White paper

Advances in MASLD and MASH: nomenclature, characteristics, and biomarkers

Metabolic dysfunction-associated steatotic liver disease (MASLD), previously known as non-alcoholic fatty liver disease (NAFLD), is a condition where excessive fat accumulates in liver cells. It has been observed that approximately 30% of MASLD patients (47 cases per 1000) develop metabolic dysfunction-associated steatohepatitis (MASH) previously known as non-alcoholic steatohepatitis (NASH). The development of the disease may lead to liver cirrhosis and associated complications, including major liver inflammation, potential scarring, and possible development of hepatocellular carcinoma. This highlights MASLD as leading cause of liver disease worldwide and its prevalence is predicted to increase by 2030 if current trends remain as today.

Nomenclature update

After years of discussions, a multisociety consensus statement has been made for the fatty liver disease nomenclature. A combined effort among the American Association for the Study of Liver Diseases (AASLD), the European Association for the Study of the Liver (EASL) and the Asociación Latinoamericana para el Estudio del Hígado (ALEH) has been made to agree on the change in nomenclature and the diagnostic criteria for the condition [1].

This work has put together professionals from around the world, including hepatologists, gastroenterologists, paediatricians, endocrinologists, hepatopathologists, public health and obesity experts along with members from industry, regulatory agencies, and patient advocacy organisations [1,2].

At the EASL summit in September 2023, the leaders of the multinational liver societies announced that steatotic liver disease (SLD) was chosen as the term to comprise the various aetiologies of steatosis. The term steatohepatitis was retained as considered to be an important pathophysiological concept. Non-alcoholic fatty liver disease (NAFLD) will now be known as metabolic dysfunction-associated steatotic liver disease (MASLD) and the condition will enclose patients who have hepatic steatosis and have at least one of five cardiometabolic risk factors [1,2].

A new category for metabolic dysfunction and alcohol associated steatotic liver disease, MetALD, was defined to describe those patients with MASLD who consume greater amounts of alcohol per week (140–350 g/week and 210–420 g/week for females and males respectively). Cryptogenic SLD includes individuals with no metabolic parameters and no known disease cause, while metabolic dysfunction-associated steatohepatitis (MASH) is the replacement term for non-alcoholic steatohepatitis (NASH) [1,2].

This new nomenclature and the consensus among the diagnostic criteria have been supported by the clinical and scientific community, aiming for non-stigmatizing patients while improving awareness and patient identification.

MASLD and MASH: an overview

“Metabolic-dysfunction-associated steatotic liver disease (MASLD) is a chronic liver disease that affects more than a quarter of the global population and whose prevalence is increasing worldwide due to the pandemic of obesity” [3]. Between 1990 and 2006, an increase was observed in the prevalence of MASLD from a starting point of 25.26% to 38%, while the ultrasound-defined MASLD prevalence increased from 25.16% to 34.59% [3]. And, as mentioned by Younossi Z. *et al*. in their 2023 article, MASLD global prevalence is 30% and increasing, highlighting that the highest MASLD prevalence was in Latin America (44.37%), followed by the Middle East and North Africa (36.53%), South Asia (33.83%), South-East Asia (33.07%), North America (31.20%), East Asia (29.71%), and Asia Pacific (28.02%) [3] (Figure 1). The data collected by researchers worldwide, addresses the urgency on developing and applying new strategies to raise individual’s awareness and address all aspects of MASLD on the different societal levels (local, regional, and global) [3].

Several risk factors have been found to be associated with the development of MASLD. These include obesity, impaired glucose metabolism, high blood pressure, and atherogenic dyslipidemia. Insulin resistance is also considered to be a significant factor that may lead to the progression of the disease. In addition, atherosclerosis is known to be the leading cause of death in

patients with MASLD[4]. Therefore, it is essential to manage these risk factors to prevent the development and progression of this disease.

Factors that can contribute to increased risk include genetics, age, race, diet, physical activity, sleep, gut microbiota, oxidative stress, type 2 diabetes, metabolic syndrome, and sarcopenia. [5]. In addition, genetic variants such as single-nucleotide polymorphisms of the patatin-like phospholipase domain-containing 3 (PNPLA-3) and the sorting and assembly machinery component 50 (SAMM-50) have been described to play a role in the incidence of MASLD[5]. Sex and age have been shown to be relevant for the study of the disease, resulting in higher frequency of the disease in men than in women. While MASLD tends to increase in middle-aged individuals and then decrease after the age of 50 in men; in women it is lower before the age of 50 and increases after post-menopause, reaching a peak at 60 years of age[5].

