



Review Article

Biomarkers of Metabolic (Dysfunction)-associated Fatty Liver Disease: An Update

Jawaher Alharthi^{1,2} and Mohammed Eslam^{1*}

¹Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Sydney, NSW, Australia; ²Department of Biotechnology, Faculty of Science, Taif University, Taif, Saudi Arabia

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Abstract

The prevalence of metabolic (dysfunction)-associated fatty liver disease (MAFLD) is rapidly increasing and affects up to two billion individuals globally, and this has also resulted in increased risks for cirrhosis, hepatocellular carcinoma, and liver transplants. In addition, it has also been linked to extrahepatic consequences, such as cardiovascular disease, diabetes, and various types of cancers. However, only a small proportion of patients with MAFLD develop these complications. Therefore, the identification of high-risk patients is paramount. Liver fibrosis is the major determinant in developing these complications. Although, liver biopsy is still considered the gold standard for the assessment of patients with MAFLD. Because of its invasive nature, among many other limitations, the search for noninvasive biomarkers for MAFLD remains an area of intensive research. In this review, we provide an update on the current and future biomarkers of MAFLD, including a discussion of the associated genetics, epigenetics, microbiota, and metabolomics. We also touch on the next wave of multiomic-based biomarkers.

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Introduction

Metabolic (dysfunction)-associated fatty liver disease (MAFLD) currently affects up to two billion people worldwide, and its prevalence has been found to be increasing.^{1,2} MAFLD is known to be associated with hepatic, cardiovascular and oncological sequelae, which means that it places an enormous

clinical and economic burden on both healthcare systems and society more generally.^{3–5}

It is now widely accepted that fatty liver disease is a heterogeneous trait shaped by the dynamic and complex interactions that occur between genetics, epigenetics and environmental factors, in addition to being impacted by both biological and chronological age, which determine the entire course of the disease.^{6–9} MAFLD is closely linked to metabolic dysfunction, and it has been recognized that a considerable proportion of MAFLD patients are non-obese.³ As a consequence of this heterogeneity at the population level, the spectrum of MAFLD varies significantly, ranging from steatosis to concomitant inflammation, which can lead to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). It is important to note, however, that only a proportion of patients with MAFLD progress to the more advanced stages of the disease.¹⁰

Therefore, efficiently identifying the subgroup of high-risk MAFLD patients represents one of the key concerns of both clinical care and drug trials. From a clinical perspective, high-risk patients could benefit from lifestyle interventions, exercise programs and follow-up in primary- or secondary-care settings, particularly given the current lack of an approved drug treatment for MAFLD. In addition, identifying patients with cirrhosis is crucial, so that they can be monitored for HCC and esophageal varices.⁴ From the perspective of those conducting drug trials, identifying high-risk patients with steatohepatitis and fibrosis is also vital in relation to formatting the inclusion criteria and assessing the endpoints.⁴

The conceptual framework behind the recent redefinition of non-alcoholic fatty liver disease (NAFLD) as MAFLD (which stresses the metabolic dysfunction aspect) aims to embrace this diagnostic and therapeutic paradigm, enhance the identification of high-risk patients and improve the referral pathways, and it also promises clinical care strategies that are tailored to the individual variability of patients.^{4,11–19} Notably, a number of studies have provided evidence that the criteria associated with MAFLD are more effective than the criteria associated with NAFLD when it comes to identifying patients with significant fibrosis as well as chronic kidney and cardiovascular diseases, including those with fatty liver disease and other concomitant liver diseases.^{20–25}

Instead of the dichotomous classification of MAFLD patients into nonalcoholic steatohepatitis (NASH) and non-NASH, the MAFLD proposal is suggesting considering hepatic inflammation as a continuous variable, similar to that with other liver diseases, which should have implications in improving patients' characterisations.¹¹ In this context, there is increasing recognition that fibrosis may develop on the ground of steatosis with little if any inflammatory changes. A recent study found that more than one-third of patients

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Abbreviations: AST, aspartate aminotransferase; AUROC, the area under the receiver operating characteristic; FIB-4, fibrosis-4 index; GWAS, genome-wide association study; HCC, hepatocellular carcinoma; MAFLD, metabolic-associated fatty liver disease; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; NFS, NAFLD fibrosis score; oxLDL, Oxidized low-density lipoprotein.

