Non-invasive screening, staging and management of metabolic dysfunction-associated fatty liver disease (MAFLD) in type 2 diabetes mellitus patients : what do we know so far ?

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Abstract

Metabolic dysfunction-associated fatty liver disease (MAFLD) is the evidence of steatosis in the setting of a metabolic risk condition such as type 2 diabetes mellitus (T2DM). Indeed, T2DM and liver steatosis share common pathophysiological mechanisms, and one can lead to the other. MAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH), fibrosis and cirrhosis as well as hepatocellular carcinoma (HCC). Because of the lack / disparity of guidelines for MAFLD screening, which is asymptomatic in its early stages, it is not rare that diabetic patients are belatedly diagnosed with NASH cirrhosis or HCC. We therefore recommend systematic non-invasive tests (NITs) that calculate an estimate of the risk based on readily available anthropometric and biological parameters. These include the fatty liver index (FLI) for steatosis detection and at least one of the following for fibrosis: non-alcoholic fatty liver disease fibrosis score (NFS), fibrosis-4 index (FIB-4) or Hepamet fibrosis score (HFS). Indeed, NFS and FIB-4 are the best predictors of liver-related events, while FIB-4 and HFS correlate with overall mortality. Systematic literature review found only few retrospective or cross-sectional studies using NITs for systematic steatosis and fibrosis screening in T2DM patients, with a crucial need for prospective studies. This screening strategy will allow targeted patients to be referred for further liver investigation (e.g. ultrasound, elastometry) and care. Current treatment modalities of MAFLD in T2DM patients range from lifestyle and dietary interventions to specific glucose-lowering drugs that recently showed some benefits regarding MAFLD, such as pioglitazone, glucagon-like peptide-1 receptor agonists and sodium-glucose cotransporter-2 inhibitors. Other treatments are currently under investigation. (Acta gastroenterol. belg., **2022,** 85, **1-12**).

Keywords: Non-invasive tests, MAFLD, NAFLD, NASH, type 2 diabetes.

Introduction

Definition of MAFLD

Metabolic dysfunction-associated fatty liver disease (MAFLD) was formerly known as non-alcoholic fatty liver disease (NAFLD), which was a diagnosis of exclusion: a chronic liver disease characterized by excessive fat build-up in the liver without another clear cause such as excessive alcohol consumption (> 210/140 g ethanol / week for male/female respectively), medications (e.g. corticosteroids, tamoxifen), total parenteral nutrition, viral and genetic diseases, etc. Recently, however, experts opted for a more accurate nomenclature based on positive diagnostic criteria (1), as shown in Table 1. Of note, the vast majority of studies discussed in this review were performed using the previous NAFLD definition.

MAFLD is thought to stem from the body's inability to store excess energy in adipocytes (considered as "healthy" storage in subcutaneous fat). The pathogenesis of MAFLD involves ectopic "unhealthy" fat accumulation, which takes place in the liver, muscle and visceral fat (2-5).

It is by definition coexisting with metabolic disorders such as insulin resistance, type 2 diabetes mellitus (T2DM), overweight or obesity. MAFLD has been commonly viewed as the hepatic manifestation of metabolic syndrome, the diagnostic criteria of which are described in Table 1.

MAFLD disease spectrum

As a disease spectrum, MAFLD can progress from simple steatosis to non-alcoholic steatohepatitis (NASH), advanced fibrosis and cirrhosis as well as hepatocellular carcinoma (HCC) (Figure 1) (6).

It is not yet known why the majority of MAFLD patients (around 90 %) remain at stage of simple steatosis, which generally has a benign course, whereas others progress to more severe disease and develop liver inflammation with hepatocyte damage (NASH), which can progress to fibrosis and cirrhosis as well as to HCC. Therefore, it is of utmost importance to determine the stage of the disease.

Steatosis was historically defined as an abnormal amount of liver fat exceeding 5% of total liver weight or 5% of hepatocytes containing lipid droplets (steatotic hepatocytes) on liver histology. This means that liver tissue samples and/or histology are needed for the diagnosis. However, as we will discuss further, in clinical practice, the presence of liver steatosis can also be detected with reasonable accuracy using biological scores and/or radiological imaging modalities (7). Moreover, these non-invasive methods suggestive of steatosis (widely used in current practice) are part of the current diagnosis of MAFLD (1).

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Table 1 – **Diagnostic criteria for MAFLD (reproduced with permission from (Lanthier & Vanuytsel, 2020) (1)) and criteria for metabolic syndrome in the Caucasian population (according to the International Diabetes Federation)**

1. Evidence of **liver steatosis** :
   – by an imaging technique,
   – and/or by the positivity of one score based on laboratory and anthropometric parameters (such as the Fatty Liver Index),
   – and/or by liver histology,
   +
2. Presence of a **metabolic risk condition** :
   – overweight/obesity,
   – and/or type 2 diabetes mellitus,
   – and/or metabolic syndrome, which diagnostic criteria are :

Waist circumference	≥ 94/80 cm for Caucasian men/women with ≥ 2 other criteria :
Arterial pressure	≥ 130/85 mmHg or treatment for hypertension
Fasting glucose	≥ 100 mg/dl (or previous type 2 diabetes mellitus diagnosis)
Serum triglycerides	≥ 150 mg/dl or treatment for dyslipidemia
HDL cholesterol	≤ 40/50 mg/dl for men/women or treatment for dyslipidemia

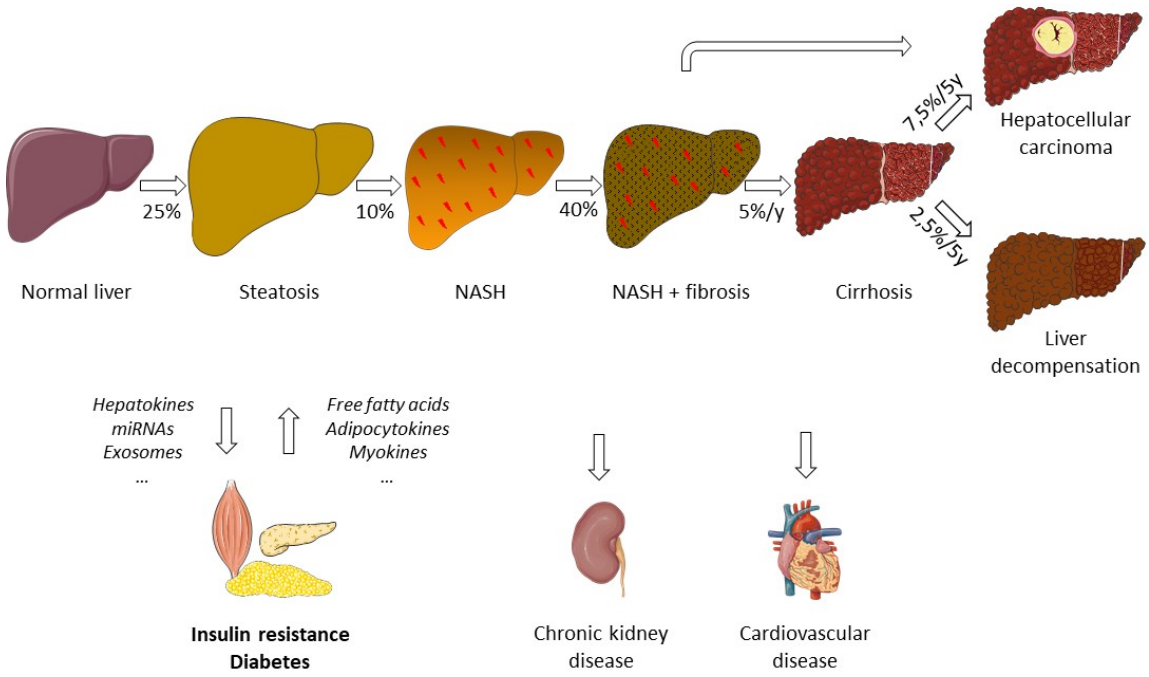

Figure 1 — MAFLD disease spectrum and its interrelationship with insulin resistance. NASH : non-alcoholic steatohepatitis. miRNA: micro-ribonucleic acid. Adapted from (Lanthier, 2018) (6) and partly created using Servier Medical Art templates, https://smart.servier.com.

NASH is defined by the coexistence of three components on liver histology: steatosis, cellular inflammation within liver lobules and hepatocyte ballooning, the latter being a feature of hepatocyte injury. This means that a diagnosis of NASH requires a liver biopsy. Further classification on disease severity (depending on the degree of necroinflammation or fibrosis) can be performed by histological scoring (7). For the biopsy report, in addition to describing the observed lesions, it is highly recommended to use the steatosis, activity and fibrosis (SAF) scoring system. The latter is probably more appropriate than the NAFLD activity score (NAS), due to better definition of ballooning, distinction between steatosis and necroinflammation, and balanced weighting between ballooning and inflammation (8). Liver biopsy also yields additional information such as the presence of microvesicular steatosis, which was shown to associate with NASH severity (9).