Considering the race and ethnicity, several studies have noticed that there is a major prevalence of the disease in Hispanics, followed by Caucasians and African Americans[5]. This has been speculated to be related to genetic predisposition.

Following the recent consensus, patients must present one of five cardiometabolic risk factors, which include (1) increase in body mass index (BMI) or waist circumference (WC); (2) impaired glucose metabolism; (3) high blood pressure; (4) high triglyceride (TG) levels; and (5) low high-density cholesterol (HDL-C) levels. Furthermore, MASLD patients might present increased cardiometabolic risk factors and may be predisposed to develop liver fibrosis as well as atherosclerotic cardiovascular disease (ASCVD)[4].

The study of the different factors and the disease progression, suggest a dynamic disease, where MASLD's progression from steatosis to MASH and fibrosis is not linear[6]. It has been mentioned that fibrosis progression into steatosis is estimated to be 14 years per stage of fibrosis. Further, fibrosis progression into MASH is estimated at 7 years per stage of fibrosis[6]. Despite the previously mentioned risk factors, MASLD is characterized by hepatic triglyceride (TG) accumulation and insulin resistance that covers a spectrum of conditions that spans from benign hepatic steatosis to MASH. Metabolic-dysfunction-associated steatotic liver (MASL) is marked by isolated steatosis while MASH is characterized by steatosis, lobular inflammation caused by inflammatory cell infiltration, and hepatocellular ballooning in the presence or absence of fibrosis [7,8]. Although simple steatosis can be considered a benign disease, the existent association with liver fibrosis can lead to the development of cirrhosis and hepatocellular carcinoma (HCC) with or without the presence of cirrhosis[7] (Figure 2).

> The prevalence of MASLD continues to rise, becoming one of the most frequent causes of cirrhosis and liver transplantation...
>
> **European Association for the Study of the Liver (EASL)**

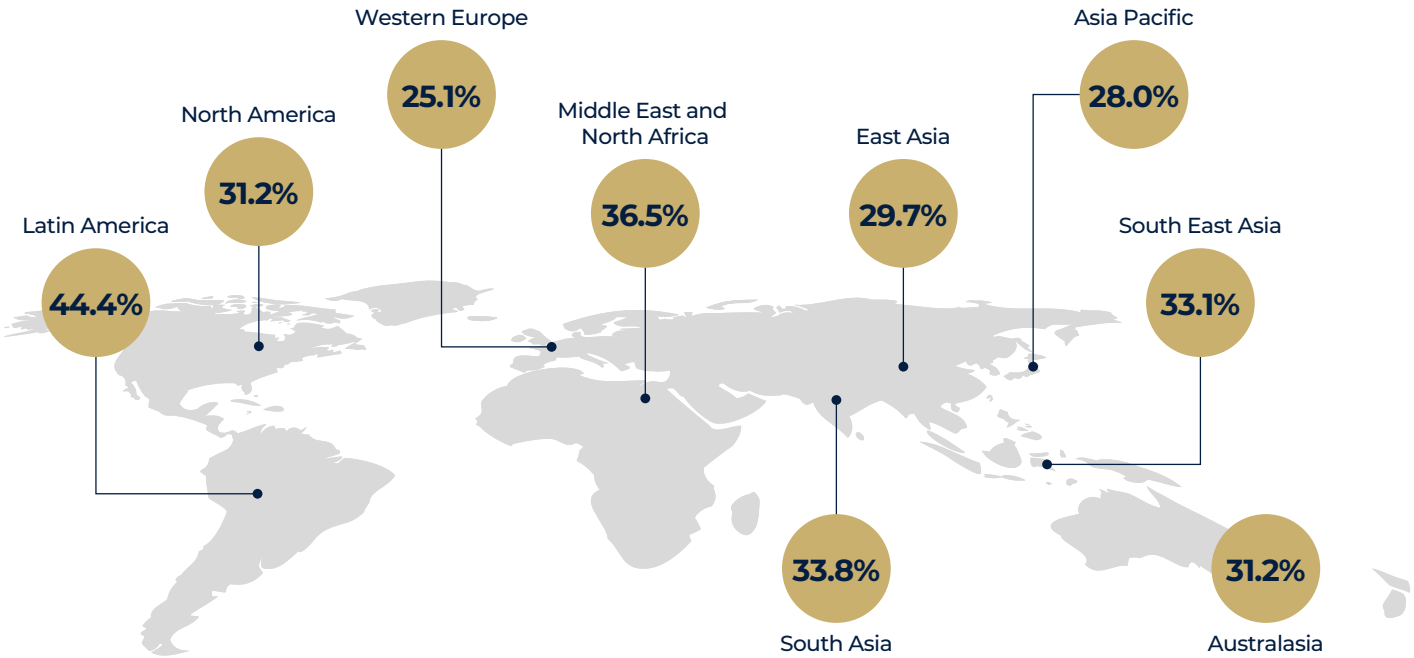

Fig 1. Global Prevalence of MASLD (Modified from[3]).