***Correspondence to:** Mohammed Eslam, Storr Liver Centre, Westmead Institute for Medical Research, Westmead Hospital and University of Sydney, Westmead 2145, NSW, Australia. ORCID: <https://orcid.org/0000-0002-4315-4144>. Tel: +61-2-8890-7705, Fax: +61-2-9635-7582, E-mail: mohammed.eslam@sydney.edu.au

with MAFLD who have significant fibrosis did not show typical histological features NASH.²⁶ On the other hand, another report demonstrated that up to 70% of patients without NASH (NAFLD activity score (NAS) <4) exhibit some inflammatory degree in the liver biopsy.²⁷ This complexity and the variance with the simple dogmatic steatosis-steatohepatitis-fibrosis chronology emphasizes the importance of the holistic view of liver injury with MAFLD, and we have witnessed some recent efforts at implementing this view in risk assessment.²⁸

However, some concerns regarding this change have been raised by some other experts; these have included the ambiguity of the term "metabolic", the need for full understanding of the pathogenesis of the disease before moving to this change, the potential consequences on drug development and ongoing clinical trials, and the risk of causing confusion for stakeholders with a subsequent impact on disease awareness among physicians and patients.^{29,30} It is noteworthy that three prior studies have demonstrated that the change from NAFLD to MAFLD has served to increase awareness of the disease among general practitioners, specialists and patients,³¹⁻³³ indicating that the opposite may be the case.

Although liver biopsy remains the gold standard for assessing the stages of liver disease in cases of MAFLD, including the histological assessment of fibrosis and steatohepatitis.⁴ As a procedure, the efficacy of liver biopsy is limited by the potential for sampling errors and suboptimal agreement among pathologists, in addition to being associated with procedural risks. Hence, non-invasive tools capable of reliably and accurately differentiating the major histologic determinants of MAFLD are required for the diagnosis of the disease, stratification according to risk, and determination of which patients would benefit from drug treatment.

The present review aims to highlight recent updates in terms of the blood-based biomarkers of MAFLD as well as other novel avenues for biomarkers. It is important to bear in mind, however, that this approach will continue to evolve as new factors that contribute to the variability of MAFLD are identified.

Blood biomarker scores and algorithms

Non-proprietary scores for MAFLD

The diagnosis of MAFLD requires the presence of hepatic steatosis accompanied by one of three other criteria, namely overweight/obesity, type 2 diabetes, or evidence of metabolic dysfunction. While the diagnosis of steatosis is commonly performed by means of ultrasound, blood scores, including the fatty liver index (FLI), may also prove useful, particularly in the case of large cohort studies. The FLI incorporates the patient's body mass index (BMI), waist circumference, and gamma-glutamyl transferase and triglyceride levels.

Among the histological features of MAFLD, the degree of liver fibrosis is typically considered the major determinant of liver-related morbidity and mortality.³⁴ Non-invasive fibrosis scores based on simple and inexpensive clinical and routine laboratory parameters, such as the NAFLD fibrosis score (NFS), the aspartate aminotransferase (AST)-to-platelet ratio index (APRI) and the fibrosis-4 index (FIB-4), are commonly used to identify or exclude significant or advanced fibrosis in patients with fatty liver disease. For this reason, such scores are particularly important in primary-care or resource-constrained settings. The overall accuracy of these scores has been determined to be modest, although they offer good negative predictive values, which can be used to exclude advanced fibrosis. The major limitation of the scores concerns the fact that their performance

varies across both the individual's lifespan and different ethnic populations.³⁵ In addition, the scores all incorporate liver enzymes into the relevant models, which can lead to false negative results.

Novel biomarkers and proprietary scores for MAFLD

A number of new biomarkers and scores have emerged in recent years. Although they are not yet widely available, such advances could prove particularly important in specialist settings, with regard to the management of patients. For example, the enhanced liver fibrosis panel, which includes the tissue inhibitor of matrix metalloproteinases-1 (TIMP-1), the aminoterminal propeptide of procollagen type III (P3NP) and hyaluronic acid (HA), has been found to exhibit good overall accuracy in multiple studies.³⁶

Fibroblasts represent the common denominator as well as the central producer of the extracellular matrix (ECM) in the interstitial matrix, which is known to be both increased in all fibrotic disorders and to drive the progression of fibrosis, thereby leading to the loss of organ function. Type III collagen, which can be quantified using PRO-C3, has been shown to be a novel biomarker capable of predicting the progression of fibrosis. The ADAPT algorithm, a PRO-C3-based algorithm that includes the patient's age, type 2 diabetes status and platelet count, has demonstrated robust diagnostic performance in relation to the prediction of advanced fibrosis (\geq F3) in cohorts from tertiary centers³⁷ and among the general population.³⁸ The area under the receiver operating characteristic (AUROC) for ADAPT was reported as 0.86 and 0.87 in discovery and validation cohorts, respectively, which was superior to other non-invasive scores, such as APRI, FIB-4 and NFS, and comparable to transient elastography.^{37,38}

