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Risk stratification and early detection biomarkers for precision hepatocellular carcinoma screening

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Abstract

Hepatocellular carcinoma (HCC) mortality remains high mainly due to late diagnosis as a consequence of failed early detection. Professional societies recommend semi-annual HCC screening in at-risk chronic liver disease patients to increase the likelihood of curative treatment receipt and improve survival. However, recent dynamic shift of HCC etiologies from viral to metabolic liver diseases has significantly increased the potential target population for the screening, whereas annual incidence rate has become substantially lower. Thus, with the contemporary HCC etiologies, the traditional screening approach might not be practical and cost-effective. HCC screening consists of (i) definition of rational at-risk population, and subsequent (ii) repeated application of early detection tests to the population at regular intervals. The suboptimal performance of the currently available HCC screening tests highlights an urgent need for new modalities and strategies to improve early HCC detection. In this review, we overview recent developments of clinical, molecular, and imaging-based tools to address the current challenge, and discuss conceptual framework and approaches of their clinical translation and implementation. These encouraging progresses are expected to transform the current "one-size-fits-all" HCC screening into individualized precision approaches to early HCC detection and ultimately improve the poor HCC prognosis in the foreseeable future.

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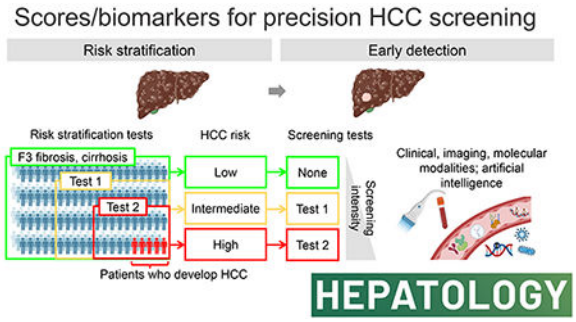
J.Y. serves as consultant for Exact Sciences, Exelixis, and Eisai. Y.H. serves as an advisory board member for Helio Genomics and Espervita Therapeutics, and share holder for Alentis Therapeutics and Espervita Therapeutics.

Graphical Abstract



Keywords

Hepatocellular carcinoma; early detection; risk stratification; biomarkers; personalized screening

INTRODUCTION

Primary liver cancer is the fourth leading cause of cancer-related death worldwide, with an estimated 0.8 million deaths in 2020.[1] More than 80% of primary liver cancers are hepatocellular carcinoma (HCC) that develop in patients with chronic infection of hepatitis B virus (HBV), hepatitis C virus (HCV), excess alcohol intake, and metabolic disorders, including non-alcoholic fatty liver disease (NAFLD)/metabolic dysregulation-associated fatty liver disease (MAFLD).[2, 3] In the U.S., the overall HCC incidence rate has been increasing in more than half of the states.[4] Despite the improvement in early HCC detection and the advance in treatment over the past decades, 5-year overall survival rate of HCC is still dismal at around 20%.[5] Given the survival benefit of diagnosing HCC at early stages amenable to potentially curative treatment, current clinical practice guidelines recommend regular HCC screening in at-risk chronic liver disease patients.[6–8] However, the recommended screening is utilized only in less than 25% of HCC patients in the U.S. due to various logistical barriers, thus effectiveness of the screening is significantly impaired.[9–11] Furthermore, application of the screening has been more challenging along with the drastic changes in the HCC etiology landscape over the past decade, namely sharp decline of active HCV infection with the widespread use of new-generation anti-HCV drugs and global epidemic of obesity and metabolic disorders.[12, 13] In addition, sensitivity of the current HCC screening test is suboptimal, and it leads to failures in early HCC diagnosis.[14, 15] Thus, new tools and strategies are urgently needed to enable more effective HCC screening with improved utilization and early HCC detection to substantially improve poor HCC mortality.

To address this urgent and growing unmet medical need, new biomarkers will have a significant role by redefining the high-risk target population for HCC screening and by enabling more sensitive and accurate detection of early-stage HCC. Cancer biomarker development is a challenging process that involves costly and lengthy test development and validation.[16] To streamline and facilitate clinical translation of experimental cancer biomarkers, several national and international efforts have been

made to develop resources for high-quality validation of promising biomarker candidates under federally-funded consortia such as the U.S. National Cancer Institute (NCI) Early Detection Research Network (EDRN).[17] In parallel, development of highly sensitive omics profiling technologies has enabled the interrogation of various cancer-associated molecular information in body fluid samples such as blood and urine, so-called “liquid biopsy”, as potential HCC screening biomarkers.[18] In this review, we outline the limitations of the current HCC screening strategy, discuss the conceptual framework of precision medicine approaches to overcome the challenges, and overview new developments on the horizon to refine HCC risk stratification and early detection with a special focus on new biomarkers that will likely impact HCC screening program and eventually reduce HCC mortality.

LIMITATIONS AND UNMET NEEDS IN HCC SCREENING

Professional societies recommend semi-annual HCC screening with abdominal ultrasound and alpha-fetoprotein (AFP) to improve early detection, curative treatment receipt, and survival in patients at risk of HCC development.[8] HCC screening consists of the following two components: (i) definition of target population, and (ii) repeated application of HCC detection tests at regular intervals (Figure 1A). A positive detection test triggers the procedure of HCC diagnosis with either contrast-enhanced dynamic computed tomography (CT)/magnetic resonance imaging (MRI) or histological assessment.[6] Efficacy of each component is limited by suboptimal performance of currently available modalities as detailed in the following sections. The complexity of the screening algorithm further compromises its effectiveness due to various logistical issues in its clinical implementation at patients, providers, and systems levels in the real-world setting.[19] Model-based simulation has been utilized to estimate efficacy and effectiveness of the HCC screening protocol based on cost-effectiveness, and revealed critical factors such as HCC incidence rates in the target population and performance of HCC detection tests.[13]

