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Authors

Hsu, Cynthia L

Loomba, Rohit

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From NAFLD to MASLD: implications of the new nomenclature for preclinical and clinical research

Cynthia L. Hsu[1,2], **Rohit Loomba**[1,3,4]

[1.]Division of Gastroenterology and Hepatology, Department of Medicine, University of California at San Diego, La Jolla, CA, USA

[2.]Department of Medicine, VA San Diego Healthcare System, San Diego, CA, USA

[3.]MASLD Research Center, Division of Gastroenterology and Hepatology, Department of Medicine, University of California at San Diego, La Jolla, CA, USA

[4.]The Herbert Wertheim School of Public Health and Human Longevity Science, University of California at San Diego, La Jolla, CA, USA

Abstract

Non-alcoholic liver disease (NAFLD) is now metabolic dysfunction-associated steatotic liver disease (MASLD), emphasizing the key metabolic factors of obesity, insulin resistance, vascular dysfunction, and dyslipidemia. Here, we discuss impacts on the existing body of clinical and preclinical liver disease research and research moving forward.

The Evolution of a Name

The term “non-alcoholic steatohepatitis” was first used to describe the phenomenon of significant liver injury characterized by lobular inflammation, steatosis, and hepatocyte ballooning observed in patients with obesity and often associated with other metabolic abnormalities by Jurgen Ludwig and colleagues in 1980. In the ensuing decade, there was increasing awareness that a large proportion of patients previously described as having “cryptogenic cirrhosis” – patients who did not have viral hepatitis, autoimmune hepatitis, hemochromatosis, Wilson’s disease, alpha-1-antitrypsin deficiency, or significant history of alcohol consumption – shared characteristics such as increased body mass index (BMI), diabetes, hypertriglyceridemia, and/or family history of these characteristics. This led to the establishment of non-alcoholic fatty liver disease (NAFLD) as a diagnosis defined by the

[†]**Corresponding Author:** Rohit Loomba, MD, MHSc, Professor of Medicine, Director, MASLD Research Center, University of California at San Diego, 9500 Gilman Drive La Jolla, CA, 92093-0887, roloomba@ucsd.edu, Web: http://fattyliver.ucsd.edu.

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presence of hepatic steatosis in over 5% of hepatocytes in the absence of significant alcohol consumption or competing etiologies for liver injury or steatosis. NAFLD can progress to non-alcoholic steatohepatitis (NASH), which is defined by steatosis, inflammatory infiltrates, and ballooning degeneration with or without Mallory bodies or pericellular/perivenular fibrosis on liver biopsy. In 2020, the term metabolic dysfunction-associated fatty liver disease (MAFLD) was proposed, defined as hepatic steatosis in the presence of an elevated BMI, type 2 diabetes mellitus, or more than one metabolic risk factors, without excluding those with significant alcohol use. However, some were concerned that this new terminology would muddle the important distinctions in pathophysiology and natural history between alcohol and metabolic disease etiologies for liver injury. In light of these concerns, a multi-society panel recommended to replace the term NAFLD with metabolic dysfunction-associated steatotic liver disease (MASLD)[1], defined by the presence of hepatic steatosis (identified via imaging or biopsy) in the context of co-existing cardiometabolic risk factors like elevated BMI, insulin resistance, hypertension, or dyslipidemia, without significant alcohol consumption history (Fig 1A). Patients who meet MASLD criteria but also present additional etiological factors for liver disease, such as heightened alcohol consumption (140 to 350 g/week and 210 to 420 g/week for females and males, respectively), are categorized as having MetALD or other combination diagnoses (Fig 1B). Clinical practice guidelines have been updated to reflect this change in nomenclature[2]. Figure 1C provides the key for the previous terminology and how it can be translated to the new terminology related to the entire spectrum of NAFLD. Here we will discuss the impact of this nomenclature change on preclinical and clinical research.

MASLD Nomenclature Impact on Clinical Research

Since the publication of the newly proposed nomenclature for steatotic liver disease, several studies have endeavoured to assess potential impacts on existing steatotic liver disease literature. Two studies – one from Europe consisting of predominantly Caucasians[3] and one from Asia consisting of predominantly Asians[4] – have assessed the impact of incorporating MASLD criteria into pre-existing biopsy-proven NAFLD study cohorts. Almost all (99.9%) of the 1,783 biopsy-proven NAFLD patients fulfilled at least one metabolic risk factor criteria, most commonly increased BMI (97.5%), followed by insulin resistance (84.7%), hypertension (82.6%), dyslipidemia (80.9%), and hypertriglyceridemia (74.5%). Over half (55.4%) fulfilled all five metabolic risk factor criteria. After incorporating a third study which included Swedish patients with NAFLD diagnosed by biopsy or non-invasive imaging test[5], the trends remained similar, with 99.6% of the 3,377 patients fulfilling at least one metabolic risk factor criteria. These patterns were similar across the cohorts despite important differences in patient ethnicity and these observations suggest that existing NAFLD research findings remain highly relevant for the diagnosis of MASLD.