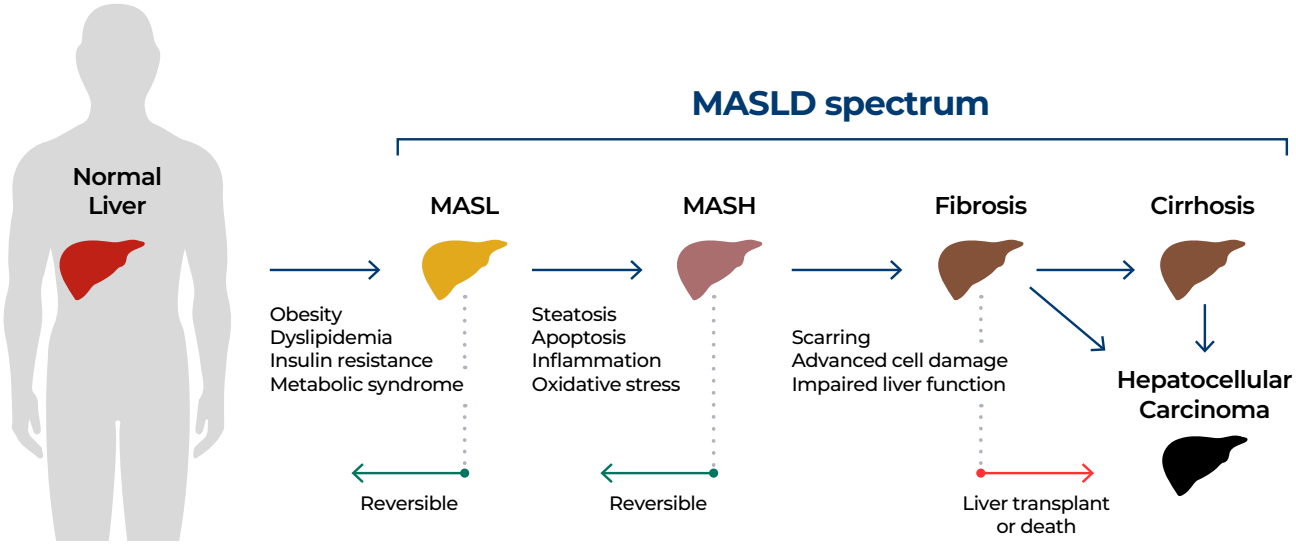

Fig 2. Progression of MASLD/MASH to Hepatocellular Carcinoma (HCC) (Modified from[7,8]).

The need of biomarkers for MASLD and MASH

With MASLD and MASH increasing globally, there is a pressing need to define biomarkers for early prognosis, detection, and election of patients for treatment and monitoring. Nevertheless, currently there are no approved diagnostic biomarkers apart from high histological activity and fibrosis stage, which is associated with higher incidence of liver-related events and mortality[9].

Despite the knowledge generated by the scientific and clinical community, and a better understanding of the disease aetiology and consequences, seeking for novel diagnostic biomarkers for MASLD and MASH remains on the loop. For this purpose, techniques have been implemented, from mass spectrometry-based proteomics to the gold standard liver biopsy[10]. Other imaging techniques remain relevant nonetheless (e.g. ultrasound, magnetic resonance imaging – MRI, magnetic resonance enterography - MRE, transient elastography - TE).

However, it remains crucial for the diagnosis and monitoring to define specific biomarkers that may be present in each of the stages of hepatic pathology, ranging from reversible steatosis and inflammation to irreversible fibrosis and eventually cirrhosis [10].

These biomarkers may be present in plasma and be secreted by the liver or because of steatosis. Some of these biomarkers are being currently studied as is the case of hemoglobin levels (associated with a higher incidence of MASLD), the fibroblast growth factor 21 - FGF21 (a protein secreted in response to peroxisome proliferator-activated receptor alpha - PPAR-α activation), cytokeratin-18 - CK18 (for the evaluation of liver inflammation), or extracellular vesicles, exosomes, and circulating microRNAs (miRNAs)[10]. Other possible biomarkers can be related to gut microbially-derived metabolomics and lipidomics, together with genetic and epigenetic markers as mentioned by Carolina Castillo Castro *et al.*[10]. Despite all the acquired knowledge, more studies are needed.