Increasingly elusive target population for HCC screening

Target population for the screening has been defined based on model-based cost-effectiveness, balancing number needed to screen (NNS) to detect one HCC case, associated net medical care costs, and net patient survival according to specific clinical context. For example, the screening was deemed cost-effective in cirrhosis patients with annual HCC incidence rate of 1.5% or greater.[6] This assumption was relevant when active HCV infection was the dominant cirrhosis etiology, where annual HCC incidence was as high as 8%.[12] However, the assumption no longer holds with the dynamic change in the landscape of liver disease etiology over the past decade, namely the sharp switching from active to cured HCV infection with the widespread use of new generation anti-virals and increase of metabolic liver diseases, particularly NAFLD.[20, 21] In these emerging at-risk populations, annual HCC incidence rate barely reaches the traditional threshold of 1.5% to justify HCC screening as a cost-effective intervention. After pharmacological cure of chronic HCV infection, i.e., sustained virologic response (SVR), annual HCC incidence rate is reduced to 0.5% to 2.1% in patients with advanced fibrosis or cirrhosis.[22] A recent simulation analysis suggested that the semi-annual screening is still cost-effective in SVR patients with advanced fibrosis or cirrhosis until age 60 to 70, but with a substantially loosened

cut-off of incremental cost-effectiveness ratio (ICER) < $150,000 that is three-times higher than the traditionally used cut-off of < $50,000, which may not be globally acceptable.[23] In histologically confirmed NAFLD cirrhosis patients, annual HCC incidence rate is only 0.1% to 0.6%.[24, 25] Of note, unlike viral hepatitis- and alcohol-related liver diseases, HCC can develop even before establishing cirrhosis in > 30% of NAFLD-related HCC patients.[26] It highlights necessity of expanding the target population for HCC screening by including patients with F3 fibrosis, although this is practically infeasible given that the guideline-recommended "one-size-fits-all" HCC screening is applied only in less than a quarter of the patients.[10] Furthermore, the NNS will become unrealistically large if we adopt the recently proposed redefinition of metabolic liver disease, namely MAFLD, which is estimated to affect half of overweight/obese adults globally (Figure 1B).[27]

In addition, given that vast majority of the patients undergoing the screening will not develop HCC during their lifetime, unnecessary harms due to over-screening patients with indolent disease will become unignorable with the large NNS.[28] Thus, HCC risk stratification is urgently and increasingly needed to redefine the target population to enable cost-effective and practically feasible HCC screening, especially with the dynamically changing landscape of liver disease etiology.

Suboptimal performance of HCC detection tests

For HCC detection at early stage amenable to potentially curative treatments, sensitivity should be sufficiently high, while maintaining specificity to minimize false positives. Ultrasound is currently the standard screening test used in clinical practice, although its sensitivity is only around 50%.[15] Even combined with AFP, sensitivity is still around 70% to detect early-stage HCC.[15] In addition, this performance may be overestimation due to inclusion of phase 2 biomarker studies in the meta-analysis. Performance of ultrasound will be further impaired due to the increase of obese NAFLD patients.[29] Other clinically available markers, AFP-L3% and des-gamma-carboxy prothrombin (DCP), show similarly suboptimal performance.

Frequency of HCC screening in the era of precision medicine

Currently, the HCC screening test is performed at 6-month interval based on clinically observed superior efficacy in comparison to longer interval and non-inferiority to shorter interval with theoretical justification according to the tumor volume doubling time.[30–32] However, this guideline-recommended "one-size-fits-all" strategy disregards considerable inter-tumor/patient heterogeneity in the doubling time and frequency of multicentric carcinogenesis; the 6-month interval may not be optimal for each individual patient.[32] Indeed, a Markov model-based simulation analysis suggested that shorter interval for high-risk patients and longer interval for low-risk patients could enable more cost-effective HCC screening compared to the uniform 6-month interval for all when overall annual HCC incidence rate is > 3%.[33] This suggests that the screening interval can be tailored according to predicted individual HCC risk.

CONCEPTUAL FRAMEWORK OF PRECISION HCC SCREENING

General principles in precision HCC screening

To address the limitations in the current HCC screening and improve its effectiveness, performance of the risk stratification and early detection tests should be improved, and the tests should be rationally embedded and sequenced in an HCC screening algorithm. To improve performance of each test, integration of multimodal information (e.g., clinical, molecular, and/or imaging variables) has been often employed for both risk stratification and early detection. In addition, for risk stratification, sequential application of multiple tests has been proposed for stepwise enrichment of high-risk population to improve efficacy and feasibility of subsequent regular application of early detection tests.[34] Early detection tests should be applied according to predicted HCC risk to avoid under-screening of high-risk patients (which can lead to failed early detection) and over-screening of low-risk patients (which can lead to unnecessary harms due to the screening tests[28]). Clinical implementation of new tests in the HCC screening protocol should be guided based on trade-offs between multiple factors, including logistical feasibility and costs of the tests, accessibility to the biospecimens and other information used in the testing algorithm, among many others, to maximize its effectiveness with improved “precision” in risk stratification and early detection.

Integrative HCC screening scores/biomarkers to improve precision

Integration of multimodal information has been attempted to improve test efficacy. It has been empirically known that AFP elevation is associated with long-term HCC risk, besides its use as an HCC detection marker, reflecting chronic liver injury and regeneration underlying carcinogenic hepatic tissue milieu.[13, 35] A blood-based Prognostic Liver Secretome signature (PLSec) was integrated with AFP to achieve robust long-term HCC risk stratification in cirrhotics.[36] Integration of etiology-specific “plug-in” biomarker with etiology-agnostic backbone biomarker is a strategy for refining HCC risk stratification according to liver disease etiology as shown in a recent proof-of-concept study.[37] Non-invasive scores (NISs) or non-invasive tests (NITs) also represent the integrative approach, combining a handful number of clinical variables (e.g., patient age, sex) and biochemical tests (e.g., AFP, hepatic transaminases). Many of these clinical variable-based NISs/NITs were originally developed for other purposes such as detection of advanced liver fibrosis and subsequently associated with adverse outcomes, including HCC development, in systematic retrospective assessment, although associated outcomes vary across studies.[38] Integration of imaging modalities (e.g., acoustic elastography, magnetic resonance elastography [MRE]) and NISs/NITs (e.g., Fibrosis-4 [FIB-4] index) have been developed for non-invasive detection of advanced fibrosis, and were subsequently associated with adverse outcomes, including HCC development.[39, 40] Germline DNA variants such as single nucleotide polymorphisms (SNPs) have been heavily studied as potential HCC risk stratification biomarkers on easily accessible biospecimens such as buccal swab. More recently, their combinations have been evaluated as polygenic risk scores (PRSs), mostly tailored for metabolic liver diseases.[41] While the genetic scores show promising HCC risk association, a recent nationwide population-level biobank study suggested that additional prognostic information gained by PRSs on top of NISs/NITs may be limited unless the target

population is carefully chosen.[42] The Liver Cancer Risk test algorithm (LCR1-LCR2) is an integration of clinical demographics and several biochemical test, which has been validated for high negative predictive value (NPV) > 99% in patients with viral hepatitis.[43] Integration of multimodal information has also been explored for early HCC detection tests such as the GALAD score, combining patient age and sex with AFP, AFP-L3%, and DCP.

