates range between 5% and 25% (150). Based on ultrasound, NAFLD is described in 2.5% of European healthy adolescents or 2.6% in 2-12 years old Japanese children. If ALT >30-40 U/L is the criterion, prevalence varies between 3.2 and 8% in Korean and United States adolescents. Prevalence is higher in male children (around 40% more likely to develop fatty liver) and increases with age. Between 1993 and 2003, NAFLD was found in post-mortem liver histology of 0.7% of 2-4 years old children as compared to 17% of teenagers.

Risk factors

Unhealthy life style associated to an at-risk genetic background are clear risk factors of NAFLD driven by visceral fat accumulation (151). Genetic susceptibility is related to single nucleotide polymorphism variants of genes involved in lipid metabolism, also described in adults, and a score based on those can predict the risk of NASH in obese children (152). Different microbiota is also demonstrated in children developing NAFLD as compared to other obese or healthy children, with more Actinobacteria and less Bacteroidetes (153). Based on age, waist circumference and triglycerides, the Paediatric NAFLD Fibrosis Index (PNFI) can be used to predict the presence of fibrosis in children with NAFLD. The ELF® panel is proposed to predict the risk of progressive fibrosis (154).

Histology

If NAFLD histological definition is the same as in adults, the pattern of the steatosis and of the associated hepatitis is often different and described as the paediatric-type of NASH or type 2 NASH, as outlined previously (155).

Clinical approach

Clinical practice guidelines are less developed for children than for adults, although screening is recommended in the paediatric population. The European Society of Paediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN) proposes screening in children above 3 years old, overweight or obese, especially if they have high waist circumference according to specific percentile (Table 8) and/or familial history of NAFLD (154). The American Academy of Pediatrics recommends screening in children older than 10 years old, overweight with risk factors (parental obesity, family medical history of NAFLD, at risk current lifestyle habits, BMI trajectory and current cardiovascular risk factors) or obese (156). Serum ALT is often used as first screening with 95th percentile of normal values at 26 U/L in boys and 22 U/L in girls (157). Differential diagnosis is broad including genetic/metabolic diseases, Wilson's disease or parenteral nutrition. When NAFLD is suspected, the interest of liver imaging is increasingly reported to detect steatosis. Liver US has shown sensitivity of 70 to 85% for fatty liver disease in children, with a specificity of 50 to 60% (158). The use of MRI PDFF is so far limited to clinical research. Presence of fibrosis can be approached by VCTE, although mainly efficient to detect advanced fibrosis (159). The diagnosis of NASH requires liver biopsy. ESPGHAN states that liver biopsy should be performed when diagnosis is uncertain, in the presence of US evidence of steatosis, or in cases of persistent elevation of ALT levels after 3 to 6 months of lifestyle intervention (154). It should also be obtained before starting any pharmacological therapy for NASH.

Comorbidities should be looked for, including metabolic syndrome and cardiovascular problems. Quality of life has also been described as impaired, although it is not clearly demonstrated if this is due to NAFLD or to overweight/obesity. Physicians have to be aware that NASH can progress to end-stage cirrhosis that will require liver transplantation in childhood (3% of 66 children in a long-term outcome follow-up study). The exact risk to develop HCC is still unknown (150).

Guidance statement : Screening for NAFLD/NASH should be performed in children above 3 years old, overweight or obese, especially if they have high waist circumference (Table 8) and/or familial history of NAFLD.
Serum ULN for ALT is 26U/L in boys and 22U/L in girls.
Liver US is the imaging modality of choice to detect steatosis.
Liver biopsy should be performed when diagnosis is uncertain, in the presence of US evidence of steatosis, or in cases of persistent elevation of ALT levels after 3 to 6 months of lifestyle intervention. Non-invasive fibrosis tests can be used but lack extensive validation. Liver biopsy should also be obtained before starting any pharmacological therapy for NASH.

Cardiometabolic co-morbidities should be looked for and treated.

Treatment

The cornerstone of NAFLD treatment is the same than in adults: improve lifestyle with focus on nutrition and exercise. Weight loss is one of the important goals (150). However, we are lacking evidence to determine the best nutritional intervention in children. Achieving persistent lifestyle changes remains difficult and has some challenges specific to children, including parental perception (160).

Pharmaceutical therapy has shown conflicting results and none of them has yet been included in the recommendations for treatment. Metformin has a good safety profile and initially showed improvement of the non-invasive parameters in small series. However, in the TONIC trial, liver histology after treatment was comparable to the placebo group (161). In the same trial, vitamin E administration was associated with decreased hepatocyte ballooning, but other studies showed no advantage when compared to lifestyle intervention. Conflicting data were also observed with omega-3 fatty acids and probiotics therapies (161). Clinical trials with a combination of molecules are ongoing. Farnesoid Receptor X (FXR) agonists are yet to be trialled in children. It is important to stress that primary endpoints should be standardised for all the studies and should mainly be based on improved liver injury on histology (162).

Bariatric surgery or temporary devices should be considered as investigational in paediatrics, although they could already be useful in very selected cases and only if appropriate long-term follow-up is available (162,163).

Primary prevention of obesity is clearly the best therapeutic option in the paediatric population.

Guidance statement : Life style intervention focussing on nutrition and exercise are the cornerstone of paediatric NAFLD treatment, with some specific challenges in the paediatric setting and, due to a lack of data, without a clear guidance on what is the best nutritional intervention.

No pharmacological treatment can currently be recommended until the results of adequately designed clinical trials become available.

Miscellaneous

Genetics

Several single nucleotide polymorphisms have been associated with the presence and severity of NASH. These genetic alterations are background disease modifiers, but their value in predicting the presence of clinically significant NASH and/or the prognosis

is limited and currently testing for these mutations is not recommended (164-166). Only in case a specific metabolic disease is suspected, genetic testing can be used, but these cases should best be referred to centres with specific expertise (167).

Lean NASH

Lean NASH is defined as the presence of NASH in patients with a normal body weight (BMI < 25 kg/m^2). In many cases, these patients present subtler metabolic disturbances, *e.g.* mild visceral adiposity, dyslipidaemia (168). These patients should be investigated at least as thoroughly as the more classical NAFLD phenotypes for both severity of the liver disease as well as for the cardiometabolic co-morbidities (169). A detailed familial history is also required. If a specific underlying metabolic defect is suspected, the patient should best be referred to a centre of expertise (167).

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