Review Article

Hepatocellular carcinoma risk prediction and early detection in patients with metabolic dysfunction associated steatotic liver disease

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Abstract: The rising prevalence of metabolic dysfunction-associated steatotic liver disease (MASLD) and its more severe form, metabolic dysfunction-associated steatohepatitis (MASH), is closely linked with a heightened risk of hepatocellular carcinoma (HCC), the fourth leading cause of cancer-related deaths worldwide. Despite the elevated risk of HCC in patients with MASLD, the existing surveillance guidelines are inadequate, particularly for those without cirrhosis. This review evaluates current HCC surveillance practices in patients with MASLD and their shortcomings. It also highlights the critical need for enhanced HCC risk stratification and diagnostic accuracy through new techniques. In this review article, we performed a comprehensive literature review of studies focusing on HCC risk factors in MASLD/MASH patients from 2000 to 2023. We discussed that demographics, comorbidities, liver fibrosis, and genetic markers play critical roles in HCC risk stratification. Additionally, non-invasive tests (NITs) for fibrosis may improve the accuracy for HCC risk stratification and diagnosis. More recently, innovative approaches, such as machine learning techniques and liquid biopsy utilizing extracellular vesicles, cell-free DNA, and circulating tumor cells show promise in redefining early HCC detection. Thus, integrating these various risk factors could optimize early detection of HCC for the growing MASLD/MASH patient population. However, further research is needed to confirm their effectiveness and practical implementation in clinical settings.

Keywords: Metabolic dysfunction-associated steatotic liver disease (MASLD); metabolic dysfunction-associated steatohepatitis (MASH); non-alcoholic fatty liver disease (NAFLD); non-alcoholic steatohepatitis (NASH); hepatocellular carcinoma (HCC)

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Introduction

Background

Metabolic dysfunction-associated steatotic liver disease (MASLD), formerly known as non-alcoholic fatty liver disease (NAFLD), is the most common form of chronic liver disease and is estimated to affect approximately 25–30% of the world's population (1-3). The incidence and prevalence of MASLD are rising (one study estimates a 15-percentage point rise from 1991 to 2019), likely

related to the increasing worldwide prevalence of metabolic syndrome (1). The more severe form of MASLD, metabolic dysfunction-associated steatohepatitis [MASH; formerly non-alcoholic steatohepatitis (NASH)], was found to be the second most common etiology of cirrhosis in patients who underwent liver transplant in the United States in 2015, and may soon become the leading cause of cirrhosis in developed countries (4).

Rationale and knowledge gap

Hepatocellular carcinoma (HCC) is the fourth-leading cause of cancer-related mortality worldwide and occurs most commonly in individuals with cirrhosis (5,6). While hepatitis C virus (HCV)-related cirrhosis confers the greatest risk for HCC, the decreasing prevalence of HCV due to increased use of direct-acting antivirals (DAAs) and the increasing prevalence of MASLD necessitates changes in the approach for HCC risk assessment and surveillance. For instance, MASLD alone in the absence of cirrhosis confers an elevated risk of HCC—an estimated 20–30% of MASLD-related HCC occur in patients without cirrhosis (6-8). Due to the low absolute incidence of HCC in patients with MASLD in the absence of cirrhosis, current guidelines from professional organizations such as the American Association for the Study of Liver Diseases (AASLD) do not recommend routine HCC surveillance in these patients (9,10). While it is likely cost-prohibitive to screen every patient with non-cirrhotic MASLD for HCC, the lack of screening limits our awareness of HCC in this population and potentially leads to late diagnoses—one meta-analysis found that individuals with non-cirrhotic MASLD-related HCC tend to have larger tumors (11). Moreover, the traditional HCC surveillance modality of abdominal ultrasound (US) with or without alpha-fetoprotein (AFP) may be less accurate in individuals with MASLD due to a greater prevalence of AFP-negative tumors and potentially worse US image quality in individuals with larger body habitus (12).