Sequential application of HCC screening scores/biomarkers to improve effectiveness

Sequential application of HCC risk assessments for stepwise enrichment of high-risk population will be a rational strategy given the explosive growth of potential at-risk population with the NAFLD/MAFLD epidemic, which has been transforming HCC screening like finding a needle in a large haystack. Indeed, stepwise enrichment of NAFLD patients who need medical attention/intervention has been actively explored,[34] and HCC risk stratification could be added as a subsequent step.[44] Desired characteristics of HCC risk stratification biomarkers would depend on target population for the tests. For instance, cheap assay costs and robust performance in less-invasively accessible specimens would be valued over high accuracy for the first step of HCC risk stratification applied to a large population (e.g., adult NAFLD patients). If the first risk assessment is performed in general population, the tests may be tailored to also cover other cancer types and chronic diseases. Subsequent step(s) of risk stratification can be performed in the narrowed target population with more expensive tests with higher accuracy to identify a substantially small subset of patients as a high-risk group for certain interventions (e.g., HCC screening, chemoprevention) with enhanced efficacy of the interventions. In a nationwide population-based study involving 266,687 individuals, a stepwise risk enrichment with first NIS/NIT followed by PRS successfully enriched individuals at risk of severe liver diseases.[45]

Model-based assessment of precision HCC screening strategies

Given that the entire HCC screening protocol is complex with many modifiable parameters, it is challenging to evaluate net benefit of new risk-stratified HCC screening algorithms in a prospective controlled clinical trial. Instead, Markov model-based simulation analysis has been widely used to estimate net survival benefit and cost-effectiveness of experimental HCC screening strategies, in which plausible ranges of model parameters such as screening utilization rate can be assessed as sensitivity analysis.[46] The first cost-effectiveness analysis of risk-stratified HCC screening strategies, comparing 2 non-risk-stratified and 14 risk-stratified strategies, showed that risk-stratified screening utilizing new tests are substantially more cost-effective than the current non-stratified screening.[33] Various key parameters such as imaging modalities, screening interval and duration, and harms from HCC screening can be incorporated in the modeling.[23, 33, 46–52] Model-based simulation also provides insight into benchmarks to meet for experimental biomarkers in development. For example, a hypothetical risk stratification biomarker enables cost-effective HCC screening for majority of top-performing risk-stratified algorithms when it achieves risk stratification at hazard ratio > 2 in cirrhosis patients dominantly affected with chronic HCV infection.

Clinical implementation of precision HCC screening

The risk-stratified approach is essentially tailoring of screening intensity, regarding test modality and frequency, according to predicted risk level; more intensive/frequent screening is offered to high-risk patients, whereas less intensive/frequent or no screening is offered to low-risk patients. Practical feasibility and acceptance from the professional societies and practitioners will be the key in clinical implementation of risk-stratified HCC screening protocol. A questionnaire-based study showed that physicians are receptive to tailoring HCC screening modality for each patient when individual HCC risk can be quantitatively estimated.[53] Alteration of screening frequency, including dropping from the screening, will need attention on specific test performance metric, e.g., high NPV to justify exclusion from the screening, balanced with physician's and patient's perspective and preference. Ethical issues and potential psychological harms such as anxiety will need to be properly considered to justify exclusion of low-risk individuals from the screening. Patients with advanced fibrosis or cirrhosis may need monitoring/care for liver failure and portal hypertension regardless of HCC risk, and it may be logistically sensible to concurrently assess presence of nodular lesions with low-cost modalities such ultrasound and/or AFP during the clinic visits. Nevertheless, the guideline-recommended semi-annual screening is currently utilized in a small subset (< 25%) of the target population due to the limited medical resources, [10] and risk stratification would help identify high-risk patients to be prioritized for the screening. Biomarker-based HCC risk level may change over time in response to influential events (e.g., antiviral therapies, body weight loss, aging) depending on the type of biological information the biomarker captures. Repeated assessment may be needed for such biomarkers, considering possibility of altering subsequent HCC screening strategy. Indeed, naturally occurring modulation of HCC risk level measured by a hepatic transcriptome signature over a median interval of 2.3 years was associated with future HCC development in a cohort of NAFLD cirrhosis patients.[37]

TECHNICAL ASPECTS OF HCC BIOMARKER DEVELOPMENT

Phases of cancer screening biomarker development

To streamline and facilitate development of cancer screening biomarkers, a five-phase conceptual framework was proposed in conjunction with the NCI EDRN (Figure 2A). [54] Phase 1 studies are preclinical exploration of candidate biomarkers in biospecimens not necessarily collected with intention of biomarker research. Phase 2 studies aim at clinical assay development, encompassing clinical assay implementation, optimization, and preliminary estimation of performance typically in cross-sectional series of HCC patients and matched controls. Analytical algorithm should be established as detailed in the next section. Clinical confounding variables such as patient sex, age, liver disease etiology and severity, particularly fibrosis stage, should be properly controlled to avoid over- or under-estimation of the test performance in anticipated target patient population. Phase 3 studies are retrospective analysis of biospecimens with longitudinal follow-up information; samples are collected before HCC development or formal HCC diagnosis and patients who develop HCC during subsequent follow-up are compared to control patients matched for confounding variables who are HCC-free over certain follow-up time. Phase 3 studies will provide more accurate estimate of biomarker performance in the screening setting.

Comparison to standard care is also within the scope of phase 3 study. As generic resources for phase 3 biomarker studies, prospectively developed patient cohorts accompanied with biorepository have been developed to enable high-quality biomarker evaluation by utilizing the prospective specimen collection, retrospective blinded evaluation (PRoBE) or "prospective-retrospective" design.[54, 55] Samples collected at the time of cancer diagnosis would allow conduct of phase 2 studies. The EDRN Hepatocellular carcinoma Early Detection Strategy (HEDS) study[56] and Texas HCC Consortium (THCCC)[57] are examples of nationwide and statewide multicenter cohorts, respectively, for phase 3 HCC biomarker validation. Phase 4 studies are prospective evaluation of candidate biomarkers in the screening setting to determine performance of the biomarkers, i.e., cancer detection rate and false referral rate based on standard-care diagnostic test's result, in the target patient population. A positive test triggers the standard-care diagnostic procedure to determine an HCC diagnosis, following practice guidelines. Phase 5 studies evaluate whether HCC screening interventions that incorporate new biomarkers reduce HCC burden and mortality in the target population. This phase will prospectively determine clinical impact of new cancer screening biomarkers measured by reduction in cancer mortality and net medical care costs.[58] These phases provide roadmap for rigorous evaluation and development of cancer screening biomarkers. However, this is a costly and lengthy process that limits cancer screening biomarker development. To overcome the challenge and accelerate clinical translation of promising candidate biomarkers, innovative approaches such as adaptive trial design are urgently needed.