Patients with “lean NAFLD/NASH”, diagnosed in individuals with NAFLD and BMI <25 kg/m2 in non-Asians or <23 kg/m2 in Asians, without metabolic risk factors would now fall into the category of cryptogenic liver disease. This subpopulation deserves more focused study to better understand disease pathogenesis. In these patients, risk factors such as human immunodeficiency virus (HIV), malnutrition, and medications such as methotrexate, amiodarone, and tamoxifen should be considered, though these factors play a role only

in a minority of cases. Other likely drivers of disease in this population, such as genetic predisposition (e.g. polymorphisms in *PNPLA3*) and gut dysbiosis, are not yet routinely tested in clinical practice. Additionally, a careful assessment of alcohol intake should be performed and incorporation of biomarker testing to quantify alcohol consumption can be considered. Patients with MetALD will also be an important population for continued study due to the increasing prevalence of both metabolic risk factors and alcohol use on a population level and the significant overlap in pathogenesis between the two etiologies[7].

Preclinical Models of MASLD

Numerous animal models exist for the study of NAFLD and have helped us broaden our understanding of disease pathogenesis, identify potentially druggable targets, and test potential therapies in preclinical trials. Existing mouse models for NAFLD broadly fall into two categories – diet-based models and genetic models, discussed in detail in a recent review[8]. Genetic models such as *ob/ob* and *db/db* mice, which are deficient in leptin signalling, exhibit multiple metabolic risk factors such as obesity, insulin resistance, and cardiovascular disease, but due to the protective effects of leptin deficiency against liver fibrosis, do not mimic liver disease progression in MASLD. On the other hand, *foz/foz* mice, which carry a mutation in *Alms1*, demonstrate obesity, insulin resistance, dyslipidemia, and hypertension, and spontaneously develop steatohepatitis and appreciable fibrosis after 24 weeks on a high-fat diet, making it an attractive model for evaluating drugs targeting MASLD.

Obesogenic diet-based models use high concentrations of fat, cholesterol, carbohydrates such as fructose, or some combination of the three to induce obesity, insulin resistance, cardiovascular disease, and hepatic steatosis and fibrosis. These models accurately represent MAFLD/MASH physiology, as the mice exhibit metabolic disease in addition to hepatic steatosis and fibrosis (Supplementary Table 1). In contrast, nutrient-deficient diet-based models such as methionine-choline deficient (MCD) and choline-deficient, l-amino acid-defined (CDAA) diets achieve moderate hepatic fibrosis with shorter feeding periods (as early as 6-9 weeks), but do not consistently recapitulate the obesity, diabetes, or other metabolic risk factors necessary to accurately reflect the natural history of MASLD (Supplementary Table 1). In humans, liver fibrosis stage is the major driver for adverse cardiovascular events and mortality in MASH, so these short models of hepatic fibrosis are inherently attractive, but important differences in metabolic risk factors make extrapolation of these results to the new MASLD diagnosis difficult.

Obesogenic diet models can be enhanced by environmental modifications such as thermoneutral housing, which eliminates chronic cold stress, or “humanization” of the mouse model to address the many inherent physiologic differences between humans and mice. One example is the humanized mouse microbiome model, which aims to address differences between the innate human and murine gut microbial composition that may be pertinent to the study of MASLD/MASH. The gut microbiome can promote liver disease through modulation of intestinal permeability and translocation of bacterial components, leading to both stimulation of proinflammatory signalling cascades and direct hepatotoxicity[9]. In a study of germ-free mice humanized with microbiota from patients with

MASH, the fungal microbiota exacerbated steatohepatitis caused by Western diet, whereas antifungal treatment improved steatohepatitis[10]. When comparing wild-type mice to germ-free mice that received fecal microbiota transplantation from patient donors with MASH, there was significantly more bacterial translocation, liver injury, and hepatic steatosis in the MASH-humanized mice[11], demonstrating the important contribution of the gut microbiota to liver disease pathogenesis. Another example of humanizing mouse models is with chimeric mice. The development of the triple-knockout Fah−/−/Rag2−/−/Il2rg−/− (FRG) mouse strain, a cross between Fah−/− mice which develop liver disease in the absence of the protective drug 2-(2-nitro-4-trifluoromethylbenzoyl)-1,3-cyclohexanedione (NTBC) and Rag2−/−/Il2rg−/− mice which lack T cells, B cells and natural killer cells, allowed for engraftment of up to 90% human hepatocytes after pretreatment with a urokinase-expressing adenovirus[12]. Subsequent advancements on this mouse model now allow simultaneous engraftment of human hepatocytes, immune cells, and non-parenchymal liver cells in the same murine host[13,14]. Taking advantage of these humanized models to study MAFLD/MASH will provide a more accurate representation of human physiology and provide an important tool for future preclinical trials.