Objective

Additional markers and diagnostic criteria are needed to (I) risk stratify HCC risk for individuals with MASLD and (II) improve the diagnostic accuracy of existing surveillance modalities. In this review article, we aim to discuss current methods for HCC risk stratification and early detection in individuals with MASLD. A brief graphical summary of the risk factor categories is provided in *Figure 1*.

Epidemiologic factors and comorbidities

Older age and male sex are known risk factors for the development of HCC in all etiologies of cirrhosis (3,13). In terms of race/ethnicity, one US-based study showed that Hispanic ethnicity likely confers a greater risk for HCC development in patients with MASLD cirrhosis compared to those with HCV or alcohol-induced cirrhosis (14). Behavioral factors—such as tobacco use and alcohol consumption—have been found to be independent predictors of HCC risk in patients with MASLD (15).

Other than cirrhosis, diabetes mellitus (DM) is the medical comorbidity associated with the greatest risk of HCC development in patients with MASLD. A study examining 354 patients from Mayo Clinic who had MASLD cirrhosis and validated on a cohort of 6,630 patients from the United Network for Organ Sharing (UNOS) database found that type II diabetes mellitus (T2DM) conferred between a 1.3–4.2-fold increased risk for developing HCC (16). The association between T2DM and HCC risk has been attributed to the effects of insulin [and insulin-like growth factor 1 (IGF-1)] on cell growth and protection from apoptosis, as well as the effect of hyperglycemia on vascular smooth muscle proliferation (17). Unsurprisingly, insulin use and diabetic retinopathy (features associated with longstanding, more severe diabetes) are independently associated with HCC (18).

Individuals with MASLD and T2DM who have additional traits associated with metabolic syndrome (hypertension, dyslipidemia, and obesity) may be at an even greater risk for HCC. In addition to the diabetes-specific risks noted above, obesity is associated with a chronic pro-inflammatory state that increases the risk of cancer (19). A cohort study examining 1,234 patients with MASLD found that lower baseline high-density lipoprotein (HDL) and greater waist circumference were associated with an elevated risk of developing HCC (20). Additionally, a multicenter study that examined over 270,000 patients with MASLD at Veterans Healthcare Administration facilities found that T2DM was the only metabolic trait that was independently associated with HCC development (approximately 2.8-fold higher risk) after taking into account demographic and metabolic features (21). However, individuals with T2DM and either two or three other metabolic traits had between a 5.55–8.63-fold greater risk for developing HCC.