Cardiometabolic biomarkers for MASLD and MASH

Mercodia keeps following the research on novel biomarkers for MASLD and MASH, keeping in mind the currently used ones for MASLD diagnosis, possible not yet validated biomarkers and novel blood-based biomarker combinations that could be promising candidates in the field. However, we also acknowledge that existing biomarkers like insulin, oxidized low-density lipoprotein (oxLDL), glucagon, and incretins are playing important roles in the disease and could be informative biomarkers in basic, preclinical and clinical research studies.

There are many challenges with diagnosis and staging of MASLD and there is an urgent need for approved biomarkers in addition to the standard procedures that currently exists. Keeping in mind the correlation between obesity, metabolic syndrome, type 2 diabetes, MASLD development and the risk of cardiovascular disease, it is possible to identify some cardiometabolic biomarkers which are relevant for the study of MASLD and MASH (Table 1).

Insulin levels can be relevant considering that obesity and insulin resistance lead to impaired lipid metabolism and chronic inflammation that can promote MASLD progression to MASH, cirrhosis, HCC, and death [7]. Glucagon may have hypolipidemic effects on hepatocytes, promote mobilization of hepatic fat and sometimes shown to be inversely related to MASLD progression[11]. Oxidized low-density lipoprotein (oxLDL) has been shown to play a role in the development od atherosclerosis, an associated disease to MASLD [12,13]. While glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP) have shown promising results in MASLD patients after treatment with dual agonists[14].

Table 1. Cardiometabolic biomarkers and their role in the study of MASLD and MASH.

Biomarker	Connection to MASLD and MASH
Insulin	Driver of disease progression.
	Insulin resistance (IR) associated with MASLD independent from diabetes and obesity.
	Measurements of insulin is an often-used parameter in MASLD/MASH to monitor disease and effects of treatment.
Glucagon	MASLD/MASH is associated with hepatic glucagon resistance.
	Key hormone in the regulation of energy homeostasis in the fasting state stimulating hepatic glucose production.
	Promotes lipid oxidation and lowers lipid synthesis.
	Increases energy expenditure.
Oxidized LDL	Marker for oxidative stress, an important pathological event in the progression of MASLD and MASH.
	Has been found trapped inside lysosomes of Kupffer cells in the liver.
	Could be a mechanistic link between MASH and atherosclerosis.
GLP-1	Reduced secretion of GLP-1 has been found in non-diabetic MASLD and MASH patients.
	A 10% body weight reduction is associated with improved hepatic steatosis, making GLP-1 an analog for weight loss a potential drug candidate for MASLD and MASH.
GIP	Increased levels of GIP associated with MASLD/MASH
	GIP plays a role in the development of MASLD in response to sugar intake.

Mercodia assays for MASLD and MASH

Mercodia offers a range of easy-to-use ELISA assays for the study of relevant biomarkers for MASLD and MASH (both for human and animal samples) with a high lot-to-lot consistency and accuracy.

Catalog no	Product name	Sample volume	Range
Insulin			
10-1113-01/10	Insulin ELISA	25 µL	3–200 mU/L
10-1132-01	Ultrasensitive Insulin ELISA	25 µL	0.15–20 mU/L
10-1247-01/10	Mouse Insulin ELISA	10 µL	0.2–6.5 µg/L
10-1249-01	Mouse Ultrasensitive Insulin ELISA	25 µL	0.025–1.5 µg/L
10-1200-01	Porcine Insulin ELISA	25 µL	2.3–173 mU/L
10-1250-01/10	Rat Insulin ELISA	10 µL	0.15–5.5 µg/L
10-1251-01	Rat Ultrasensitive Insulin ELISA	25 µL	0.02–1 µg/L
10-1291-01	Lispro NL-ELISA	10 µL	1–500 mU/L
Glucagon			
10-1271-01	Glucagon ELISA	25 µL	1.5–130 pmol/L
10-1281-01	Glucagon ELISA -10 µL	10 µL	2–180 pmol/L
Oxidized LDL			
10-1143-01	Oxidized LDL ELISA	10 µL	6.56–164 U/L
Incretins			
10-1278-01	Total GLP-1 NL ELISA	25 µL	0.9–940 pmol/L
10-1258-01	Total GIP NL-ELISA	25 µL	2.7–1000 pmol/L

The assays have been validated not only by Mercodia but also by independent researchers using different methods. Among them mass spectrometry for some of the most polemic biomarkers, proving their efficiency and specificity, and their usefulness in basic and clinical research.

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