Macrophages play a pivotal role in the progression of liver disease in cases of MAFLD.³⁹ Soluble CD163, a macrophage activation marker, is another biomarker that has been found to predict advanced fibrosis (\geq F3), and its performance can be enhanced by combining it with the NFS score (AUROC of 0.83).⁴⁰ Another study reported that the circulating activity of another macrophage marker, namely macrophage-derived deaminase, can predict advanced fibrosis (\geq F3), with an AUROC of 0.82.⁴¹

Other novel avenues for biomarkers

Genetic factors as biomarkers

MAFLD is a heritable polygenic disease that shares a genetic basis with other metabolic diseases.^{6,8} Multiple variants have been determined to be implicated in MAFLD susceptibility and progression, which are involved in the regulation of key pathways, such as those involved in lipid metabolism, insulin signaling, immune cell activation, adipocytokine and myokine activities, oxidative stress and inflammation responses.^{6,42-48} This has been the subject of other recent reviews.^{6,8,49}

Despite the initial optimism that genetic discoveries would demonstrate immediate utility as diagnostic biomarkers, none of the discovered genetic variants for complex diseases such as MAFLD, including major genetic determinants such as of *PNPLA3* and *TM6SF2* (even with their observed relatively high effect sizes), can now be used as biomarkers for diagnostic clinical implementation.⁶ Currently, the Asian Pacific Association for the Study of the Liver (APASL) guidelines do not yet recommend the use of this variant in routine clinical practice to assess the risk

of fibrosis and HCC in MAFLD.⁴ However, the development of polygenic risk scores with and without the incorporation of clinical risk variables provides hope for improving patient stratification and management. For example, a recent study reported improved prediction of steatosis in MAFLD based on a combination of genetic risk variants in *PNPLA3*, *TM6SF2*, and *FNDC5*.⁴³ Similarly, another study showed that a combination of genetic risk variants in *PNPLA3*, *TM6SF2* and *MBOAT7* enhanced the prediction accuracy of HCC in MAFLD.⁵⁰ Another study incorporated the *IFNL3* variant with other simple clinical variables in a decision tree model, and yielded a negative predictive value of >0.96 to exclude cirrhosis.⁴⁶ More recently, genome-wide polygenic scores have been developed and have demonstrated promising results for the prediction of increased risk of developing cardiovascular diseases and obesity.^{51,52} Similar approaches may also be useful for predicting the risk for MAFLD progression.

These genetic-based score approaches can inform therapeutic intervention, surveillance programs and life planning. However, there are also multiple challenges for the implementation of polygenic scores. First, the best approach for the interpretation of these scores has yet to be defined. For example, are risk stratification or risk prediction tools the most suitable approaches? Initially, in the post-genome-wide association study (GWAS) era, the utility of genetic risk variants has been evaluated on the basis of their ability to differentiate between diseased and healthy individuals, which is usually estimated by the AUROC.^{6,8} However, this was perhaps inappropriate, and genetic risk information may be better obtained via the prediction of the likelihood of a certain outcome, such as the onset of complications in a particular individual or subgroup of subjects. In this regard, changing disease probabilities for individuals at the extremes of the distribution does not impact AUROC measures markedly; rather, it affects results in risk estimates with varying utility implications.⁵³

Second, there is also a concern that these scores may exacerbate health inequalities, with a recent study revealing that the performance of polygenic scores for the prediction of 17 blood quantitative traits was substantially lower in all other ethnicities compared to Europeans. These results could be attributed to the fact that nearly 80% of every GWAS in the GWAS catalogue have been conducted in European populations.⁵⁴ Therefore, increasing the involvement of multiethnic populations in genetic studies is crucial to extend the utility of these scores to all populations. Third, the other concern is the uncertainty of the performance of these scores at individual levels. This uncertainty is mainly due to missing heritability, as the proportion of explained heritability can impact the diagnostic accuracy of the genetic classifier tested. These limitations suggest that further studies exploring the role of other rare or modest size effect variants, gene-gene and gene-environment interactions, types of genetic variation rather than single-nucleotide polymorphisms, and epigenetics are required to make up for this missing heritability.⁵⁵