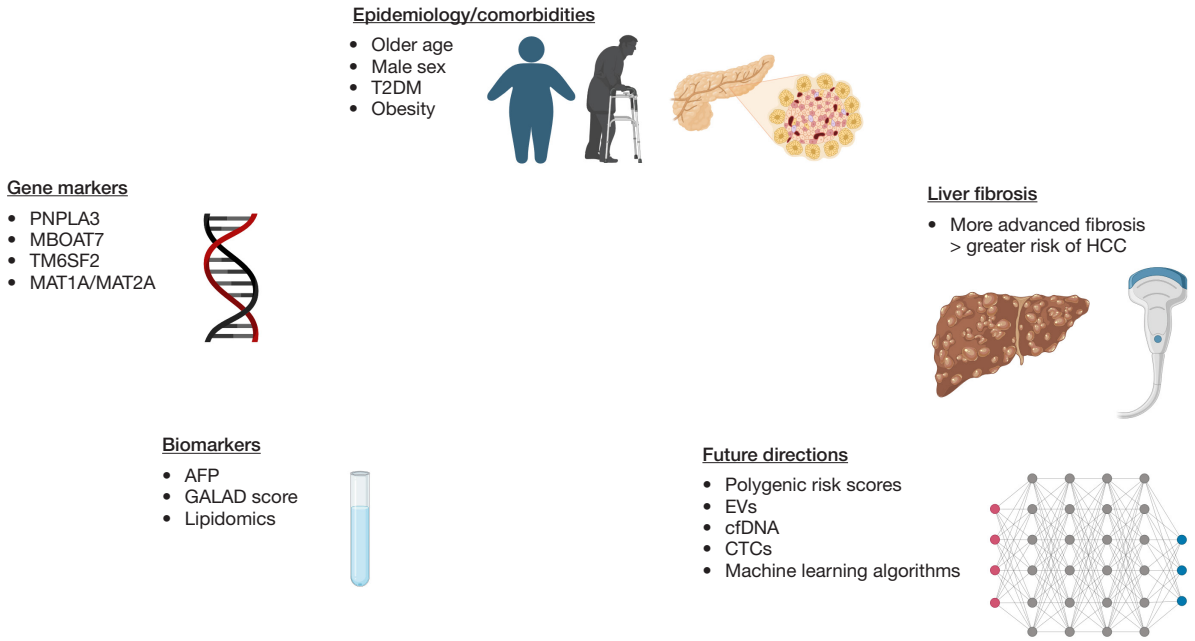

Figure 1 Risk factors for hepatocellular carcinoma in MASLD. PNPLA3, patatin-like phospholipase domain-containing 3; MBOAT7, membrane bound O-acyltransferase domain containing 7; TM6SF2, transmembrane 6 superfamily member 2; AFP, alpha-fetoprotein; GALAD, gender, age, AFP-L3%, AFP, DCP; DCP, des-gamma carboxy prothrombin; EVs, extracellular vesicles; cfDNA, cell-free DNA; CTCs, circulating tumor cells; HCC, hepatocellular carcinoma; T2DM, type II diabetes mellitus; MASLD, metabolic dysfunction-associated steatotic liver disease.

Liver fibrosis

The majority of HCC associated with MASLD still occur in individuals with advanced liver fibrosis/cirrhosis, and progression to cirrhosis is associated with increasing risk of HCC. Therefore, accurate fibrosis evaluation is vital for determining cancer risk. While guidelines recommend HCC screening in patients with cirrhosis, whether patients with non-cirrhotic MASLD (e.g., stage 3 fibrosis) require HCC surveillance remains controversial. The American Gastroenterological Association (AGA) guidelines suggest screening should be considered in patients with MASLD and advanced fibrosis (Metavir stage F3–F4); however, the AASLD does not currently recommend HCC surveillance in patients with F3 fibrosis (9,22). This section briefly summarizes the modalities for evaluating the fibrosis stage and reviews the current evidence correlating fibrosis scores with HCC risk.

While liver biopsy is the gold standard for the diagnosis of MASLD, non-invasive tests (NITs) are preferred for fibrosis staging because they are safer and generally cheaper. Additionally, imaging NITs are able to map the entire liver whereas the liver biopsy can only evaluate limited areas. Options for NITs include serum-based biomarker scores or imaging [either US or magnetic resonance elastography (MRE)]; since most NITs tend to have high false-positive rates, the diagnosis of MASLD advanced fibrosis should be confirmed with at least two different testing modalities.

Several serum-based markers/scores were originally developed to predict fibrosis in hepatitis C—some of these can be easily calculated using routine lab tests that act as surrogate markers for hepatic inflammation [i.e., aspartate aminotransferase (AST)/alanine transaminase (ALT) ratio, AST to platelet ratio index (APRI score), fibrosis 4 (FIB-4)], while others measure specific markers that are directly produced during fibrosis [enhanced liver fibrosis (ELF) panel] (23,24). The NAFLD fibrosis score (NFS) and body mass index (BMI), AST, ALT, and diabetes (BARD) score were developed specifically for staging fibrosis for MASLD (25). The FIB-4 score is the most widely validated serum biomarker for ruling out advanced fibrosis (26). Additionally, proprietary tests such as the ELF panel, FibroSPECT II and FibroSURE, have been developed and are undergoing trials to determine their accuracy (27,28). Studies comparing