MASLD Nomenclature Impact on Preclinical Research

Preclinical researchers must take care when applying existing mouse models of NAFLD to study MASLD physiology. Because the MASLD diagnosis is defined by hepatic steatosis and concurrent cardiometabolic risk factors, only rodent models such as the obesogenic diet-based models of steatotic liver disease and the *foz/foz* mouse model which recapitulate factors such as elevated BMI, insulin resistance, hypertension, and dyslipidemia in addition to liver disease are representative of MASLD pathophysiology. Rodent models using nutrient-deficient diets to achieve steatohepatitis and fibrosis do not consistently elicit metabolic dysfunction and as such may not be described as MASLD/MASH models. Instead, they should be described using the terms “nutrient-deficient diet-induced steatotic liver disease” or “nutrient-deficient diet-induced steatohepatitis”. The prior term NAFLD was based on exclusionary criteria which lack relevance to rodent models – in tightly controlled lab environments, rodents would not consume alcohol or develop secondary etiologies of liver injury unless deliberately introduced by the researcher. Therefore, preclinical researchers who intend to study the pathophysiology of steatotic liver disease associated with metabolic dysfunction are advised to exclusively use the terms MASLD and MASH.

Summary

The new MASLD nomenclature emphasizes the important influence of cardiometabolic risk factors on the pathogenesis and progression of steatotic liver disease, which will guide both clinical practice and preclinical trials. While rodent models innately cannot recapitulate all aspects of human MASLD pathophysiology, the development of novel techniques together with obesogenic diets now allow preclinical researchers to study the role of the human gut microbiome, immune system, hepatocytes, and non-parenchymal liver cells in MASLD in rodent models. These models provide a platform for testing new therapeutic approaches or

even evaluating the potential use of existing drugs targeting cardiometabolic risk factors, now redirected towards the treatment of MASLD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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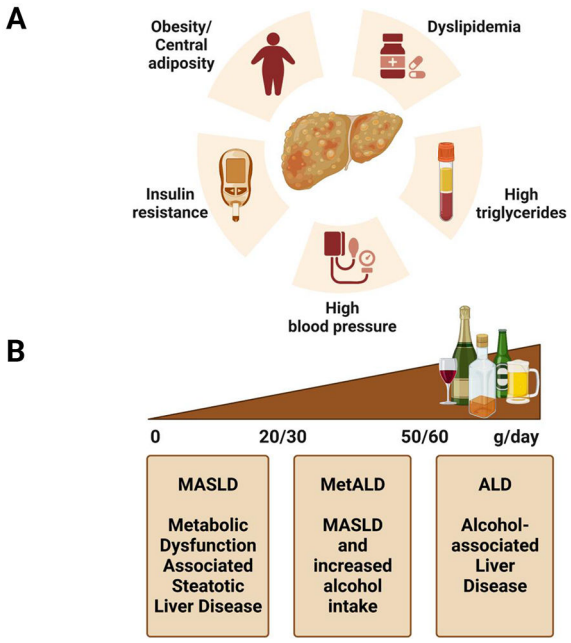

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Prior terminology	New terminology	Clinical relevance
NAFLD	MASLD (metabolic dysfunction-associated steatotic liver disease)	Fatty liver in individuals who consume little or no alcohol plus at least one metabolic risk factor
NAFL	MASL (metabolic dysfunction-associated steatotic liver)	Non-progressive sub-type of MASLD
NASH	MASH (metabolic dysfunction-associated steatohepatitis)	Progressive sub-type of MASLD
At-risk NASH	At-risk MASH	Patients with MASH with ≥ stage 2 fibrosis

Figure 1. Summary of new nomenclature for steatotic liver disease

(A) Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by co-existing cardiometabolic risk factors such as elevated BMI, insulin resistance, hypertension, or dyslipidemia.

(B) MetALD is defined by the concurrence of MASLD and alcohol consumption between 20 to 50 g/day for females or 30 to 60 g/day for males, while alcohol consumption over these limits is diagnostic of alcohol-associated liver disease (ALD).

(C) Table for the translation of previous terminology to new terminology and the clinical relevance of each term for the entire spectrum of NAFLD.