Epigenetics as biomarkers

The human epigenome plays a crucial role in mediating gene-environment interactions, with a recent meta-analysis of twin studies indicating a nearly equal contribution of the environment and genes to the heritability of complex traits.⁵⁶ The plasticity of epigenetic markers and their tissue and context-based specificity suggest that they can serve as biomarkers for diagnosis, disease monitoring and prediction of treatment response.⁷

Multiple epigenetic markers have been suggested as potential candidate biomarkers, although they are yet to be

validated clinically. For instance, plasma DNA methylation of the promoter of the peroxisome proliferator-activated receptor gamma (PPAR γ) gene was reported to have an AUROC of 0.91 for the prediction of advanced liver fibrosis (F3-F4) in MAFLD.⁵⁷ Similarly, a meta-analysis suggested that some micro (mi)RNAs, predominantly miRNA-122, miRNA-34a and miRNA-192, could serve as circulating biomarkers to differentiate steatosis and steatohepatitis; however, the diagnostic accuracy appeared to be modest, with substantial heterogeneity in results among studies.⁵⁸ Hopefully, applying more sensitive tools to estimate miRNA (e.g., droplet digital PCR and standardized assays) can enhance the utility of these biomarkers, as has recently been suggested.⁵⁹ In addition, there is potential merit in utilizing non-coding RNAs as biomarkers for MAFLD, although this needs to be validated in larger cohorts.

Metabolomic factors as biomarkers

As MAFLD pathogenesis is intimately linked to metabolic dysfunction,^{11,13} there is growing interest in leveraging metabolomics as biomarkers for MAFLD, particularly since the metabolome demonstrates high plasticity in response to genetic and environmental factors.

Multiple studies have reported metabolic variables and metabolomic-based scores for differentiated steatosis and steatohepatitis and for predicting advanced fibrosis, portal hypertension and HCC.⁶⁰ For example, the 'oxNASH', an algorithm that incorporates the ratio 13-hydroxyoctadecadienoic acid (13-HODE) to linoleic acid besides age, BMI and AST, was demonstrated as having the ability to identify the presence of steatohepatitis with an AUROC of 0.83 in the discovery cohort and 0.74 in the independent validation cohort.⁶¹ In a subsequent study, the same score was found to have modest diagnostic accuracy for detection of steatosis, inflammation and fibrosis in biopsy-tested patients with MAFLD, with AUROC of 0.70, 0.73 and 0.67, respectively.⁶² Another example includes a predictive score that combined a panel of 32 serum metabolite panels, which were identified using an untargeted metabolic approach to predict advanced fibrosis (stages 3–4) in MAFLD patients and was validated in two other independent cohorts. This score showed an AUROC of 0.94, which was higher than that for FIB-4 and NFS (AUROC: 0.84; 95% confidence interval [CI]: 0.724–0.929).⁶³

Moreover, a panel of 28 triglyceride species was found to have utility in discriminating between individuals who were healthy and those with MAFLD, as well as differentiating steatohepatitis from steatosis.⁶⁴ The 'NASH ClinLipMet Score' is another interesting example that integrated five metabolites (i.e. glutamate, isoleucine, glycine, lysophosphatidylcholine [16:0] and phosphatidylethanolamine [40:6]), *PNPLA3* genotype and simple clinical variables (e.g., AST and fasting insulin) into a novel score and could differentiate steatohepatitis from steatosis with an AUROC of 0.866.⁶⁵ Oxidized low-density lipoprotein (oxLDL) can be defined as a particle derivative from circulating LDL that has been undergone oxidative changes. The accumulation of oxLDL in the coronary arteries is the hallmark of coronary disease pathogenesis. It has been recently demonstrated that oxLDL accumulation in the wall of the portal vein is involved in plaque formation, endothelium deformation, and portal venous inflammation and fibrosis in MAFLD.⁶⁶ These findings could represent a link between cardiovascular disease and MAFLD.