Table 1 Summary of common serum-based NITs for MASLD fibrosis

Biomarker test	Components	Cutoffs (for advanced fibrosis)
AST/ALT ratio (24)	AST, ALT	>0.8: sensitivity 52%, specificity 90%
APRI score (29)	AST (including cutoff for upper limit of normal: usually set at 40), platelet count	>0.7: significant fibrosis (sensitivity 77%, specificity 72%)
		>1.0: severe fibrosis (sensitivity 61%, specificity 64%)
		>1.0: cirrhosis (sensitivity 76%, specificity 72%)
FIB-4 score (23,24,26,29)	Age, AST, ALT, platelets	<1.30: sensitivity 85%, specificity 65%
		>2.67: sensitivity 26%, specificity 98%
NFS (25,29)	Age, BMI, impaired fasting glucose/DM, AST/ALT ratio, platelets, albumin	>−1.455: sensitivity 78%, specificity 58%
		>0.676: sensitivity 33%, specificity 98%
BARD score (25,29)	BMI >28 kg/m^2, AST/ALT ratio, DM	>2: sensitivity 89%, specificity 44%
HFS (29,30)	Age, sex, homeostatic model assessment score (fasting insulin × fasting glucose/405), T2DM, AST, albumin, platelets	<0.12: sensitivity 70.7%, specificity 80.9%
		≥0.47: sensitivity 38.0%, specificity 98.0%
Enhanced liver fibrosis panel (27)	Hyaluronic acid, PIIINP, TIMP-1	>7.7: sensitivity >90%, specificity approx. 30%
		>9.8: sensitivity 65%, specificity 86%

NITs, non-invasive tests; MASLD, metabolic dysfunction-associated steatotic liver disease; AST, aspartate aminotransferase; ALT, alanine transaminase; APRI, aspartate aminotransferase to platelet ratio index; FIB-4, fibrosis 4; NFS, NAFLD fibrosis score; NAFLD, nonalcoholic fatty liver disease; BMI, body mass index; DM, diabetes mellitus; BARD, BMI, AST, ALT, and diabetes; HFS, Hepamet fibrosis score; T2DM, type II diabetes mellitus; PIIINP, type III procollagen peptide; TIMP-1, tissue inhibitor of matrix metalloproteinase 1; approx., approximately.

the non-proprietary biomarker scores found that the FIB-4, NFS and Hepamet fibrosis score (HFS) scores performed similarly in distinguishing F0–F2 *vs.* F3–F4 fibrosis and were superior to the AST:ALT ratio and APRI scores (29). The various tests' components, cutoffs, and estimated sensitivity/specificity for detecting advanced fibrosis (defined as Metavir stage F3–F4) in MASLD are summarized in *Table 1*.

A low score based on serum-based NITs (see cutoffs above) is useful for ruling out advanced fibrosis due to their high sensitivity (24); however, they are limited by a high false-positivity rate. Imaging NITs are more expensive than serum-based tests, but have greater accuracy. The most commonly used modalities are vibration-controlled transient elastography (VCTE)/Fibroscan and MRE—both techniques yield a "liver stiffness" score, which can then be interpreted as advanced fibrosis *vs.* non-advanced fibrosis based on a pre-specified cutoff (31,32). While MRE has been shown to be more accurate for diagnosing cirrhosis (F4 stage) compared to Fibroscan, there was no difference in the performance of these tests in detecting advanced (F3–F4 stage) fibrosis *vs.* no/non-advanced (F0–F2 stage) fibrosis (sensitivity and specificity approximately 80% for both) (33,34). Additionally, Fibroscan is cheaper and more accessible compared to MRE.

Several studies have examined the association between NIT fibrosis scores and HCC risk. An elevated FIB-4 score (>1.30–1.45) is associated with a greater likelihood of developing HCC in patients with MASLD [hazard ratio (HR): 8.46–13.99] (35-37). A Japanese study that examined the predictivity of the APRI and BARD scores in over 6,500 patients with MASLD found that patients with an APRI score of >1.50 were at greater risk of developing HCC (HR: 25.03), but there was no association between the BARD score and HCC (38). A longitudinal study that directly compared the APRI, FIB-4, NFS, BARD and HFS scores found that NFS performed best in predicting the development of HCC [area under the receiver operating characteristic curve (AUROC): 0.901] (29).