Analytical validity and clinical utility of cancer screening biomarker

Analytical validity of new cancer biomarkers should be established in clinically applicable assays. For each molecular probe in the assays, reproducibility of its measurement should be confirmed, and magnitude of variation should be determined across day-to-day and inter-operator/laboratory variations measured by correlation coefficient, coefficient of variation, and/or other relevant statistics in technical and/or biological replicates. Reference standards will ensure proper adjustment of the measurements for experimental batch difference as needed. Cut-off values and/or analytical algorithms to call positivity of the tests should be pre-determined in derivation/training dataset(s), which should be applied in independent validation dataset(s) without any modification based on information from the validation set(s) to avoid information leak. For biomarkers that provide quantitative estimates (e.g., predicted probability of HCC incidence), proper calibration should be performed to ensure agreement between predicted and observed measures.

Clinical utility is critical in determining which candidate biomarkers warrant further clinical development and translation to ensure that the biomarkers provide clinically actionable information. Clinically meaningful effect size (e.g., magnitude of HCC risk association measured by hazard ratio, performance of early HCC detection measured by area under receiver operating characteristic [AUROC] curve) should be defined a priori, and sample size to detect the effect size should be defined for independent validation of a candidate biomarker. Comparison to or integration with existing clinical scores and/or biomarkers should be performed to determine whether additional information gained by the new biomarker justifies costs and efforts of its clinical development.

Performance metrics for risk stratification biomarker include Harrel's C-index (a.k.a. concordance index), time-dependent AUROC curve, explained variation (R^2), Brier score, Royston's D index, Akaike information criterion (AIC), and Bayesian information criterion (BIC) to assess discrimination and/or goodness of fit. Performance metrics for early detection biomarker include contingency table statistics such as sensitivity, specificity, positive/negative-predictive values, AUROC curve. Reporting guidelines help ensure proper assessment for diagnostic/prognostic biomarkers (e.g., STARD, REMARK, TRIPOD) available via the enhancing the quality and transparency of health research (equator) network (www.equator-network.org/reporting-guidelines).

Issues in clinical deployment and implementation of cancer screening biomarkers

Analytically and clinically validated biomarkers would undergo the process of clinical deployment and implementation, including commercial product development, regulatory approval, coding for health insurance coverage, and incorporation in clinical practice guidelines, which can hugely vary across geographic regions and countries. In the U.S., while it keeps evolving, there are two major paths under oversight by the FDA: (i) in vitro diagnostic devices (IVDs) as commercial medical devices with 510(k) clearance, and (ii) laboratory developed tests (LDTs) as home-grown tests performed at each diagnostic lab.[59] FDA guidance documents are available for several relevant types of biomarkers and topics such as circulating tumor DNA (ctDNA)-based tests and LDTs (www.fda.gov/regulatory-information). Clinical biomarker tests must be conducted in diagnostic laboratories certified for Clinical Laboratory Improvement Amendments (CLIA) and in accordance with state-specific regulations. Coverage by health insurance is critical for physicians to order the tests. Other local/regional agencies such as European Medicines Agency (EMA) employ similar but their own procedure.[60] Coding for the tests, e.g., current procedural terminology (CPT) codes, is needed for insurance coverage as billable medical procedures. Centers for Medicare & Medicaid Services (CMS) regularly updates the billing and coding policies according to specific indications (www.cms.gov/medicare-coverage-database).

For decision of payers and policy makers, incorporation of the tests into clinical practice guidelines/guidance is important, which should be based on the level of available evidence (Figure 2B). Public organizations such as the Biomarkers Compendium of National Comprehensive Cancer Network (NCCN) (www.nccn.org) and the U.S. Preventive Services Task Force (USPSTF) (www.uspreventiveservicestaskforce.org) also provide regularly updated guidelines and recommendations for cancer biomarkers and screening algorithms graded by quality of available evidence (Figure 2C, D).[61] Post-marketing clinical utility validation, including the phase 5 biomarker validation study, will further support the use of biomarker tests and may result in indication for additional diseases and/or clinical scenarios. With the sharply expanding clinical and commercial interests especially in circulating cancer biomarkers so-called "liquid biopsy", several federally-funded and private consortia have been established to facilitate clinical translation of this type of biomarkers, including Blood Profiling Atlas in Cancer (BloodPAC) and NCI Division of Cancer Prevention's Liquid Biopsy Consortium.[62] Further, engagement of practitioners who order the tests and medical staffs via education, training, and/or incentive will be important to ensure proper adherence to the new biomarker-based care.

Emerging technologies/methodologies with potential utility in HCC screening

The requirement of clinic visits at 6-month interval is a significant logistical hurdle in the current ultrasound-based HCC screening protocol.[11, 63] Body fluid (e.g., plasma, urine)-based tests are expected to be available in clinic in near future and alleviate the burden as overviewed in subsequent sections. A functional *in vivo* genetic screening suggested that there may be a new class of HCC risk-associated DNA variants, somatic DNA mutations in *PKD1*, *KMT2D*, and *ARID1A* genes in cirrhotic liver that confers protective effect against carcinogenesis.[64] Point-of-care (POC) biochemical tests and imaging devices have been actively explored as potential options to substantially improve receipt of the regular screening examination particularly in developing regions with limited access to medical care.[65–68] These new technologies could be combined with software as a medical device (SaMD), incorporating artificial intelligence (AI) and machine learning/deep learning (ML/DL) for widespread application.[69] Several promising examples are overviewed in the following sections.

HCC RISK STRATIFICATION SCORES AND BIOMARKERS

Numerous HCC risk-associated clinical and molecular scores and biomarkers have been reported to date. None of them has been adopted into clinical practice yet, but some scores/biomarkers have shown promising performance in more advanced stages of clinical validation as summarized below (Table 1, Supplementary table 1).

HCC risk scores based on clinical variables

Many clinical HCC risk scores have been proposed in various regional populations, representing diverse HCC etiology and race/ethnicity, based on etiology-agnostic clinical variables such as age, sex, hepatic transaminases, and platelet count with or without etiology-specific variables such as status of viral hepatitis, alcohol abuse, and metabolic disorders. These scores are readily available and could be useful as the initial step of risk enrichment followed by application of more accurate molecular risk biomarkers tailored for specific clinical context. Some of the scores were developed in a cohort of patients with various HCC etiologies within a specific region, which may compromise general applicability of the scores to other regions with distinct etiology. Some scores were developed in more homogeneous population such as patients with HBV infection, in which head-to-head comparison between the scores clarified superior performance of several scores such as REAL-B and PAGE-B.[70, 71] Toronto HCC risk index[72] and aMAP risk score are examples of externally validated etiology-agnostic risk scores.[73] In a systematic comparison between six clinical HCC risk scores in HCV-cured cirrhosis patients in the U.K., aMAP score outperformed other scores.[74] This study also found that age plays a substantial role in the risk prediction, and their performance was suboptimal in the older patient subgroup. In viral hepatitis patients, quickly evolving anti-viral therapies will be critical confounding factors in the risk score performance. New-generation anti-HBV drugs under development may have a significant impact in predicting HBV-related HCC risk, while viral control/cure may not eliminate the risk as observed in HCV-cured cirrhosis patients who are at risk for nearly a decade.[75] Serum AFP is currently used as an HCC