Liver fibrosis staging primarily relies on histology. However, as we will discuss hereunder, clinico-biological scores or imaging techniques can be used to rule out advanced fibrosis using appropriate and age-adjusted cut-offs (7).

Cirrhosis is characterized by diffuse nodular regeneration surrounded by dense fibrotic septa with subsequent parenchymal extinction and collapse of liver

Table 2 — **Prevalence of MAFLD disease spectrum in type 2 diabetes mellitus patients (T2DM), according to (Younossi et al, 2019) (17). US: ultrasound. H-MRS: proton magnetic resonance spectroscopy**

Disease stage	Number of studies included (and method of diagnosis)		Number of patients (interstudy range)	Estimated global prevalence in T2DM
MAFLD	80	74 (US)	875 (55 - 234)	**55.5%** (95% CI 47.3 - 63.7)
		6 (H-MRS)	48,544 (35 - 8,571)	
NASH	10 (biopsy)		892	**37.3%** (95% CI 24.7 - 50.0%)
Advanced fibrosis	7 (biopsy)		439	**4.80%** (95% CI 0.0 - 17.5)

structures, together causing pronounced distortion of hepatic vascular architecture (10). This distortion results in increased resistance to portal blood flow and hence in portal hypertension and hepatic synthetic dysfunction. Cirrhosis is notoriously asymptomatic until clinical decompensation occurs, with e.g. ascites, sepsis, variceal bleeding, encephalopathy and jaundice. Imaging of an irregular and nodular liver together with impaired liver synthetic function is sufficient for the diagnosis of cirrhosis.

HCC accounts for the majority of primary liver cancers. The majority of HCC occur in patients with underlying cirrhosis, mostly as a result of chronic hepatitis B or C viruses (HBV or HCV) infection, alcohol abuse or NASH. Rarely, as seen in patients with chronic HBV infection, patients with NASH can develop HCC without underlying cirrhosis (Figure 1).

Epidemiology and morbi-mortality

MAFLD is the most common liver disease worldwide and particularly in Western countries where it affects roughly a quarter of the population (11). Its prevalence is still on the rise, paralleling obesity and diabetes epidemics. NASH concerns 2.5-5% of the adult population. Among those patients with NASH, approximately 40% will develop progressive fibrosis (11) (Figure 1).

MAFLD is responsible for a high morbi-mortality. First of all, it is the source of hepatic complications (NASH, cirrhosis (12), HCC). NASH has become the leading cause for liver transplantation in the United States, due to its rising prevalence alongside highly-effective therapies for hepatitis C (13,14). Secondly and probably more importantly, MAFLD is associated with extra-hepatic morbi-mortality, mainly from insulin resistance and cardiovascular disease (CVD) (Figure 1). The majority of deaths in MAFLD patients are related to CVD and, beyond the risk factors in common, MAFLD independently increases the risk of CVD. Other strong evidence exists for a causal link between MAFLD and T2DM or chronic kidney disease (CKD) (Figure 1). Increasing evidence indeed supports the fact that MAFLD itself participates in the pathogenesis of these complications, rather than being a simple marker of shared metabolic risk factors (15). MAFLD has also been shown to be independently associated with obstructive sleep apnea syndrome or colorectal cancer (16).

Because MAFLD, NASH and NASH with advanced fibrosis are closely associated with T2DM, it is important to describe their global prevalence rates in that specific population. Younossi *et al* made a systematic review and meta-analysis of the global epidemiology of MAFLD in T2DM (17). Their study provides evidence of the high prevalence of MAFLD (globally 55.5 % but it was region-specific and likely underestimated as ultrasound has low performance for the detection of mild steatosis) (18), NASH (37.3 %) and advanced fibrosis (4.8 %) in patients with T2DM (Table 2).

In another report, these authors estimated the major economic burden of MAFLD using a Markov's model: it was estimated that 18.2 million people in the United States were living with both T2DM and MAFLD, of which 6.4 million have NASH. Twenty-year costs for MAFLD in these patients were $55.8 billion. Over the next 20 years, NASH with T2DM would account for 65,000 transplants, 1.37 million cardiovascular-related deaths, and 812,000 liver-related deaths (19). Similar alarming predictions were done in Belgium with direct annual medical cost due to NASH estimated to range between 100 to 400 million euro (20).

Interrelationship between MAFLD and T2DM

The liver constitutes a key organ in systemic metabolism, contributing substantially to the development of insulin resistance and T2DM (Figure 1). The mechanisms underlying these processes are not entirely understood, but involve hepatic fat accumulation, alterations of energy metabolism and inflammatory signals derived from various cell types including immune cells. In addition, chronic hyperinsulinemia from systemic (skeletal) insulin resistance promotes hepatic fat accretion and triglyceride-rich lipoproteins export, the former promoting liver insulin resistance and the latter driving atherogenic dyslipidemia, the combination of low high-density lipoprotein cholesterol (HDL-C) and hypertriglyceridemia, a key driver of residual cardiovascular risk in obesity and T2DM. Lipotoxins, mitochondrial dysfunction, cytokines and adipocytokines have been proposed to play a major part in both MAFLD and T2DM (21) (Figure 1).

Through the use of advanced mass spectrometry "omics" approaches and detailed experimentation in

cells, mice, and humans, there is better understanding of the mechanisms by which the liver secretes a wide array of proteins (hepatokines), metabolites, and noncoding ribonucleic acids (microRNAs), and how many of these liver-derived factors exert powerful effects on metabolic processes both in the liver and in peripheral tissues (e.g. fetuin-A and selenoprotein-P promote insulin resistance in adipose tissue and muscle respectively). There is also some evidence that extracellular vesicles, and in particular exosomes, may be an important mechanism for intratissue communication (e.g. fibrosis progression) but also for intertissue communication in promoting metabolic dysregulation in MAFLD (22) (Figure 1).

Conversely, insulin resistance in adipocytes results in lipolysis with excess release of free fatty acids (FFA) and glycerol into the bloodstream, which predisposes to **lipotoxicity** e.g. via excess lipid uptake by tissues such as the liver, muscle and pancreas, impairing insulin secretion in the latter (23) (Figure 1). FFA also activate a fibrogenic response in hepatic stellate cells that can promote progression to NASH and cirrhosis, and production of reactive oxygen species. Moreover, dysfunctional adipose tissue releases adipokines that activate pro-inflammatory pathways in the liver, muscle and pancreas (24) (Figure 1).

T2DM is also a chronic condition of **glucotoxicity** which, alongside lipotoxicity, contributes to insulin resistance, ectopic fat accumulation and beta-cell dysfunction (and vice versa) (24).

T2DM and MAFLD share insulin resistance and compensatory portal or systemic hyperinsulinemia as a common pathophysiological mechanism. Each of these conditions not only increases the risk of developing the other, but will also affect the course of each other. Clinically, the mutual interrelationship between these conditions is shown by findings suggesting that **liver steatosis is a rapid consequence of an unbalanced diet and is associated with hepatic insulin resistance, a major step to developing T2DM** (25-27)**. T2DM can exacerbate MAFLD by promoting progression to NASH or fibrosis**, while **MAFLD causes the natural course of diabetic complications (both micro- and macrovascular) to worsen in T2DM patients** (2,28)**.** For example, in a study by Targher *et al*, MAFLD was associated with increased rates of CKD (odds ratio 1.87; 95% CI 1.3-4.1, p=0.020) and proliferative/laser-treated retinopathy (odds ratio 1.75; 1.1-3.7, p=0.031) independently of age, sex, body mass index (BMI), waist circumference, hypertension, diabetes duration, diabetes control, lipids, smoking status and medications use (29). Moreover, in a study by Ciardullo *et al*, liver steatosis has been associated with higher prevalence of microalbuminuria (FLI: OR: 3.49; 95% CI 2.05 to 5.94, p<0.01) whereas liver fibrosis has been associated with CKD (FIB-4: OR: 6.39; 95% CI 4.05 to 10.08, p<0.01) and CVD (FIB-4: OR: 2.62; 95% CI 1.69 to 4.04, p<0.01) (30). However, contradictory results emerged from other studies, such as Gninkoun *et al.* that describe an inverse association between MAFLD (diagnosed by ultrasound) and the presence of diabetic retinopathy, cataract and ocular hypertonia (31). Therefore, prospective studies are urgently needed to clarify the course of microvascular complications in the setting of MAFLD.