Models incorporating imaging features and serum-markers have been developed for predicting HCC. One study built a multivariate regression model that included liver stiffness ≥9.3 kPa as a predictor; this variable was associated with a HR of 13.8 (39). While this model performed well in its derivation cohort [area under the

curve (AUC) >0.93], it was less accurate in a validation cohort (AUC approximately 0.78), demonstrating the need for further studies in this area.

Genetic markers

Several genetic mutations associated with hepatic steatosis have also been found to be predictive for HCC development; however, the underlying physiologic mechanism connecting these genetic changes to HCC is not well-described. We briefly summarize the common genetic mutations associated with HCC in the setting of MASLD/MASH (genetic mutations commonly seen in all cancers are not included), and review some risk scores that have developed to incorporate these genetic markers.

- *PNPLA3*: the I148M mutation in patatin-like phospholipase domain-containing 3 (PNPLA3) gene confers a 2.2 to 3.1-fold increased risk for developing HCC, after adjusting for other risk factors such as T2DM and obesity (40). Despite the strong association between PNPLA3 and HCC risk, this marker when used by itself has poor specificity for confirming HCC diagnosis (40,41). It has been suggested that PNPLA3 may have a role in triglyceride transport out of the liver and the I148M mutation induces loss of function that leads to increased hepatic steatosis; however, the exact mechanism of hepatocarcinogenesis related to this mutation remains to be elucidated (41).
- *MBOAT*7 and *TM6SF2*: the MBOAT7 rs641738 variant and TM6SF2 E167K variant are two other genetic mutations associated with increased HCC risk in MASLD patients (42,43). One study suggests that these two genetic variants can be helpful in risk-stratifying patients with MASLD and non-advanced fibrosis—on multivariate regression, both genetic mutations conferred an odds ratio (OR) of approximately 2 for developing HCC, even after accounting for severe fibrosis (44). However, other cohort studies on MBOAT7 have shown mixed results, and the function of the TM6SF2 gene and its association with HCC is not well-studied. Further investigation is needed before genetic testing can be used in routine clinical practice (45).
- *MAT1A/MAT2A*: downregulation of the MAT1A gene and upregulation of MAT2A is known to occur in HCC progression (46). *In vitro* and animal studies have shown that these changes are associated with DNA hypomethylation, impaired DNA repair/genome stability and upregulation of oncogenes. These genes have not been studied in the context of HCC risk stratification. However, the MAT1A gene encodes S-adenosylmethionine (SAMe), and studies in mice suggest that administration of SAMe may inhibit hepatocarcinogenesis (47). While similar studies have not been performed in humans, this pathway represents a promising therapeutic target.
- Interleukin-13 (IL-13): IL-13 is a pro-inflammatory cytokine that promotes liver fibrosis via the JAK-STAT pathway and carcinogenesis via upregulation of TGFβ-1. IL-13 has been identified as the cytokine with the greatest role in liver fibrogenesis (48). A retrospective cohort study of 134 patients with MASH cirrhosis examined different single-nucleotide polymorphisms (SNPs) in the genes encoding IL-13, IL-13 receptors and downstream molecules. Elevated IL-13 levels were associated with greater HCC risk, while SNPs in two downstream molecules (STAT6 and YAP1) were inversely related to HCC development (49).
- Genetic scores: due to the limitations around each individual genetic marker, some studies have developed models combining multiple genetic markers. The results of three such studies are summarized in *Table 2*. While each study showed some promising results, many genetics-based studies are limited by poor positive predictive value, small population size, and/or retrospective nature. A recent study examined the benefit of incorporating a genetic risk score into models that predicted HCC using clinical data and scores derived from standard lab values [i.e., age, male sex, albumin-bilirubin, platelets (aMAP)]; the results showed addition of the genetic risk score only modestly improved HCC detection (C-index from 0.769 to 0.78) (52). These findings may suggest that the HCC risk conferred by these genotypic markers are already accounted for by more easily measured phenotypic (i.e., clinical and lab features) characteristics. However, this study only included the seven most commonly-found SNPs associated with MASLD-HCC; genetic markers detected in the future may still provide incremental benefit in predicting HCC risk.