detection tumor marker, while it is frequently selected as a variable in HCC risk scores. It is empirically known that mild AFP elevation is often observed when hepatic injury and regeneration occur following a transient flare of hepatic inflammation due to active HCV infection even in the absence of HCC. Indeed, AFP elevation can be observed more than a decade before HCC diagnosis.[36, 76] Interestingly, baseline AFP levels decrease along with a resolution of hepatic inflammation after achieving HCV cure, namely SVR, and AFP elevation post-SVR is more specifically associated with HCC risk.[77]

Combinations of clinical variables have been explored to develop NIS/NITs mostly to detect liver disease severity such as fibrosis stage in viral hepatitis and NAFLD.[34] Not surprisingly, some of the NISs/NITs such as the FIB-4 were associated with future HCC risk in retrospective assessment (Table 1). In regional and national NAFLD cohorts, aspartate aminotransferase to platelet ratio index (APRI) and FIB-4 showed the highest association with cirrhosis-related morbidity, including HCC development, among 20 NISs/NITs.[42] Together with the scores specifically developed for HCC risk, the NISs/NITs may enable convenient risk enrichment in large patient population for further biomarker-based risk stratification and/or indication for chemopreventive interventions.

While most of the clinical risk scores were derived from conventional regression modeling, AI/ML/DL-based approaches have also been emerging. In 48,151 patients with HCV cirrhosis, recurrent neural network models outperformed logistic regression-based model in predicting 3-year HCC risk.[78] These promising results demonstrate utility of the new approaches, whereas there are several caveats such as overfitting to specific datasets/cohorts and the black-box nature of the DL/AI models that precludes adjustment guided by human interpretation. To avoid the issues and ensure transparency in model building, reproducible performance, and general applicability of DL/AI-based diagnostic/prognostic models, methodological and reporting guidelines have been developed.[79]

Germline DNA variants

As indicators of genetic susceptibility to HCC, SNPs have been extensively studied in the settings of genome-wide association study (GWAS) or hypothesis-driven single-gene analysis. The major logistical advantages of SNPs include easy access via readily available biospecimens such as buccal swab and the discrete measurement of genotypes less affected by assay conditions.[80] Prevalence of risk alleles/genotypes often varies across patient populations, and therefore may be associated with racial/ethnic and/or other disparities. Vast majority of the SNPs were evaluated in comparison between HCC cases and matched controls, and thus phase 3 validation (i.e., analysis of samples obtained before HCC development) is needed. SNPs in *EGF*, *IFNL3*, and *MICA* genes were associated with viral HCC risk, whereas SNPs in *PNPLA3*, *TM6SF2*, and *HSD17B13* genes were associated mainly with metabolic etiology-related HCC.[81–86] A SNP in *WNT3A-WNT9A* was recently identified for its association with alcohol-related HCC.[87] Despite the logistical advantages, magnitude of HCC risk association for these individual SNPs is generally modest with odds ratio of 1.5 or less. To overcome the limited risk association of single SNP and improve risk enrichment, combinations of multiple SNPs have been explored as polygenic risk scores in HCV-SVR and NAFLD patients.[83, 88] However, a recent

national biorepository-based study reported that additional prognostic information gained by such multi-SNP scores beyond readily available NISs/NITs is likely minimal.[41] This may not necessarily indicate that the SNP-based risk assessment is useless given that information about several confounding factors was not available in the population-based study, but suggest that specific clinical contexts/scenarios should be carefully considered when applying the SNP-based scores to maximize their utility.

Tissue-based molecular HCC risk biomarkers

Tissue transcriptome has been extensively studied as a direct source to interrogate molecular aberrations that drive HCC development.[80] Prognostic Liver Signature (PLS) is an example of hepatic transcriptome signature predictive of long-term HCC risk in all major viral and metabolic HCC etiologies.[89–93] Of note, PLS can be induced by HBV, HCV, ethanol, or free fatty acids in a cell culture model called cell culture-derived PLS (cPLS) for high-throughput drug screening and functional study.[94, 95] Such transcriptomic signatures can capture various types of molecular dysregulations involved in the mechanisms of hepatocarcinogenesis, including hepatic injury and regeneration,[96] HCC-promoting status of hepatic stellate cells,[97–99] and presence of pathogenic histological structures such as ectopic lymphoid structure as a niche supporting malignant transformation.[100]

Tissue-based histopathological HCC risk scores/biomarkers

Histological fibrosis stage is associated with magnitude of future HCC risk, although sampling bias in liver biopsy and low inter-observer agreement impair its reproducibility.[13] Collagen proportionate area based on immunostaining of fibrous tissue enables more robust and quantitative measurement of fibrosis severity and reliable HCC risk estimation.[101] Second harmonic generation/two-photon excitation fluorescence microscopy combined with artificial intelligence enables more precise quantification and characterization of collagen in liver tissue to monitor subtle change in fibrosis,[102] which may refine HCC risk prediction. Infiltrating HCC risk-driving immune cell types, e.g., $CXCR6^+$ $PD\text{-}1^+$ CD8 T cells and $IDO1^+$ conventional dendritic cells, can be conveniently estimated based on tissue transcriptome in NAFLD-affected livers.[37]

Body fluid-based HCC risk biomarkers

Body fluid such as blood, urine, ascites, and bile can serve as windows to detect hepatic or systemic molecular dysregulations associated with HCC risk less invasively compared to liver tissue biopsy. Serum cytokines such as interleukin (IL)-6, IL-17, and IL-27 and serum proteins such as laminin $\gamma 2$ monomer and insulin-like growth factor (IGF)-I were reported as correlates of HCC risk.[103–108] A serum surrogate of tissue-based PLS, Prognostic Liver Secretome signature (PLSec), was developed as a “liquid liver biopsy”, and its combination with AFP (PLSec-AFP) was validated as an etiology-agnostic HCC risk biomarker in cirrhosis from mixed etiologies and HCV-SVR.[36, 109] PLSec-AFP also predicted development of hepatic decompensation in cirrhosis patients.[110] NAFLD-specific “plug-in” module, PLSec-NAFLD, refined HCC risk prediction with the etiology-agnostic PLSec-AFP as a proof of concept of integrative test to optimize prognostic performance according to specific clinical context.[37] Tissue transcriptome signatures can be converted by a generic computational pipeline, TexSEC (www.texsec-app.org), to facilitate development of non-

invasive biomarkers reflecting hepatic tissue-based molecular information.[36, 111] Chemical modifications of serum proteins such as glycomics-based GlycoCirrhoTest represent another type of proteome-based HCC risk biomarker.[112] Metabolomic and lipidomic profiling by mass spectrometry (MS) and/or nuclear magnetic resonance spectroscopy in body fluid samples can also be non-invasive HCC risk biomarkers.[113] Liquid-chromatography-MS analysis identified serum metabolites associated with HCC risk in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort and a Korean prospective cohort. [114, 115]. Plasma phenylalanine and glutamine levels were associated with HCC incidence in Asian patients mainly affected with viral hepatitis.[116] Phenylalanyl-tryptophan and glycocholate were also identified as a serum metabolite biomarker in combination with AFP to detect pre-clinical HCC.[117]