MAFLD screening and disease severity evaluation

Recommendations from several international entities (European Association for the Study of the Liver, National

Table 3 — **Non-invasive score for steatosis. BMI: body mass index. WBC: white blood cell count. HT: hypertension. LR-/+: negative/ positive likelihood ratio**

Non-invasive score for steatosis	Reference	Formula	Interpretation		
Fatty Liver Index (FLI)	Bedogni *et al*, 2006[33]	FLI = e^y / (1 + e^y) × 100 Where y = 0.953 × ln(triglycerides, mg/dL) + 0.139 × BMI, kg/m^2 + 0.718 × ln (GGT, U/L) + 0.053 × waist circumference, cm – 15.745	< 30	Low risk	Fatty liver ruled out
			30 - 60	Indeterminate	Fatty liver neither ruled in nor ruled out
			> 60	High risk	Fatty liver ruled in
Hepatic Steatosis Index (HSI)	Lee *et al*, 2010[34]	HSI = 8 x (ALT/AST ratio) + BMI (+2, if female; +2, if diabetes mellitus)	< 30.0	Low risk	Fatty liver ruled out
			30.0 - 36.0	Indeterminate	Fatty liver neither ruled in nor ruled out
			> 36.0	High risk	Fatty liver ruled in
NAFLD Ridge Score (NRS)	Yip *et al*, 2017[xx]	NRS = -0,614 + 0,007 x ALT - 0,214 x HDL + 0,053 x triglycerides + 0,144 x HbA1c + 0,032 x WBC + 0,132 x HT	< 0.24	Low risk	Fatty liver ruled out
			0.24 - 0.44	Indeterminate	Fatty liver neither ruled in nor ruled out
			> 0.44	High risk	Fatty liver ruled in

Table 4 — **Non-invasive scores for fibrosis. IFG: impaired fasting glucose. DM: diabetes mellitus. AST: aspartate aminotransferase. ALAT: alanine aminotransferase. Fibrosis Severity Scale: F0 = no fibrosis ; F1 = mild fibrosis ; F2 = moderate fibrosis ; F3 = severe fibrosis; F4 = cirrhosis**

Non-invasive score for fibrosis	Reference	Formula	Interpretation
NAFLD Fibrosis Score (NFS)	Angulo *et al*, 2007[23]	NFS = -1.675 + (0.037 x age [years]) + (0.094 x BMI [kg/m²]) + (1.13 x IFG/DM [yes = 1, no = 0]) + (0.99 x AST/ALT ratio) – (0.013 x platelet count [×10⁹/L]) – (0.066 x albumin [g/l])	NFS: < -1.455 -1.455 – 0.675 > 0.675 Correlated Fibrosis Severity F0 - F2 Indeterminant score F3 - F4
FIB-4 index	Sterling *et al*, 2006[24]	FIB-4 = (Age* x AST) / (Platelet count x √(ALT))	*Use with caution in patients < 35 years old, as the score has been shown to be less reliable in these patients. *Use age-adjusted cut-offs in patients ≥ 65 years old. (McPherson et al, 2017) [xx] FIB-4 score: 36 – 64 yo / ≥ 65 yo < 1.3 / < 2.0 — Advanced fibrosis excluded 1.3 – 2.67 / 2.0 – 2.67 — Further investigation needed > 2.67 / > 2.67 — Advanced fibrosis (F3 – F4) likely
Hepamet Fibrosis Score (HFS)	Ampuero *et al*, 2018[21]	1 / (1 + e (5.390 - 0.986 x Age [45-64 years old] – 1.719 x Age [>65 years old] + 0.875 x Male sex– 0.896 x AST – 2.126 x AST – 0.027 x Albumin – 0.897 x Albumin – 0.899 x HOMA [2-3.99 with no DM] – 1.497 x HOMA [>4 with no DM] – 2.184 x DM – 0.882 x platelet count [155-219] – 2.233 x platelets [<155]).	HFS < 0.12 0.12 – 0.47 > 0.47 Correlated Fibrosis Severity Low risk Intermediate risk High risk for advanced fibrosis (F3 - F4)

Institute for health and Care Excellence, Asia-Pacific, American Association for the Study of Liver Disease) differ and there remains a lot of uncertainty on how to handle MAFLD in practice. In this setting, Francque *et al* published the *Belgian Association for Study of the Liver Guidance Document on the Management of Adult and Paediatric Non-Alcoholic Fatty Liver Disease* (Acta Gastroenterol Belg, 2018) (7), that offers algorithms for personalized screening, follow-up and management. In this paper, we wanted to thoroughly review the screening and diagnostic approaches for MAFLD in the specific population of T2DM patients. Indeed, whereas diabetic patients routinely benefit from an annual eye fundus (to detect retinopathy), urinalysis (looking for microalbuminuria) and cardiac stress test above 50 years old (to rule out silent myocardial ischemia), it is not rare that they are belatedly diagnosed with NASH cirrhosis or HCC because of the lack / disparity of guidelines for MAFLD screening, which is asymptomatic in its early stages.

Appropriate identification of patients at increased risk of NASH and advanced fibrosis is a critical step in the assessment of MAFLD. Since liver biopsy is invasive, expensive and prone to sampling error, it is difficult to justify its use in all high-risk patients such as those suffering from metabolic syndrome, obesity, T2DM or ischemic CVD.

Non-invasive tests (NITs)

Several clinical prediction rules and blood-based biomarkers were developed as attractive and affordable alternatives for identification of patients at high risk of NASH and significant fibrosis (≥ F2). They are called NITs (non-invasive tests). Current biomarkers constitute either predictive models (e.g. NAFLD fibrosis score and FIB-4 index) or direct measures of inflammation (e.g. circulating keratin 18 fragments) or fibrosis (e.g. FibroTest®, Enhanced Liver Fibrosis (ELF™) test or N-terminal type III collagen (Pro-C3) dosage). In the clinical setting, use of biomarkers-based NITs might discriminate between patients with NASH or advanced fibrosis, predict dynamic changes in NASH/fibrosis over time, and provide long-term prognostic information. Although clinically useful, current biomarker-based predictions can be influenced by hepatic and extrahepatic conditions (e.g. age, patient comorbidities, and fibrosis or NASH prevalence), which could lead to inaccurate estimates in subsamples of patients. Unfortunately, no highly-sensitive and specific tests are yet available to differentiate NASH from simple steatosis (32).

Non-invasive clinico-biological tests for steatosis (Table 3) include the fatty liver index (FLI) introduced by Bedogni *et al* in 2006 (33) (Area Under the Receiving Operating Characteristics (AUROC) 85 %; Sensitivity (Sn) 87% and Specificity (Sp) 86% with dual cut-offs of 30 and 60) and the hepatic steatosis index (HSI) introduced by Lee et al in 2010 (34) (AUROC 81%; Sn 93% and Sp 92% with dual cut-offs of 30 and 36) which were both developed in comparison to ultrasound. Conversely, the NAFLD Ridge score, introduced by Yip *et al* in 2017 (AUROC 87 %; Sn 91 % and Sp 90 % with dual cut-offs of 0.24 and 0.44), was developed in comparison

with magnetic resonance spectroscopy. All three will allow steatosis diagnosis with good accuracy but cannot however provide a quantitative evaluation of liver fat. Other tests include the SteatoTest™ (patented) and the NAFLD liver fat score, but these will not be discussed in this review since both require using laboratory data not available in routine. Moreover, the SteatoTest™ was developed and validated in non-diabetic cohorts, and was shown to underperform when applied to a large cohort of patients with T2DM (35).

Non-invasive clinico-biological tests for advanced fibrosis (Table 4) include the NAFLD fibrosis score (NFS) (AUROC 84%; Sn 82 % and Sp 98 % with dual cut-offs of -1.455 and 0.676) (36) and the fibrosis-4 index (FIB-4) (AUROC 86%; cut-off 1.3; Sn 85 % Sp 95 % NPV 95%) (37) which are based on readily available biochemical surrogates and clinical risk factors, and can be used to rule in or rule out advanced fibrosis using appropriate age-ajusted cut-offs (38). Their combined use can increase accuracy. An important restriction is that their accuracy is unacceptably low in patients below 35 years old. Even though guidelines do not specify which test to use in specific populations, NFS in patients with T2DM seems to identify most of the patients in intermediate or high risk groups, probably due to the variable hyperglycemia being included in the formula, leading to spectrum bias and potentially over-referral (30). In addition, it also requires the assessment of serum albumin concentration, which is not a routine test in a diabetes clinic. The FIB-4 index might therefore be more relevant in T2DM populations.

The NFS and FIB-4 tend to be less accurate than more 'complex' serum tests, which incorporate direct measures of fibrogenesis or fibrolysis (e.g. hyaluronic acid, N-terminal propeptide of type III collagen). Such tests (ELF™, FibroTest®, ...) can be used according to local expertise, but are proprietary and not reimbursed by the National Health Service. Pro-C3 has however been shown to perform well for the detection of fibrosis in T2DM and showed promising results for the prediction of histological changes in fibrosis stage with treatment (39).

The Hepamet fibrosis score (HFS) (AUROC 85 % for advanced fibrosis, Sn 71 % and Sp 98 % for dual cut-offs of 0.12 and 0.47) (40) was recently proposed to bring the greatest benefit in identifying patients who should undergo liver biopsy analysis and lead to significant improvements in reclassification, reducing the number of patients with undetermined results to 20% from 30% for the FIB-4 and NFS systems ($p < 0.05$). One of its limitations is that the score calculation requires an homeostasis model assessment of insulin resistance (HOMA-IR test with fasting insulin and glucose measurements). However, this is not the case in T2DM patients for whom the test is validated with an arbitrary high HOMA-IR value. Another limitation is the inapplicability of the HOMA model equations in patients with cirrhosis and redistribution of hepatic blood flow.