Biomarkers

AFP is classically used for routine HCC screening;

Table 2 Accuracy of polygenic scores in predicting MASLD/MASH HCC

Study authors	Population size	Study design	Study results
Bianco *et al.* (50)	Two cohorts: (I) NAFLD cohort: n=2,566 (226 with HCC) and replication cohort of 427 patients with NAFLD; (II) general population: n=364,048 (202 with HCC)	Retrospective cohort study. Genes examined: *PNPLA3*, *TM6SF2*, *GCKR*, *MBOAT7*. Model: polygenic risk score [PRS-HFC and PRS-5 (latter score accounts for HSD17B13 gene variant, which is protective against HCC)]. Model cutoff: 0.532 (PRS-HFC), 0.495 (PRS-5)	PRS-HFC: AUROC, 0.64; sensitivity, 0.43; specificity, 0.80; PPV, 0.16; NPV, 0.94. PRS-5: AUROC, 0.65; sensitivity, 0.43; specificity, 0.79; PPV, 0.17; NPV, 0.93
Pelusi *et al.* (44)	Two cohorts: (I) HCC cases: discovery cohort (n=72) and validation cohort (n=70); (II) controls: healthy individuals from 1,000 G project (n=404), phenotyped healthy individuals (n=50), individuals with advanced fibrosis but no HCC (n=59)	Retrospective cohort. Genes examined: 181 candidate genes associated with HCC, all cancer, liver disease, dysmetabolism. Narrowed to 39 genes that were "significantly enriched" in MASLD-HCC. Model: GRS. Derived by including 655 patients and validated on the same dataset using the jack-knife method. Model cutoff: 0.22	Overall: AUROC, 0.74; sensitivity, 0.61; specificity, 0.76; PPV, 0.42; NPV, 0.88
Fujiwara *et al.* (51)	Derivation cohort (n=48; underwent curative ablation with follow-up). Validation cohort: tissue from patients who are HCC-naïve (n=106) and had prior HCC (n=59). 2nd validation: genetic markers converted to serum protein panel using TexSEC algorithm tested on an independent serum validation set (n=59)	Longitudinal study. Genes examined: 133-gene signature. Model: PLS-NAFLD; genetic signature then transformed into a protein signature (PLSec-NAFLD)	Patients divided into "high-risk" and "low-risk" PLS signatures. HCC naïve patients: 15-year HCC risk was 22.7% in high-risk patients, 0% in low risk. Patients with prior HCC: 5-year recurrence rate was 71.8% in high-risk, 42.9% in low risk. Independent HCC naïve panel (for serum protein validation): 15-year HCC risk was 37.6% in high-risk, 0% in low-risk

MASLD, metabolic dysfunction-associated steatotic liver disease; MASH, metabolic dysfunction-associated steatohepatitis; HCC, hepatocellular carcinoma; NAFLD, nonalcoholic fatty liver disease; PNPLA3, patatin-like phospholipase domain-containing 3; TM6SF2, transmembrane 6 superfamily member 2; GCKR, glucokinase regulatory protein; MBOAT7, membrane bound O-acyltransferase domain containing 7; PRS-HFC, polygenic risk score for hepatic fat content; PRS-5, polygenic risk score considering 5 risk variants; HSD17B13, hydroxysteroid 17-beta dehydrogenase type 13; AUROC, area under the receiver operating characteristic curve; PPV, positive predictive value; NPV, negative predictive value; GRS, genetic risk score; TexSEC algorithm, translation of tissue gene expression to secretome; PLS, prognostic liver signature; PLSec-NAFLD, serum protein-based surrogate marker of PLS-NAFLD.