Imaging-based HCC risk scores/biomarkers

The Liver Imaging Reporting and Data System (LI-RADS) category 3 and 4 (LR-3, LR-4) indicate suspicious hepatic nodules with no definite features of HCC, which are observed in one-fourth of the patients enrolled in HCC screening program.[118] Presence of these intermediate lesions is associated with elevated risk of HCC development not necessarily from the index lesions; 32% and 21% of HCC diagnoses following detection of LR-3 and LR-4 lesions were made elsewhere in the liver, respectively.[119, 120] These data suggest that the presence of LR-3/LR-4 legions may have utility for HCC risk stratification. An MRI radiomic feature-based model was developed to predict 3-year HCC risk in HBV cirrhosis patients (AUROC 0.64 in external validation).[121] This study supports radiomics as a promising tool for HCC risk stratification, although its reproducibility across different MRI systems is low.[122] Deep learning model of radiomic elastography features was used to determine liver fibrosis stage in chronic hepatitis B patients.[123] Hepatic venous pressure gradient (HVPG) is an interventional radiology-based measure of liver disease severity, which was correlated with HCC risk.[124] To circumvent the transcatheter-based procedure to measure HVPG, CT-based radiomics model, auto-machine-learning HVPG, was developed to non-invasively detect HVPG ≥ 20 mmHg (AUROC, 0.81 in internal test set).[125] Integrative scores combining imaging modalities and clinical variables/scores have also been actively explored mainly as tools to measure disease severity in NAFLD, and then assessed for risk of developing lethal complications, including HCC. FibroScan-AST (FAST) score was initially developed to detect significant disease activity and fibrosis in NAFLD patients,[126] The score was later shown to be associated with HCC risk in HCV-cured patients, but not in NAFLD patients.[127, 128] Similarly, MRE-FIB-4 (MEFIB) index was developed to estimate fibrosis severity in NAFLD patients, and later was found to be associated with adverse outcomes, including HCC development.[40]

Pathogen-related HCC risk biomarkers

Microbiome in the digestive tract and changes in its composition, namely dysbiosis, are associated with exacerbating or protective effects on liver disease severity and HCC risk via cellular signaling such as toll-like receptor pathway, metabolites, bile acids, fatty acids, lipopolysaccharide, and other biomolecules.[129–131] Several intestinal bacteria such as Enterococcus, Limnobacter, and Phyllobacterium, oral Cyanobacteria, and duodenal Alloprevotella were associated with elevated HCC risk, whereas probiotic bacteria may

attenuate HCC risk.[132–136] These reported HCC risk associations are likely influenced by variations between patient populations defined by dietary habits, host genetics/race, and geographic environmental factors, which need to be addressed before their application as HCC risk biomarkers. History of viral exposure measured by a viral exposure signature was associated with future HCC development.[137, 138]. Genomic integrations of HBV and adeno-associated virus 2 were associated with HCC risk even after seroclearance of hepatitis B surface antigen.[139] These pathogen-related features may serve as a new class of HCC risk biomarkers upon successful high-quality validation.

Environmental exposure-related HCC risk biomarkers

Food contamination with carcinogens such as aflatoxin B1 and aristolochic acid is known to increase HCC risk, not exclusively in developing countries.[2, 140] Several genetic aberrations have been reported as characteristic molecular features of dietary carcinogen exposure such as C>A transversions, hotspot somatic mutations in *TP53*, *ADGRB1*, and *NEIL1* genes, high-level mutation-associated neoantigens, and infiltrating lymphocytes, and PD-L1 over-expression.[141–143] Prevalence of the aflatoxin exposure-related features in HCC patients was 9.8% in China, whereas the prevalence in patients from other regions was 0.4%-3.5%. A mutational signature of aristolochic acid exposure was observed in nearly 80% of Taiwanese HCC patients.[144] Prevalence of the mutational signature of aristolochic acid exposure in HCC patients ranged from 2.7% to 47% in Asia and from 1.7% to 4.8% in North America and Europe. These features may serve as HCC risk biomarkers according to their regional prevalence and magnitude of risk association that influence cost-effectiveness of HCC screening with the assays. The hotspot *TP53* R249S was frequently observed in Hispanic HCC patients in South Texas, but its detection in cfDNA was not useful as HCC risk biomarker.[145]

Therapeutically modifiable HCC risk biomarkers

The HCC risk scores and/or biomarkers may identify at-risk liver disease patients who should be considered for preventive interventions because of elevated HCC risk (prognostic enrichment) and/or anticipated benefit of such intervention (predictive enrichment)[146] (Figure 3A). Many HCC risk scores based on readily available clinical variables (e.g., sex, age) and SNPs will allow convenient and low-cost enrichment of target population for HCC chemopreventive therapies. However, these features are not therapeutically modifiable, and therefore cannot be used to monitor therapeutic response. In contrast, other types of HCC risk biomarkers measuring abundance of functional biomolecules such as transcripts and proteins may enable real-time monitoring of dynamic change in HCC risk status in response to medical interventions. Such biomarkers may allow monitoring of biological response to chemopreventive therapies to gauge therapeutic modulation of HCC risk level in hepatic tissue milieu and/or systemic condition, which is distinct from measuring effect on direct molecular target of the therapy (Figure 3B). If the biomarker measurement is quantitatively correlated with future HCC incidence, the modulation may serve as surrogate biological endpoints in HCC chemoprevention clinical trials to infer anticipated reduction of future HCC incidence (Figure 3C). This is distinct from a surrogate biological endpoint that measures effect of tested agent on direct molecular targets (i.e., on-target effect). Such functional HCC risk biomarkers may resolve the long-standing logistical hurdle for

chemoprevention clinical trials that typically require a large sample size and lengthy follow-up time exceeding the timeframe of typical clinical trials and studies.[13] In a previous HCC chemoprevention trial with S-adenosylmethionine (SAMe) in HCV cirrhosis patients, modulation of AFP was assessed as surrogate endpoint of HCC risk.[147] This trial failed to show decrease of AFP levels, and the concept of surriogate biomarkers for HCC risk is yet to be demonstrated.