As regards future developments, recent studies have demonstrated lipidomic, proteomic and gut microbiome profiles (41,42), as well as microRNA signatures, to be promising techniques for fibrosis assessment (43).

Literature review and discussion regarding the use of NITs for MAFLD screening and staging in T2DM patients

There is a crucial need for prospective studies of MAFLD screening and severity assessment in high-risk populations, as a systematic literature review revealed only few retrospective or cross-sectional studies using non-invasive scores for systematic steatosis and fibrosis screening in T2DM populations.

The first study in this setting was an Italian retrospective study by Ciardullo *et al* (30) of T2DM patients attending ambulatory care between 2013 and 2018 (n = 2770). Steatosis was assessed using FLI, HSI and NAFLD Ridge Score. The prevalence of steatosis using FLI was 65 % (cut-off ≥ 60) with 24% of indeterminate results (30-60). Fibrosis was assessed using NFS, FIB-4, APRI and AST/ALT ratio. Outcome measures were altered albumin excretion rate, CKD and CVD. The prevalence of advanced fibrosis varied from 1% (APRI), 7 % (FIB-4) to 33% (NFS). The application of the guidelines using a sequential combination of FLI and FIB-4 would lead to referral of 28.3% of patients when using standard FIB-4 cut-offs, while this number dropped to 13.4% when age-adjusted FIB-4 thresholds were applied. A higher prevalence of (micro)albuminuria was associated with liver steatosis (FLI: OR 3.49 [95% CI 2.05- 5.94]; $p<0.01$), whereas liver fibrosis was associated with CKD (FIB-4: OR 6.39 [95% CI 4.05-10.08]; $p<0.01$) and CVD (FIB-4: OR 2.62 [95% CI 1.69-4.04]; $p<0.01$).

The second report was an Italian retrospective multicenter study by Morieri *et al* (44) who collected data from 46 diabetes clinics (n = 281381 patients), extracted data to calculate the HSI in 78895 patients and validated it against ultrasound-detected hepatic steatosis in 2179 patients. MAFLD (defined by HSI > 36) was present in 76.3% of the included population and was associated with macroangiopathy and nephropathy, while only 2.7% had HSI < 30 (low probability of steatosis). Treatment with dapagliflozin or incretin-based therapies resulted in a significant decrease of HSI after one year.

Limitations of those studies include mainly their retrospective design, which means the non-invasive scores might not have been appropriately calculated in all patients because of the lack of crucial information (e.g. abdominal circumference for the FLI) or because of non-concomitant collection of anthropometric and biological parameters. Secondly, they did not systematically assess the presence of other etiologies for steatosis such as excessive alcohol consumption. Furthermore, there was no systematic research of other causes (e.g. viral) of liver disease in case of elevated liver tests and the exclusion was based on what was reported by the patients or on available medical records. Thirdly, intermediate or

high-risk non-invasive scores did not lead to medical counseling and/or further investigation (e.g. imaging studies, liver biopsy) as recommended in guidelines.

Regarding the detection of advanced fibrosis, a cross-sectional study by Bril *et al* (45) compared the performance of several of the hereabove described non-invasive tools against liver histology in the specific T2DM population. The tests performed as follow : pro-C3 (AUROC 0.90 [95% CI 0.85 - 0.95]) > aspartate aminotransferase (AST) to platelet ratio index (APRI) (46) (AUROC 0.86 [95% CI 0.80 - 0.91]) > AST (AUROC 0.85 [95% CI 0.80 - 0.91]) > FIB-4 (AUROC 0.78 [95% CI 0.69 - 0.86]) > Fibrotest® (AUROC 0.70 [95% CI 0.59 - 0.81]) > NFS (AUROC 0.64 [95% CI 0.54 - 0.75]). Surprisingly, none of the studied approaches did significantly better than plasma AST (45).

Several reasons may explain why these tests under-performed in cohorts of patients with T2DM. First, patients with T2DM may only represent a relatively small part of the whole spectrum of MAFLD severity, resulting in potential spread effect. Second, certain glucose-lowering agents may significantly affect liver fat accumulation and/or measurements used to calculate these scores (e.g. plasma alanine aminotransferase (ALT), AST, BMI, ...). In addition, many of these diagnostic tools have been developed and validated in Caucasian populations and they might not necessarily apply to other ethnicities. Finally, observational studies are always prone to some selection bias that can affect generalizability of the study.

An option to reduce indeterminate results rate is therefore to use sequentially different biomarkers of liver fibrosis. Srivastava *et al* recently applied a two-step strategy in a primary care setting in which patients with a FIB-4 in the indeterminate range were evaluated using the ELF test (47). This approach led to a reduction in indeterminate results and in unnecessary referrals to hepatology clinics and rescued several patients with significant fibrosis that would have been falsely reassured by the FIB-4 score. Although the setting was different, a similar approach could potentially be more effective than a single step algorithm in diabetes clinics as well, although it remains to be proven. Bril *et al* studied the use of sequential tools in the identification of advanced fibrosis in the T2DM population (45). They assessed which tool allowed for exclusion of more patients with a negative predictive value (NPV) of 100%. The cut-off of 26 units/L for plasma AST excluded 44% of the patients. In the remaining population, PRO-C3 (<10 ng/mL) excluded an additional 19% of the cohort with an NPV of 100%. This resulted in only 37% of the initial cohort requiring liver biopsy, from whom 41% would have advanced fibrosis. No patient with advanced fibrosis was missed to diagnosis with this approach. The application of this approach using FIB-4 (< 0.87) after AST allowed for reducing the rate of biopsies to 48% of the entire cohort. APRI and NFS showed similar results to FIB-4, but the FibroTest® was unable to exclude any additional patients on top of plasma AST measurement. While one sees some trends toward improvement in sensitivity and specificity when these diagnostic approaches are combined, larger studies are required to assess whether these improvements are cost effective.

Imaging techniques

NITs are valuable for excluding steatosis or advanced fibrosis, but are unfortunately not sufficiently predictive when used alone. Moreover, NITs have not yet been demonstrated to accurately reflect changes in fibrosis following therapies targeting MAFLD, therefore limiting their role in disease monitoring (43). In the setting of intermediate or high NITs scores, further exams are thus needed e.g. Doppler-ultrasound and/or 1-dimensional ultrasound vibration controlled transient elastography (VCTE) (Fibroscan®, Echosens, Paris, France). Combining serum and ultrasound / elastography techniques increases diagnostic accuracy and can be used as screening and confirmatory tests, respectively.

Conventional B-mode ultrasound with color Doppler is the first line imaging modality frequently used to assess the presence of MAFLD. It allows for assessment of liver surface, parenchyma and vasculature along with the other abdominal organs, particularly the spleen and splenic vein. It is however insufficient to evaluate steatosis severity (not sensitive enough for mild steatosis and not precise enough for steatosis quantification) or fibrosis (it will only allow for detection of signs of cirrhosis and portal hypertension) (7).

VCTE is the most validated ultrasound-based technique for measuring liver stiffness as a non-invasive surrogate for fibrosis assessment. Its precise usefulness for screening still needs to be determined, but taking into account its availability and current lack of specific reimbursement, it is probably to be positioned in second line in case of non-invasive scores that show intermediate or high probability of advanced fibrosis. VCTE was shown to allow for excluding severe fibrosis with high negative predictive value in MAFLD patients. The technique suffers however from increasing rates of unreliability with more severe obesity (7).

New imaging techniques were further developed, including **acoustic radiation force impulse** (ARFI) in 2009 and more recently **supersonic shear wave imaging** (SSI) in 2011. Both techniques allow the selection of the surface of interest (through a coupled standard two dimension ultrasound image) to perform fibrosis quantification. When compared to VCTE, those techniques do not benefit from sufficient evidence for their implementation in clinical practice (48).