however, its utility is limited in cases of MASLD cirrhosis due to a higher percentage of AFP-negative tumors (12). Several studies have attempted to improve the predictive value of AFP by developing models using other common serum markers (53-55). For example, low platelet count is associated with a greater likelihood of developing HCC in patients with MASLD (53). The HCC Early Detection Screening (HES) algorithm, which offers a slight improvement over AFP alone, incorporates AFP, patient age, platelet count, ALT, two interaction terms (AFP×ALT, AFP×platelets) and rate of change of AFP over one year (54). Other markers, such as des-gamma carboxy prothrombin [DCP; also referred to as Vitamin K Absence or Antagonist II (PIVKA-II)] have been used in HCC screening, and one small retrospective study suggests that it may be more accurate than AFP for identifying HCC in patients with MASLD (55). This section reviews common biomarker-derived scores, metabolic models, and novel markers and techniques that are currently being investigated for use in HCC screening.

- GALAD score: the GALAD score, which incorporates patient gender, age, and the three serum biomarkers AFP, AFP isoform L3 (AFP-L3) and DCP, has been found to have good predictive accuracy for HCC (56-58). An international, multi-center case-control study that specifically examined patients

with MASLD confirmed that the GALAD score had good accuracy in this population (AUROC: 0.96, sensitivity: 0.91, and specificity: 0.91 at the optimal cutoff) and had superior performance compared to the individual biomarkers alone. Moreover, the GALAD score accurately detected early tumors, as well as tumors that developed in cases of non-cirrhotic MASLD (59).

- Lipid metabolism markers: alterations in lipidomics/lipid metabolism may be seen in patients with MASLD and HCC compared to MASLD alone. One multi-center, retrospective study assessed 273 lipid and small molecule markers in 257 patients and found 21 metabolites that accurately differentiated HCC from non-cancer cases (AUROC: 0.79), which they termed the predictive metabolite vector (PMV) (60). Metabolites associated with phosphocholine, fatty acylcarnitine, and asymmetric dimethylarginine were elevated in cases of HCC, while those associated with sphingomyelin, lysophosphatidylcholine, and phosphatidylinositol were inversely correlated with HCC. The PMV was positively correlated with more advanced BCLC stage and worse overall survival. Another study found that individuals with MASLD and HCC had depletion in unsaturated fatty acids and acylcarnitines (61). Using a cohort of 249 patients (27 of whom had MASLD-HCC), they analyzed nearly 1,300 lipidome-associated serum metabolites and ultimately selected 4 [linoleic acid, osbond acid, monounsaturated fatty acid (14:1n-5trans) and phosphatidylcholine (18:2/0:0)] metabolites to generate the NAFLD-HCC diagnostic score (NHDS). This score performed well in HCC detection (AUROC: 0.87).
- CD163: serum soluble CD163 (sCD163) is produced by macrophages, which are involved in the development of liver fibrosis. One retrospective study measured sCD163 levels in patients at the time of MASLD diagnosis; those with HCC at the time of diagnosis had greater sCD163 levels (62). Moreover, those with an incident sCD163 level >800 ng/mL had nearly four-fold greater 10-year HCC risk and lower 10-year survival. Larger sample prospective studies that trend sCD163 levels over time are needed to validate these findings and determine the clinical utility of this marker.
- *Wisteria*: The glycoprotein *Wisteria floribunda* is a marker of liver fibrosis; in a retrospective study of 331 patients with MASLD, *Wisteria* levels were higher in HCC *vs.* non-HCC patients who had advanced fibrosis (63). This marker had a AUROC of 0.806 with sensitivity and specificity of 0.704 and 0.784, respectively, at its optimal cutoff.
- Machine learning models: machine learning techniques, particularly neural networks, have been shown to have good accuracy for predicting HCC based on common patient demographic features (age, gender and comorbidities) and basic labs. One study examined 175 patients with MASLD (n=34 with HCC) and input 12 basic demographic and lab characteristics into a neural network to generate the HCC-Scope model, which was then validated on an independent dataset of 55 patients (n=19 with HCC) (64). The model had excellent predictive accuracy (AUROC >0.98 for both exploration and validation cohorts, and sensitivity and specificity both approaching nearly 100% in both cohorts). However, this study is limited by its small validation cohort and retrospective nature; additionally, as with all neural network models, the relative importance for each of the 12 input variables cannot be determined.
- Liquid biopsy: the liquid biopsy involves identifying cancer genomic profile from a patient's serum sample using extracellular vesicles (EVs), cell-free DNA (cfDNA) or circulating tumor cells (CTCs), and represents a novel area of research with the promise of increasing precision in early HCC diagnosis (65-67). cfDNA markers are already being used for treatment response assessment in some cancers; however, the clinical utility of the liquid biopsy is currently limited by (I) the small sample size and retrospective nature of most studies, leading to highly variable results and (II) challenges with distinguishing cancer markers from EVs and cell-free released by normal cells (68). Here, we briefly review EVs, cfDNA and CTC in the general context of HCC diagnosis, since few studies have examined the accuracy of these markers specifically in the context of MASLD.