Therapeutic modulation of hepatic transcriptome signatures were associated with magnitude of future HCC risk and prognosis in chronic liver disease patients treated with anti-HCV, bariatric surgery, and lipophilic statin.[37, 92, 93, 148] Of note, such transcriptome signatures can be modeled in cell culture model for in vitro high-throughput screening and functional assessment of experimental chemopreventive agents.[94, 95] Similarly, abundance of proteins in blood circulation was associated with reduction of HCC risk level after successful HCV cure by direct-acting antivirals that reflect reduced HCC incidence in subsequent clinical follow-up.[36] These promising observations have led to ongoing and planned HCC chemoprevention clinical trials of various agents using HCC risk biomarkers as surrogate endpoints for HCC incidence (NCT02273362, NCT05028829).

HCC EARLY DETECTION SCORES AND BIOMARKERS

Performance of the current standard-care HCC early detection tests, ultrasound and AFP, is suboptimal and needs improvement. To address the unmet need, new approaches have been explored by developing new biomarkers and imaging techniques integrated with existing tests (Table 2, Supplementary table 2), many of which are under active clinical testing (Table 3).

Clinical HCC tumor markers

AFP is the most commonly used HCC tumor marker currently incorporated in practice guideline-recommended HCC screening protocol.[7] In a recent meta-analysis of phase 2-4 biomarker studies, sensitivity of AFP for early-stage HCC is only 49% with specificity of 88%.[15] AFP can elevate due to non-malignant hepatic inflammation due to chronic hepatitis that limits specificity.[149] In the setting of HCC screening, addition of AFP improved sensitivity of ultrasound for detection of early-stage HCC from 53% to 74%.[15] AFP is the only HCC tumor marker assessed for its survival impact (i.e., phase 5 study) as a part of the recommended HCC screening protocol together with ultrasound.[8] It is ethically infeasible to conduct randomized controlled trial (RCT) comparing HCC screening vs. no screening, but one RCT conducted in China showed a 37% reduction in HCC mortality.[150] AFP-L3% is lens culinaris agglutinin-reactive fraction of AFP, which showed high specificity of 84%-98%, while sensitivity is limited to 13%-49%.[151–154] DCP, also known as protein induced by vitamin k absence or antagonist-II (PIVKA-II), showed similarly suboptimal sensitivity of 64% and specificity of 87% in a meta-analysis of mostly phase 2 studies.[155] In phase 3 studies for early-stage HCC detection, its sensitivity dropped to 12-26%.[154, 156] Given the complementary positivity of these tumor markers, their combination has been explored to improve their performance.[154, 157–159] In phase

3 studies testing their combinations, sensitivity ranged from 31% to 77% and specificity between 66% and 91% for early-stage HCC detection.[154, 159]

HCC risk scores based on tumor markers and clinical variables

Gender, Age, AFP-L3%, AFP, and DCP (GALAD) score was developed by using patient gender, age, AFP, AFP-L3%, and DCP to predict presence of HCC in 833 patients with chronic liver disease in the U.K.[160] Since the initial report, GALAD score has been extensively validated in global viral and metabolic liver disease patients from Germany, Hong Kong, Japan, China, and the U.S.,[161–168] which allowed us to perform meta-analysis by the phase of biomarker development. In meta-analysis of seven phase 2 biomarker studies, sensitivity, specificity, and AUROC for detection early-stage HCC were 69%, 91%, and 0.83, respectively, at the original cutoff of −0.63 (Figure 4, Table 2). In meta-analysis of two phase 3 studies, sensitivity, specificity, and AUROC for detection early-stage HCC were 58%, 83%, and 0.73, respectively, reiterating general limitation of phase 2 studies that can overestimate test performance. Subgroup analysis suggested that the score's performance measured by AUROC is comparable across the HCC etiologies and geographic regions. Despite the superiority to the individual tumor markers, high false-positive rate (14% to 22%) raises concerns of potential harm and cost.[154, 156]. More recent studies have attempted to further improve performance of the score. Longitudinal measurement of GALAD achieved higher sensitivity (69%) compared to cross-sectional single-timepoint measurement (54%).[156] Integration of ultrasound (GALADUS) yielded sensitivity, specificity, and AUROC of 88%, 94%, and 0.97, respectively, for detection of early-stage HCC (Barcelona Clinic Liver Cancer [BCLC] stage 0/A).[165]

Hepatocellular Carcinoma Early Detection Screening (HES) algorithm is another integrative composed of AFP, rate of AFP change within the last year, age, alanine aminotransferase (ALT), platelets, etiology, and interaction terms (AFP and ALT, and AFP and platelets) for HCC diagnosis in 6 months.[169, 170] The HES algorithm has serially validated in multiple phase 2 studies.[169–173] One of the largest studies in 709 patients reported sensitivity of 51% and specificity of 90% for early-stage HCC.[173] Phase 3 studies reported sensitivity ranging from 39% to 42% at fixed specificity of 90%.[154, 156] Its superiority to the GALAD score and individual tumor markers is yet to be conclusively determined.[154, 156]

Doylestown algorithm, comprised of age, gender, log AFP, alkaline phosphatase, and ALT, was developed for HCC detection and validated in serial phase 2 studies.[174, 175] With the addition of polyethylene glycol-precipitated IgG and fucosylated kininogen, a newer version, Doylestown Plus algorithm, was tested in a phase 3 study of 29 HCC patients and 58 matched cirrhosis controls and showed sensitivity of 80% at specificity of 90% and AUROC of 0.92 for early-stage (BCLC stage 0/A) HCC.[175, 176] Larger phase 2 study is ongoing to further validate the algorithm (NCT03878550).

Plasma cell-free DNA (cfDNA) and circulating tumor DNA (ctDNA)

cfDNA/ctDNA are fragmented DNA in circulation that are likely released from and/or associated with HCC cells and therefore may serve as a sensitive measure to detect presence of malignant cell in and/or outside the liver.[18, 177] cfDNA/ctDNA may reflect various

types of biological information from the tumor, and may serve as sensitive tools to non-invasively detect early-stage HCC. Methylated cfDNA/ctDNA is cancer-specific circulating DNA fragments, and represents one of the most advanced types of HCC early detection biomarker toward clinical translation.