Magnetic resonance elastography appears more accurate than sonographic elastography and is considered the most accurate non-invasive technique in diagnosing fibrosis and cirrhosis in patients with chronic liver disease. One noticeable advantage is that it is not impacted by obesity. Limitations include its cost, test duration, pre-

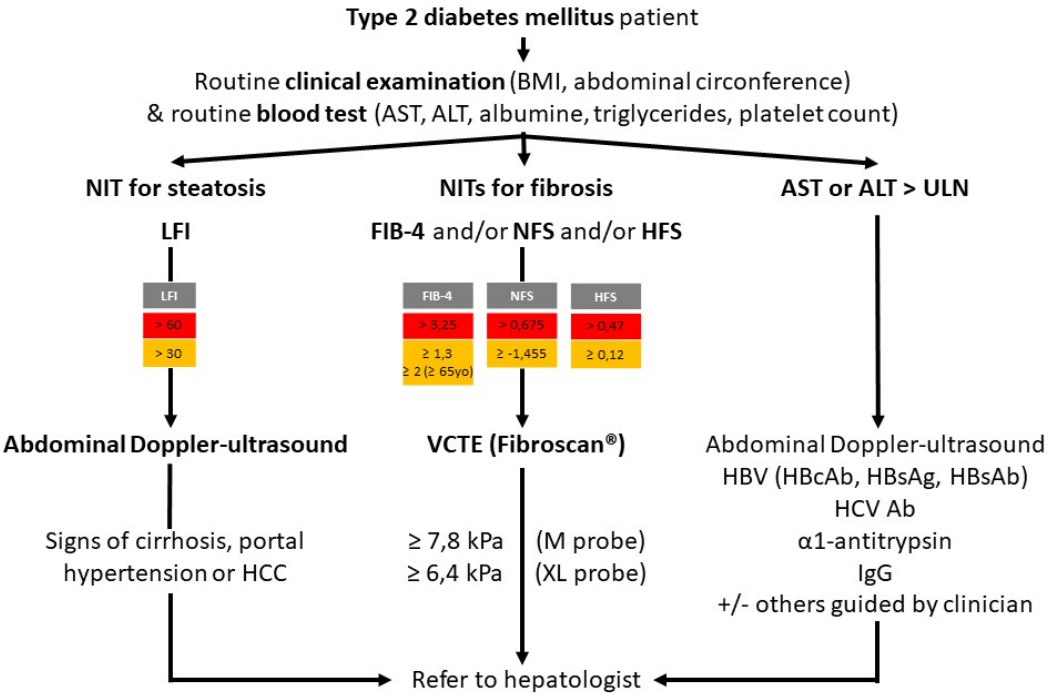

Figure 2 — Screening algorithm for MAFLD in type 2 diabetes mellitus patients. BMI: Body Mass Index. AST: Aspartate aminotransferase. ALT: Alanine aminotransferase. NIT: non-invasive test. ULN: upper limit of normal values. LFI: liver fatty index. FIB-4: fibrosis-4. NFS: NAFLD fibrosis score. HFS: Hepamet fibrosis score. HCC: Hepatocellular carcinoma. VCTE : vibration-controlled transient elastography. M and XL probes: medium and extra-large. HBV and HCV: hepatitis B and C viruses. Ab: antibody. Ag: antigen. IgG: immunoglobulin G.

sence of contraindications such as claustrophobia or presence of a pacemaker, and more importantly limited availability (49,50). Moreover, it was not specifically studied in T2DM populations.

Screening algorithm

Based on the discussion hereabove, we suggest a simple algorithm combining serum and ultrasound / elastography techniques as screening and confirmatory tests respectively, in order to increase diagnostic accuracy (Figure 2). Moreover, in case of elevated liver enzymes, other causes should be excluded; we recommend an initial screening including at least HBc antibody, HBs antigen, HBs antibody, HCV antibody, alpha-1-antitrypsin, immunoglobulin G and others guided by clinician, patient background and age.

Limitations of screening algorithms based on fibrosis

An approach which bases referrals only on fibrosis stage is insufficient.

First of all, it is likely to miss a subset of patients with NASH and early fibrosis, who still need referral to a specialist since they are at high risk of developing advanced fibrosis or cirrhosis in the upcoming years. This underlines the need for reliable non-invasive biomarkers of NASH, which are still not available in clinical practice. Several non-invasive clinical models/scores and plasma biomarkers have been studied to identify NASH in the T2DM population (ALT, cytokeratin-18, NashTest 2 (BioPredictive), ActiTest (BioPredictive), HAIR (51), BARD (52), and OWLiver (53)). None of the noninvasive tools assessed for the diagnosis of NASH in T2DM had an optimum performance (all AUROCs <0.80) (35,45). Of note, none of the panels or biomarkers outperformed plasma ALT (AUROC 0.78 [95% CI 0.71-0.84]).

Secondly the reliability of those scores in predicting long-term outcomes for hepatic/extra-hepatic complications or death and their concordance in cross-sectional and longitudinal risk stratification remains uncertain. The largest study in a European cohort of biopsy-proven MAFLD patients with a considerable follow-up is the recent study by Younes *et al* (54), which assessed the most common non-invasive scores (NFS, FIB-4, BARD, APRI) and the HFS in 1173 MAFLD European patients from tertiary centres (of which 331 also had T2DM). Cross-sectional analysis revealed HFS as the best performer for the identification of significant (F0-1 vs F2-4, AUC=0.758) and advanced fibrosis (F0-2 vs F3-4, AUC=0.805), while NFS and FIB-4 showed the highest performance in detecting histological cirrhosis (range AUCs 0.85-0.88). Considering longitudinal data (follow-up between 62 and 110 months), NFS and FIB-4 were the best at predicting liver-related events (end-stage cirrhosis and cirrhosis decompensation including ascites, encephalopathy and variceal bleeding), NFS performed best for HCC, while FIB-4 and HFS showed the best performance for overall mortality. All non-invasive scoring systems showed scarce performance for extra-hepatic events. Overall, NFS, HFS and FIB-4

outperformed APRI and BARD in both cross-sectional identification of fibrosis and prediction of long-term outcomes, showing to be useful tools for clinicians in managing MAFLD patients at increased fibrosis risk and liver-related complications or death. In diabetic MAFLD patients, scores including diabetes in the algorithm had inferior performance whereas APRI or FIB-4 performed better for significant fibrosis and FIB-4 for overall fibrosis risk predictions. However, the scores including diabetes were more effective compared to the other scores in predicting cardiovascular and extra-hepatic cancer events, despite overall performance for these events is low.

MAFLD management in T2DM patients

Unfortunately, therapeutic disease modifying options for patients with MAFLD are currently still limited but a lot of research is going on, focusing on fibrosing NASH (8,55). Currently, lifestyle therapeutic changes remain the cornerstone of treatment for T2DM patients with MAFLD, who should be informed that these measures are necessary to improve the condition of their liver. The main strategies in the treatment of MAFLD are summarized as follows :

(1) **Lifestyle modifications** : physical activity, caloric restriction, Mediterranean diet, reduction of fructose intake, > 7 - 10 % weight loss (56), alcohol abstinence…

(2) **Treatment of metabolic traits** e.g. dyslipidemia, glycemic control, obesity (including bariatric surgery when meeting local reimbursement criteria)…

(3) Mostly off-label **pharmacological treatments** for the treatment of NASH, but approved in specific circumstances as glucose-lowering drugs in the treatment of T2DM.

Pharmacological treatment of MAFLD should for now on be limited to patients with comorbidities (e.g. T2DM, persistently elevated ALT, metabolic syndrome) or with histologically proved NASH with a NASH activity score (NAS) ≥ 4-5 and fibrosis (F) ≥ 1-2 according to the NASH-CRN scoring (7). When T2DM and MAFLD co-exist, there are published data that can help inform the clinician as to the most appropriate oral hypoglycemic agent or injectable therapy that may improve MAFLD, however most of these data are drawn from observations in retrospective series and there is a paucity of well-designed randomized double blind placebo controlled studies with gold-standard endpoints. Data from a few prospective phase 2 studies exist in the treatment of fibrosing NASH but these studies included patients with and without T2DM (57-61). Furthermore, given the heterogeneity of inclusion criteria and primary outcomes, as well as duration of follow-up, it is difficult to draw robust conclusions that are applicable across the entire spectrum of MAFLD and diabetes.

Thiazolodinediones or glitazones (peroxisome proliferator-activated receptor gamma or PPARɣ agonists) have shown to be effective in improving histological lesions of NASH. Indeed, PPARɣ activation reverses insulin resistance and improves glucose and lipid metabolism, with redistribution of excess triglyceride accumulation from the liver to adipose tissue, explaining the frequent weight gain (of 3 to 5% in placebo-controlled trials lasting 6 months to 3 years) observed. Other mechanisms include an increase in plasma adiponectin and an amelioration of subclinical inflammation (62). For example, pioglitazone has showed a greater clinical benefit in NASH (related to its partial PPARα agonism, unlike pure PPARɣ agonists such as rosiglitazone) (63) and a more favorable safety profile than other molecules of the same family regarding cardiovascular risk. Its reimboursement in Belgium is restricted to uncontrolled T2DM despite metformin or sulfamide treatment (34). The clinical practice guidelines recognize that pioglitazone may also be prescribed for patients with biopsy-proven NASH, but those will not get reimbursement in Belgium (64).

Glucagon-like peptide-1 receptor agonists (GLP1-RAs), that belong to the incretin mimetics family, improve glycemic control and reduce weight. They became, along with SGLT2 inhibitors, the second line therapy for T2DM after metformin, because of their potent glucose-lowering effects as well as antiproteinuric effects and a substantial effect (for some GLP1-RAs) in reducing residual cardiovascular risk, irrespective of glucose lowering or weight loss. They also have numerous seemingly-beneficial metabolic effects relevant to the pathophysiology of MAFLD, likely to go beyond weight loss or glucose lowering (65). For example, liraglutide, the current indications of which include obesity (BMI > 30 kg/m²) or overweight (BMI > 27 kg/m²) associated with comorbidities (T2DM, arterial hypertension, dyslipidemia, obstructive sleep apnea syndrome, NASH), has been reported to beneficially affect liver histology. Whereas weight loss is thought to be the main driver of the histological benefit, direct intrahepatic effects cannot be excluded (66). Another agent is semaglutide which also showed significant beneficial effects on NASH resolution but not on fibrosis stage improvement (58).