EVs are exosomes and microvesicles that contain proteins, lipids and/or genetic markers (DNA, mRNA, miRNA and non-coding RNA) that are released by cells and function in intercellular communication (69). Studies have identified unique patterns of EV excretion from tumor or microenvironment cells (65,69). Between the three aforementioned liquid biopsy techniques, EVs are elevated

in early-stage tumors and persist across different cancer stages (70). Total serum EVs are increased in patients with HCC, and multiple studies have linked specific EVs to tumor proliferation, angiogenesis, metastasis and recurrence in HCC. Pilot studies examining the predictive utility of different EVs as biomarkers for early HCC detection have shown promising results, many with AUROC of >0.9 (70). However, most of these biomarker studies are limited by (I) small patient sample size and (II) examining EVs that can be produced by different types of cells instead of only by hepatocytes, which may lead to false positive results in the setting of other chronic illnesses. Access to EV purification and measurement methods is another limitation of its clinical utility (65).

cfDNA refers to short strands (usually around 100–200 base pairs) of double-stranded DNA detectable in serum; circulating tumor DNA (ctDNA) refers to the proportion of cfDNA released from tumor cells, either via apoptosis or active transport (i.e., as exosomes) (71,72). ctDNA can be measured quantitatively—either via total concentration in serum or integrity, which refers to the concentration of long-strand to short-strand DNA fragments—or qualitatively via SNPs and epigenetic changes (primarily methylation) (71). Small retrospective cohort studies have shown that ctDNA profiles, especially when combined with other markers such as AFP, have good accuracy for early tumor diagnosis and disease prognosis (66). One study examined cfDNA of the *TERT* gene with C228T mutation, and found that it had greater accuracy than the AFP and DCP biomarkers in detecting MASLD-HCC (73).

CTCs are released into the bloodstream during the process of tumor invasion through the basement membrane into the vasculature (67). CTCs that are able to survive after entering the bloodstream are generally characterized by epithelial-to-mesenchymal transition (EMT). Studies have shown that CTCs markers and receptors may be significantly different from those of the primary tumor. Combined CTC markers (such as EpCAM, ASGPR, and GPC3) have demonstrated high accuracy for detecting HCC in small cohort studies (67). Additionally, mesenchymal cell markers and cellular aneuploidy are associated with poor prognosis (74,75).

Conclusions

The rising incidence of MASLD will lead to a greater population at risk for HCC, highlighting the urgent need for more accurate HCC surveillance and early detection methods. While the absolute risk of HCC in patients with MASLD is smaller than the risk in HCV, high prevalence of MASLD necessitates new tests that are both more sensitive and specific for risk stratification and early detection of HCC. Demographic features and NIT for fibrosis are easily accessible characteristics that may improve the accuracy for HCC risk stratification and diagnosis. Genetic markers and serum biomarkers can potentially be useful screening tools for HCC risk, especially in patients with non-cirrhotic MASLD who do not undergo routine HCC surveillance. Finally, machine learning techniques and liquid biopsy have the potential to greatly improve early detection, treatment response monitoring and prognosis assessment for HCC—however, these techniques are still under investigation, and larger prospective trials are needed to determine their clinical utility.

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