Gut microbiome factors as biomarkers

Given the association between gut dysbiosis and clinical

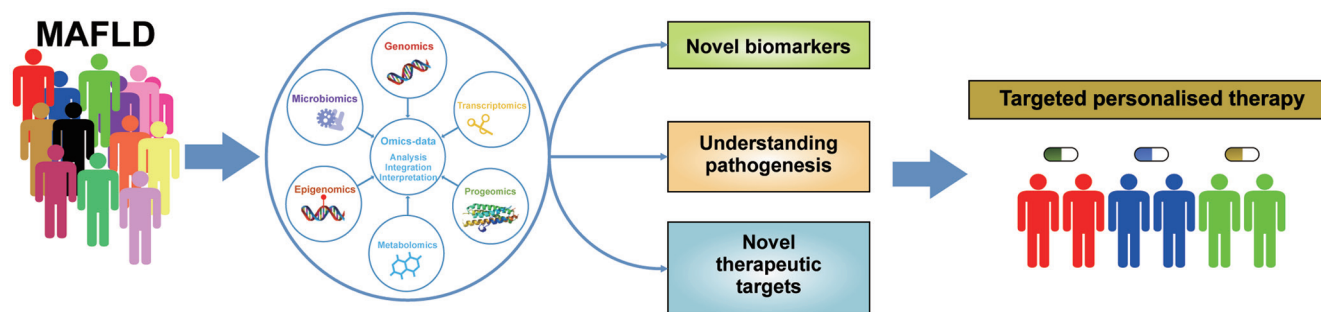


Fig. 1. Framework for the multiomics integration for the precision of management of MAFLD. MAFLD, metabolic-associated fatty liver disease.

phenotypes of MAFLD severity, a clinical consideration includes determining whether the fecal markers of disease based on perturbation in gut microbiota composition may serve as noninvasive diagnostic and/or prognostic biomarkers of disease phenotype and progression risk. Multiple studies have explored this possibility, e.g., fecal-microbiome-derived metagenomic signatures that incorporated a microbial diversity index with age and BMI displayed an AU-ROC of 0.936 for detection of advanced fibrosis.⁶⁷ Another study demonstrated that a prediction model integrating the metagenome profile with age and serum albumin levels had an AUROC of 0.91 for predicting cirrhosis.⁶⁸

A recent study revealed that lean and obese individuals belonged to distinct phenotypes that displayed differential gut microbiota.⁶⁹ Although the use of fecal samples as a biomarker source has some merit, it also presents several challenges. The gut microbiota is impacted by a multitude of factors, including age, sex, diet, medication, lifestyle factors, geographic localization and hormonal cycles, which renders generalizability across populations based on a single assay questionable. In addition, the variation in the utilized technical methodology for characterizing gut microbiome may have implications for the generalizability of findings. Furthermore, as most human data has been obtained in cross-sectional studies, it is unclear whether microbial markers could predict risk for disease progression and the development of complications. Finally, given the heterogeneity of MAFLD, it is likely that more than one microbiome profile for the disease could exist.

Multiomic integration as the future of biomarker identification

Given the complexity and heterogeneity of MAFLD, the predictive value of various potential biomarkers (i.e. genetic, epigenetic, metabolomic and gut microbiota factors) for the MAFLD disease phenotype is likely to become more robust through an integration strategies for individual's multilayered data (Fig. 1). These components are dynamic, multidimensional and interactive, and integrating and analyzing multiomic datasets presents several challenges, including bioinformatics and the need for extensive computational resources and storage capabilities. Although we are still in the infantile phase and it will take time before such data are processed meaningfully, this approach is promising in terms of its ability to provide personalized insights for each individual.

Conclusion

The increase in MAFLD prevalence and the consequent related hepatic and extrahepatic complications represent ma-

yor health, economic and societal burdens. As only a small proportion of patients with MAFLD will develop these complications, identifying high-risk patients is paramount. The presence of advanced fibrosis is the major risk factor for both hepatic and extrahepatic manifestations of MAFLD. Although liver biopsy remains the gold standard for characterizing histological severity in these patients, due to the numerous problems with it and its unsuitability for use in large populations, finding robust noninvasive biomarkers for MAFLD remains an area of intense research. Various proprietary and nonproprietary biomarkers and scores have been identified and are used widely. The advancement of our understanding of MAFLD pathogenesis and characterizing the role of genetics, epigenetics, microbiota and metabolomics in disease pathogenesis provides an avenue for identifying and developing novel biomarkers. The next wave of emerging research in this area will likely feature the integration of multilayered information that will enable more precise stratification of patients and personalized management approaches. Further evidence, including that from cost-effectiveness studies, will be required before these novel biomarkers or multiomics approaches can be recommended for incorporation in clinical practice.

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Conflict of interest

ME reports personal fees from Pfizer and honoraria payment from Sanofi outside the submitted work. ME has been an associate editor of *Journal of Clinical and Translational Hepatology* since 2021. JA has no competing interests or any conflict of interest relevant to this work.

Author contributions

Both authors equally contributed to this work.

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