Dipeptidyl peptidase 4 (DPP-4) inhibitors exert their glucose-lowering effects by blocking the DPP-4 enzyme that is involved in the degradation of incretins i.e. GLP-1 and glucose-dependent insulinotropic polypeptide (GIP). Among DPP-4 inhibitors, sitagliptin has been most widely studied in relation to its effects on MAFLD/NASH. Besides the weight loss and reduction in serum HbA1c, data are available from studies with a small number of subjects but no large phase IIb trial with histological endpoints has been reported (67).

Sodium-glucose cotransporter-2 (SGLT2) inhibitors have been shown to improve glucose homeostasis as well as conferring cardiorenal protection via multiple mechanisms (68), placing them along with GLP1-RAs as second-line agents for the management of T2DM. There is also a growing expectation that this class of agents will assist in the treatment of NASH as several studies

report significant benefits regarding the reversal of liver steatosis, hepatocyte necrosis, inflammation and fibrosis. Dedicated prospective studies with histological outcomes are needed to confirm supposed beneficial effects in the treatment of NASH. Examples include dapaglifozin, shown to significantly reduce steatosis and fibrosis in T2DM patients with MAFLD (69) and empaglifozin, shown to significantly reduce steatosis and ALT levels in T2DM patients with MAFLD (70).

Other molecules that have no place in the treatment of NASH in T2DM patients include metformin, vitamin E and ursodeoxycholic acid. Metformin, although often considered as indirect insulin sensitizer, does not significantly impact resolution of NASH or fibrosis and is considered neutral by current guidelines (64). Vitamin E has shown beneficial effects on liver histology in non-diabetic and non-cirrhotic NASH patients, so its use should be restricted to this group (and shall therefore not be discussed in this paper) (71). However, the potential of increased prostate cancer in men is an unresolved issue of concern (72). Ursodeoxycholic acid has been shown to improve liver tests and histological features but fails to maintain long-term histological benefit, and is therefore not recommended (73).

Promising molecules that deserve mentioning are resmetirom (MGL-3196), lanifibranor (IVA-337) and pemafibrate. Resmetirom is a liver-directed, orally active, selective thyroid hormone receptor-β agonist designed to improve NASH by increasing hepatic fat metabolism and reducing lipotoxicity. This treatment resulted in significant reduction in hepatic fat after 12 weeks and 36 weeks of treatment in patients with NASH. Further studies of resmetirom will allow assessment of its safety and effectiveness in a larger number of patients with NASH with the possibility of documenting associations between histological effects and changes in non-invasive markers and imaging (57). Lanifibranor is a panPPAR agonist studied in the phase II NATIVE trial (59), that showed impressive results on NASH resolution, fibrosis regression and both lipid profile and insulin sensitivity improvement in adult non-cirrhotic NASH patients, with only 24 weeks of treatment. Lastly, pemafibrate, a selective peroxisome proliferator-activated receptor α modulator (SPPARMα), may also offer benefits on liver function and glucose metabolism based on preliminary studies (74,75).

All in all, adequate management relies on a multi-layer prevention strategy where MAFLD staging plays a central role. Professional health care providers should put more efforts in **primary prevention** using a behavioral therapy needing a multidisciplinary approach, in **secondary prevention** applying on a regular basis in the clinical setting available predictive algorithms to identify the patients at higher cardiovascular and hepatologic risk (Figure 2), and in **tertiary prevention** treating, when not contraindicated, the diabetic patients preferentially with drugs with proven benefit on MAFLD/NASH. Effective and appropriate disease staging will not only allow for prevention measures and targeted treatment but also allow for a **personalized follow-up** and **early detection of complications of cirrhosis**, such as hepatic encephalopathy, spontaneous bacterial peritonitis, portal vein thrombosis and hepatocellular carcinoma (76). It is therefore crucial to measure insulin resistance and seek for the presence of T2DM in a patient with MAFLD, but also to look for MAFLD and assess its severity (NASH? fibrosing disease?) in a patient with T2DM.

Conclusion

MAFLD is now recognized as the most prevalent chronic liver disease worldwide. T2DM is an important risk factor for MAFLD and vice-versa, and seems to accelerate the progression of liver disease. Despite the high prevalence and serious clinical implications of MAFLD in patients with T2DM, it is usually overlooked in clinical practice.

Because MAFLD is responsible for an important (extra-)hepatic morbi-mortality, there is a need for increased awareness among all important stakeholders (primary care physicians, specialists, and health policy makers) on **adding MAFLD as a frequent end-organ complication of T2DM**, next to well-known micro- and macrovascular complications. It is feasible to routinely triage patients with potentially severe liver disease using **simple non-invasive tools** so that only few selected T2DM patients will eventually benefit from a liver biopsy which remains the gold standard for MAFLD staging.

In clinical practice and for the specific population of T2DM patients, we would recommend the systematic calculation of the FLI for steatosis detection and at least one of the following non-invasive tests for fibrosis: NFS, FIB-4 and HFS. These scores are based on readily available clinical and biological parameters, and could therefore routinely be calculated by general practitioners and diabetologists to detect patients that might benefit from further investigation and counseling in an hepatology consultation. In the setting of indeterminate results for fibrosis or suspected advanced fibrosis, patients should benefit from a VCTE for confirmation and an abdominal Doppler-ultrasound to assess liver surface, parenchyma and vasculature. In case of elevated liver enzymes, other causes should be excluded. In order to confirm and assess the feasibility and accuracy of such a systematic screening, there is a crucial need for prospective studies of MAFLD screening in high-risk patients such as those with T2DM.

Management of patients with MAFLD and T2DM relies on lifestyle optimization to achieve significant weight loss. Currently, there is no drug approved for the treatment of NASH in T2DM although pioglitazone might be considered in selected patients. Approved glucose-lowering medications (e.g. GLP1-RAs, SGLT2 inhibitors) hold promise for NASH treatment and several liver-specific drugs are in evaluation clinical trials. A

combination approach will likely represent the future of MAFLD therapeutics.

Conflict of interest statement

The authors declare that they have no competing interests.

References

1. LANTHIER N, VANUYTSEL T. Metabolic dysfunction-associated fatty liver disease: A new clearer nomenclature with positive diagnostic criteria. *Acta Gastroenterol Belg.* 2020; **83**(4): 513-5.
2. RHEE E-J. Nonalcoholic Fatty Liver Disease and Diabetes: An Epidemiological Perspective. *Endocrinol Metab.* 2019; **34**(3): 226.
3. LANTHIER N, LECLERCQ IA. Adipose tissues as endocrine target organs. *Best Pract Res Clin Gastroenterol.* 2014; **28**(4): 545-58.
4. NACHIT M, LANTHIER N, RODRIGUEZ J, NEYRINCK AM, CANI PD, BINDELS LB, *et al.* A dynamic association between myosteatosis and liver stiffness: Results from a prospective interventional study in obese patients. *JHEP Reports*. 2021; **3**(4): 100323.
5. LANTHIER N, RODRIGUEZ J, NACHIT M, HIEL S, TREFOIS P, NEYRINCK AM, *et al.* Microbiota analysis and transient elastography reveal new extra-hepatic components of liver steatosis and fibrosis in obese patients. *Sci Rep.* 2021 Jan; **11**(1): 659.
6. LANTHIER N. La NASH en 2018. *Louv Med.* 2018; **137**(5): 308-13.
7. FRANCQUE SM, LANTHIER N, VERBEKE L, REYNAERT H, VAN STEENKISTE C, VONGHIA L, *et al.* The Belgian association for study of the liver guidance document on the management of adult and paediatric non-alcoholic fatty liver disease. *Acta Gastroenterol Belg.* 2018; **81**(1): 55-81.
8. HEYENS LJM, BUSSCHOTS D, KOEK GH, ROBAEYS G, FRANCQUE S. Liver Fibrosis in Non-alcoholic Fatty Liver Disease: From Liver Biopsy to Non-invasive Biomarkers in Diagnosis and Treatment. *Front Med.* 2021 Apr; **8**: 615978.
9. CELEBI G, CICEK AF, GUREL H, GENC H, KIRIK A, ERCIN CN, *et al.* Microvesicular steatosis: A missed item in the management of nonalcoholic fatty liver disease? *Acta Gastroenterol Belg.* 2020; **83**(4): 565-70.
10. CLAREMBEAU F, BALE G, LANTHIER N. Cirrhosis and insulin resistance: Current knowledge, pathophysiological mechanisms, complications and potential treatments. *Clin Sci.* 2020; **134**(16): 2117-35.
11. YOUNOSSI ZM, KOENIG AB, ABDELATIF D, FAZEL Y, HENRY L, WYMER M. Global epidemiology of nonalcoholic fatty liver disease – Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016 Jul; **64**(1): 73-84.
12. KAZE E, DESCAMPS OS, HENRION J. The changing pattern of cirrhosis in belgium: A study based on two cohorts pro-spectively collected 15 years apart. *Acta Gastroenterol Belg.* 2020; **83**(4): 559-63.
13. SHIRAZI F, WANG J, WONG RJ. Nonalcoholic Steatohepatitis Becomes the Leading Indication for Liver Transplant Registrants Among US Adults Born Between 1945 and 1965. *J Clin Exp Hepatol.* 2020 Jan; **10**(1): 30-6.
14. HAMOIR C, HORSMANS Y, STÄRKEL P, DAHLQVIST G, NEGRIN DASTIS S, LANTHIER N. Risk of hepatocellular carcinoma and fibrosis evolution in hepatitis c patients with severe fibrosis or cirrhosis treated with direct acting antiviral agents. *Acta Gastroenterol Belg.* 2021 Jan-Mar; **84**(1): 25-32.
15. ADAMS LA, ANSTEE QM, TILG H, TARGHER G. Non-Alcoholic fatty liver disease and its relationship with cardiovascular disease and other extrahepatic diseases. *Gut*. 2017 Jun; **66**(6): 1138-53.
16. TARIQ R, AXLEY P, SINGAL AK. Extra-Hepatic Manifestations of Nonalcoholic Fatty Liver Disease: A Review. *J Clin Exp Hepatol.* 2020 Jan; **10**(1): 81-7.
17. YOUNOSSI ZM, GOLABI P, DE AVILA L, PAIK JM, SRISHORD M, FUKUI N, *et al.* The global epidemiology of NAFLD and NASH in patients with type 2 diabetes: A systematic review and meta-analysis. *J Hepatol.* 2019 Oct; **71**(4): 793-801.
18. FERRAIOLI G, MONTEIRO LBS. Ultrasound-based techniques for the diagnosis of liver steatosis. *World J Gastroenterol.* 2019 Oct; **25**(40): 6053-62.
19. YOUNOSSI ZM, TAMPI RP, RACILA A, QIU Y, BURNS L, YOUNOSSI I, *et al.* Economic and clinical burden of nonalcoholic steatohepatitis in patients with type 2 diabetes in the U.S. *Diabetes Care*. 2020 Feb; **43**(2): 283-9.
20. VANDEKERCKHOVE P, VAN DAMME B, ANNEMANS L. How will the welfare state cope with welfare diseases such as nash? *Acta Gastroenterol Belg.* 2019; **82**(4): 548-9.
21. TILG H, MOSCHEN AR, RODEN M. NAFLD and diabetes mellitus. *Nat Rev Gastroenterol Hepatol.* 2017 Jan; **14**(1): 32-42.
22. WATT MJ, MIOTTO PM, DE NARDO W, MONTGOMERY MK. The Liver as an Endocrine Organ—Linking NAFLD and Insulin Resistance. *Endocr Rev*. 2019 Oct; **40**(5): 1367-93.
23. OLIVEROS-MONTIEL A, SANTOS-LÓPEZ G, SEDEÑO-MONGE V. Proteins involved in lipid metabolism as possible biomarkers or predisposing factors for non-alcoholic fatty liver disease. *Acta Gastroenterol Belg.* 2020; **83**(4): 622-30.
24. GASTALDELLI A, CUSI K. From NASH to diabetes and from diabetes to NASH: Mechanisms and treatment options. *JHEP Reports.* 2019 Oct; **1**(4): 312-28.
25. LANTHIER N. Targeting Kupffer cells in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis: Why and how? *World J Hepatol.* 2015; **7**(19): 2184.
26. LANTHIER N, LECLERCQ IA. Liver and systemic insulin resistance. *Hepatology*. 2014; **60**(3): 1113-4.
27. LANTHIER N. The role of the liver in insulin resistance. *Treat Strateg - Hepatol*. 2014; **1**(1): 89-95.
28. HAZLEHURST JM, WOODS C, MARJOT T, COBBOLD JF, TOMLINSON JW. Non-alcoholic fatty liver disease and diabetes. *Metabolism*. 2016 Aug; **65**(8): 1096-108.
29. TARGHER G, BERTOLINI L, RODELLA S, ZOPPINI G, LIPPI G, DAY C, *et al.* Non-alcoholic fatty liver disease is independently associated with an increased prevalence of chronic kidney disease and proliferative/laser-treated retinopathy in type 2 diabetic patients. *Diabetologia*. 2008 Mar; **51**(3): 444-50.
30. CIARDULLO S, MURACA E, PERRA S, BIANCONI E, ZERBINI F, OLTOLINI A, *et al.* Screening for non-alcoholic fatty liver disease in type 2 diabetes using non-invasive scores and association with diabetic complications. *BMJ Open Diabetes Res Care*. 2020 Feb; **8**(1): e000904.
31. GNINKOUN CJ, AHN SA, AMOUSSOU-GUENOU KD, BOUENIZABILA E, ROUSSEAU MF, HERMANS MP. Fatty Liver Linked to Reduced Frequency of Ocular Complications in T2DM. *J Diabetes Mellit*. 2020; **10**(03): 154-68.
32. VILAR-GOMEZ E, CHALASANI N. Non-invasive assessment of non-alcoholic fatty liver disease: Clinical prediction rules and blood-based biomarkers. *J Hepatol.* 2018 Feb; **68**(2): 305-15.
33. BEDOGNI G, BELLENTANI S, MIGLIOLI L, MASUTTI F, PASSALACQUA M, CASTIGLIONE A, *et al.* The fatty liver index: A simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol.* 2006 Dec; **6**(1): 33.
34. LIAO H-W, SAVER JL, WU Y-L, CHEN T-H, LEE M, OVBIAGELE B. Pioglitazone and cardiovascular outcomes in patients with insulin resistance, pre-diabetes and type 2 diabetes: a systematic review and meta-analysis. *BMJ Open*. 2017 Jan; **7**(1): e013927.
35. BRIL F, MCPHAUL MJ, CAULFIELD MP, CASTILLE JM, POYNARD T, SOLDEVILA-PICO C, *et al.* Performance of the SteatoTest, ActiTest, NashTest and FibroTest in a multiethnic cohort of patients with type 2 diabetes mellitus. *J Investig Med.* 2019 Feb; **67**(2): 303-11.
36. ANGULO P, HUI JM, MARCHESINI G, BUGIANESI E, GEORGE J, FARRELL GC, *et al.* The NAFLD fibrosis score: A noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology*. 2007 Apr; **45**(4): 846-54.
37. STERLING RK, LISSEN E, CLUMECK N, SOLA R, CORREA MC, MONTANER J, *et al.* Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV coinfection. *Hepatology*. 2006 Jun; **43**(6): 1317-25.
38. MCPHERSON S, HARDY T, DUFOUR JF, PETTA S, ROMERO-GOMEZ M, ALLISON M, *et al.* Age as a Confounding Factor for the Accurate Non-Invasive Diagnosis of Advanced NAFLD Fibrosis. *Am J Gastroenterol.* 2017 May; **112**(5): 740-51.
39. BRIL F, LEEMING DJ, KARSDAL MA, KALAVALAPALLI S, BARB D, LAI J, *et al.* Use of plasma fragments of propeptides of Type III, V, and VI procollagen for the detection of liver fibrosis in type 2 diabetes. *Diabetes Care*. 2019 Jul; **42**(7): 1348-51.
40. AMPUERO J, PAIS R, ALLER R, GALLEGO-DURÁN R, CRESPO J, GARCÍA-MONZÓN C, *et al.* Development and Validation of Hepamet Fibrosis Scoring System-A Simple, Noninvasive Test to Identify Patients With Nonalcoholic Fatty Liver Disease With Advanced Fibrosis. *Clin Gastroenterol Hepatol*. 2020 Jan; **18**(1): 216-225.e5.
41. LANTHIER N, RODRIGUEZ J, NACHIT M, HIEL S, TREFOIS P, BINDELS LB, *et al.* New determinants of liver steatosis and fibrosis in obese patients: results of a prospective clinical study. *Clin Nutr ESPEN*. 2020; **40**: 438-9.
42. KNUDSEN C, NEYRINCK AM, LANTHIER N, DELZENNE NM. Microbiota and nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care*. 2019 Sep; **22**(5): 393-400.

43. LOOMBA R, ADAMS LA. Advances in non-invasive assessment of hepatic fibrosis. *Gut*. 2020 Jul; **69**(7): 1343-52.
44. MORIERI ML, VITTURI N, AVOGARO A, TARGHER G, FADINI GP. Prevalence of hepatic steatosis in patients with type 2 diabetes and response to glucose-lowering treatments. A multicenter retrospective study in Italian specialist care. *J Endocrinol Invest.* 2021 Sep; **44**(9): 1879-1889.
45. BRIL F, MCPHAUL MJ, CAULFIELD MP, CLARK VC, SOLDEVILLA-PICO C, FIRPI-MORELL RJ, *et al.* Performance of plasma biomarkers and diagnostic panels for nonalcoholic steatohepatitis and advanced fibrosis in patients with type 2 diabetes. *Diabetes Care.* 2020 Feb; **43**(2): 290-7.
46. WAI C. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology*. 2003 Aug; **38**(2): 518-26.
47. SRIVASTAVA A, GAILER R, TANWAR S, TREMBLING P, PARKES J, RODGER A, *et al.* Prospective evaluation of a primary care referral pathway for patients with non-alcoholic fatty liver disease. *J Hepatol.* 2019 Aug; **71**(2): 371-8.
48. LANTHIER N, STÄRKEL P, HORSMANS Y. New imaging assisted methods for liver fibrosis quantification: Is it really favorable to classical transient elastography? *J Hepatol.* 2015 Sep; **63**(3): 765-6.
49. LI J, VENKATESH SK, YIN M. Advances in Magnetic Resonance Elastography of Liver. *Magn Reson Imaging Clin N Am.* 2020 Aug; **28**(3): 331-40.
50. LEFEBVRE T, WARTELLE-BLADOU C, WONG P, SEBASTIANI G, GIARD JM, CASTEL H, *et al.* Prospective comparison of transient, point shear wave, and magnetic resonance elastography for staging liver fibrosis. *Eur Radiol.* 2019 Dec; **29**(12): 6477-88.
51. DIXON JB, BHATHAL PS, O'BRIEN PE. Nonalcoholic Fatty Liver Disease: Predictors of Nonalcoholic Steatohepatitis and Liver Fibrosis in the Severely Obese. *Gastroenterology*. 2001 Jul; **121**(1): 91-100.
52. HARRISON SA, OLIVER D, ARNOLD HL, GOGIA S, NEUSCHWANDER-TETRI BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut*. 2008 Apr; **57**(10): 1441-7.
53. MAYO R, CRESPO J, MARTÍNEZ-ARRANZ I, BANALES JM, ARIAS M, MINCHOLÉ I, *et al.* Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: Results from discovery and validation cohorts. *Hepatol Commun.* 2018 Jul; **2**(7): 807-20.
54. YOUNES R, CAVIGLIA GP, GOVAERE O, ROSSO C, ARMANDI A, SANAVIA T, *et al.* Long-term outcomes and predictive ability of non-invasive scoring systems in patients with non-alcoholic fatty liver disease. *J Hepatol.* 2021 Oct; **75**(4): 786-794.
55. LANTHIER N. New therapies in non-alcoholic steatohepatitis. *Nutr Clin Metab*. 2020 Oct; **34**(3): 216-22.
56. VILAR-GOMEZ E, MARTINEZ-PEREZ Y, CALZADILLA-BERTOT L, TORRES-GONZALEZ A, GRA-ORAMAS B, GONZALEZ-FABIAN L, *et al.* Weight loss through lifestyle modification significantly reduces features of nonalcoholic steatohepatitis. *Gastroenterology*. 2015; **149**(2): 367-378.e5.
57. HARRISON SA, BASHIR MR, GUY CD, ZHOU R, MOYLAN CA, FRIAS JP, *et al.* Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet*. 2019; **394**(10213): 2012-24.
58. NEWSOME PN, BUCHHOLTZ K, CUSI K, LINDER M, OKANOUE T, RATZIU V, *et al.* A Placebo-Controlled Trial of Subcutaneous Semaglutide in Nonalcoholic Steatohepatitis. *N Engl J Med.* 2021 Mar; **384**(12): 1113-24.
59. FRANCQUE SM, BEDOSSA P, RATZIU V, ANSTEE QM, BUGIANESI E, SANYAL AJ, *et al.* A Randomized, Controlled Trial of the Pan-PPAR Agonist Lanifibranor in NASH. *N Engl J Med.* 2021 Oct; **385**(17): 1547-58.
60. SIDDIQUI MS, IDOWU MO, PARMAR D, BORG BB, DENHAM D, LOO NM, *et al.* A Phase 2 Double Blinded, Randomized Controlled Trial of Saroglitazar in Patients With Nonalcoholic Steatohepatitis. *Clin Gastroenterol Hepatol*. 2020 Nov 2; **S1542-3565**(20)31509-3.
61. GAWRIEH S, NOUREDDIN M, LOO N, MOHSENI R, AWASTY V, CUSI K, *et al.* Saroglitazar, a PPAR-α/γ Agonist, for Treatment of NAFLD : A Randomized Controlled Double-Blind Phase 2 Trial. *Hepatology*. 2021 Oct; **74**(4): 1809-1824.
62. MUSSO G, CASSADER M, PASCHETTA E, GAMBINO R. Thiazolidinediones and Advanced Liver Fibrosis in Nonalcoholic Steato-hepatitis. *JAMA Intern Med.* 2017 May; **177**(5): 633.
63. KINTSCHER U. Pharmacological Differences of Glitazones. *J Am Coll Cardiol.* 2008 Sep; **52**(10): 882-4.
64. MARCHESINI G, DAY CP, DUFOUR JF, CANBAY A, NOBILI V, RATZIU V, *et al.* EASL-EASD-EASO Clinical Practice Guidelines for the management of non-alcoholic fatty liver disease. *J Hepatol.* 2016 Jun; **64**(6): 1388-402.
65. DAVIES MJ, D'ALESSIO DA, FRADKIN J, KERNAN WN, MATHIEU C, MINGRONE G, *et al.* Management of hyperglycemia in type 2 diabetes, 2018. A consensus report by the American Diabetes Association (ADA) and the european association for the study of diabetes (EASD). *Diabetes Care.* 2018 Dec; **41**(12): 2669-701.
66. ARMSTRONG MJ, GAUNT P, AITHAL GP, BARTON D, HULL D, PARKER R, *et al.* Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): A multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet*. 2016 Feb; **387**(10019): 679-90.
67. VAN GAAL LF, MERTENS J, FRANCQUE S, DE BLOCK C. Therapeutic approaches for non-alcoholic steatohepatitis. *Ther Adv Endocrinol Metab.* 2021 Sep; **12**: 20420188211034300.
68. VERMA S, MCMURRAY JJV. SGLT2 inhibitors and mechanisms of cardiovascular benefit: a state-of-the-art review. *Diabetologia*. 2018 Oct; **61**(10): 2108-17.
69. SHIMIZU M, SUZUKI K, KATO K, JOJIMA T, IIJIMA T, MUROHISA T, *et al.* Evaluation of the effects of dapagliflozin, a sodium-glucose co-transporter-2 inhibitor, on hepatic steatosis and fibrosis using transient elastography in patients with type 2 diabetes and non-alcoholic fatty liver disease. *Diabetes, Obes Metab.* 2019 Feb; **21**(2): 285-92.
70. KUCHAY MS, KRISHAN S, MISHRA SK, FAROOQUI KJ, SINGH MK, WASIR JS, *et al.* Effect of empagliflozin on liver fat in patients with type 2 diabetes and nonalcoholic fatty liver disease: A randomized controlled trial (E-LIFT Trial). *Diabetes Care*. 2018 Aug; **41**(8): 1801-8.
71. SANYAL AJ, CHALASANI N, KOWDLEY K V., MCCULLOUGH A, DIEHL AM, BASS NM, *et al.* Pioglitazone, Vitamin E, or Placebo for Nonalcoholic Steatohepatitis. *N Engl J Med.* 2010 May; **362**(18): 1675-85.
72. KEY TJ, APPLEBY PN, TRAVIS RC, ALBANES D, ALBERG AJ, BARRICARTE A, *et al.* Carotenoids, retinol, tocopherols, and prostate cancer risk: Pooled analysis of 15 studies. *Am J Clin Nutr.* 2015 Nov; **102**(5): 1142-57.
73. DULAI PS, SINGH S, PATEL J, SONI M, PROKOP LJ, YOUNOSSI Z, *et al.* Increased risk of mortality by fibrosis stage in nonalcoholic fatty liver disease: Systematic review and meta-analysis. *Hepatology*. 2017 May; **65**(5): 1557-65.
74. SHINOZAKI S, TAHARA T, KAWARAI LEFOR A, OGURA M. Pemafibrate improves hepatic inflammation, function and fibrosis in patients with non-alcoholic fatty liver disease: a one-year observational study. *Clin Exp Hepatol*. 2021; **7**(2): 172-7.
75. YOKOTE K, YAMASHITA S, ARAI H, ARAKI E, MATSUSHITA M, NOJIMA T, *et al.* Effects of pemafibrate on glucose metabolism markers and liver function tests in patients with hypertriglyceridemia: a pooled analysis of six phase 2 and phase 3 randomized double-blind placebo-controlled clinical trials. *Cardiovasc Diabetol.* 2021 Dec; **20**(1): 96.
76. LANTHIER N, FRANCQUE S. Nash: A welfare disease with emerging questions and adequate answer attempts. *Acta Gastroenterol Belg.* 2020; **83**(2): 339.