A 28-gene (covering 77 CpG sites) methylated cfDNA panel combined with AFP, AFP-L3%, DCP, age, and sex (HelioLiver Test) showed superior sensitivity (76%) for early-stage HCC (AJCC stage I/II) detection compared to AFP alone (57% at cutoff of 20 ng/mL) and the GALAD score (65% at cutoff of −0.63) in a phase 2 study.[178] AUROC for early-stage HCC detection for the HelioLiver Test, AFP, and the GALAD score were 0.92, 0.81, and 0.84, respectively. Another methylated cfDNA markers in three genes (*HOXA1*, *TSPYL5*, and *B3GALT6*) combined with AFP and sex (multi-target HCC blood test [mt-HBT] algorithm) showed sensitivity of 82% for early-stage HCC (BCLC stage 0/A) detection, which was higher than AFP (40%) and GALAD score (71%) in a phase 2 study.[167, 179, 180] AUROC for early-stage HCC detection for the mt-HBT algorithm, AFP, and the GALAD score were 0.92, 0.84, and 0.89 for GALAD, respectively. *SEPT9* is involved in the process of liver carcinogenesis, and its methylation level in cfDNA showed a pooled sensitivity of 80% and specificity of 90% for all-stage HCC detection in a meta-analysis of six case-control studies conducted in Europe, Asia, and the U.S.[181–186] A 32-gene 5-hydroxymethylcytosine (5hmC) markers in cfDNA selected from genome-wide profiling showed AUROCs of 0.85 and 0.92 in detecting early-stage (BCLC stage 0/A) HCC in a phase 2 study Chinese patients with HBV infection or cirrhosis.[187]

HCC-specific somatic DNA mutations in *TP53*, *CTNNB1*, *AXIN1*, and *TERT* promoter, HBV integration breakpoint, combined with serum AFP and DCP (HCCscreen) distinguished 65 HCC patients from 70 HBV-infected patients with AUROC, sensitivity, and specificity of 0.93, 85%, and 93%, respectively, in a phase 2 study.[188] HCCscreen was positive in four patients 6-8 months prior to early-stage HCC diagnosis among 331 HBV-infected patients under HCC screening. Despite this encouraging result, the sample size was small and positive predictive value only 17%, which may cause unnecessary harms from HCC screening.[189] A new approach utilized HCC-specific length of cfDNA fragments from shallow-read whole-genome sequencing data as fragmentomics profile to detect early-stage HCC and intrahepatic cholangiocarcinoma in a phase 1/2 study.[190] Integration of four genomic features (i.e., 5hmC, motif, fragmentation, and nucleosome footprint [HIFI]) yielded AUROC of 0.996 for all-stage HCC detection.[191]

Circulating tumor cell (CTC)

CTC has shown promising capability in prognostication of HCC patients.[192] For HCC screening, sensitivity of CTC count for detecting HCC is low at 60% despite the high specificity of 95% across different CTC platforms.[193] To address the suboptimal sensitivity and overcome technical limitations in CTC enumeration, RNA-based CTC detection methods were proposed and evaluated in phase 2 studies.[194–196] Leveraging a negative enrichment platform with quantitative real-time PCR, an mRNA panel, including *EPCAM*, *THY1* (encoding CD90), *PROM1* (encoding CD133), and *KRT19*, for identifying a CTC subpopulation with stem-like cell features in a large multicenter cohort comprising 1,006

patients.[196] The CTC detection panel distinguished early-stage (BCLC stage 0/A) HCC from cirrhosis and HBV-infected patients with AUROC of 0.93 in a phase 2 study.[196] Of note, the AUROC remained high (0.92) in AFP-negative subgroup. Phase 3 study for CTC-based test is still lacking.

Circulating noncoding RNA (ncRNA), extracellular vesicle (EV)

Non-coding RNA such as microRNA (miRNA), long non-coding RNA (lncRNA), and circular RNA (circRNA) are regulatory RNA species involved in a wide variety of biological processes in HCC.[197] A comprehensive miRNA profiling in serum samples from 345 HCC, 139 chronic hepatitis or cirrhosis, and 1,033 non-cancer controls derived an 8-miRNA panel, which demonstrated sensitivity of 98% for detecting early-stage HCC, outperforming sensitivity of AFP (59%) and DCP (40%), in this phase 1/2 study.[198] In patients with chronic hepatitis B, a combination of *miR-10a* and *miR-125b*,[199] and *lncRNA-AF085935*[200, 201] were suggested as potential HCC detection biomarkers, which showed AUROC of 0.99 and 0.81-0.86, respectively, for detecting all-stage HCC. A large multi-center Chinese study proposed a 3-circRNA panel (CircPanel) for screening HBV-related HCC.[202] CircPanel showed superior performance in detecting small HCC (single and ≤ 3 cm) in three independent cohorts of HBV-related cirrhosis and chronic hepatitis patients (AUROC, 0.81 to 0.87) compared to AFP (0.65 to 0.73), which was maintained in AFP-negative cases.

Extracellular vesicles (EVs), lipid bilayer-enclosed particles released from tumor and normal cells, can serve as cargos for various biomolecules, including mRNA, ncRNA, proteins, and lipids.[203] *LINC00853* and *miR-10b-5p* were upregulated in HCC tissues and EVs with AUROC of 0.96 and 0.94 for single small (< 2 cm) HCC, respectively[204, 205] EV-derived *LINC00853* was detectable in 97% of AFP-negative HCC patients. Whole RNA sequencing of EVs identified three small RNA clusters (smRC) specific to HCC, which showed sensitivity, specificity, and AUROC of 86%. 91%, and 0.87, respectively in 105 early-stage (BCLC stage 0/A) HCC and 85 chronic liver disease patients.[206] Microarray-based screening identified three lncRNAs, and their combination with AFP yielded AUROC of 0.87, although half the HCC cases were advanced metastatic disease.[207] An HCC-specific 10-EV-mRNA panel was identified by using microfluidics combination with reverse-transcription droplet digital PCR yielded sensitivity, specificity, and AUROC of 94%, 89%, and 0.93, respectively in 36 early-stage (BCLC stage 0/A) HCC patients and 26 cirrhosis controls.[208] EV-lipidome biomarkers were identified by using ultra-high-resolution mass spectrometry to distinguish HCC and cirrhotic patients.[209] A recent phase 2 study showed that HCC EV ECG score based on $EpCAM^+$ $CD63^+$, $CD147^+$ $CD63^+$, and $GPC3^+$ $CD63^+$ HCC EVs, yielded AUROCs of 0.95 and 0.93 for early-stage HCC (BCLC stage 0/A) detection in the training and validation cohorts, respectively.[210] EV-based biomolecules may have a potential role in HCC early detection, although its clinical assessment is still in early phase. These promising results warrant subsequent larger phase 2 studies.

Serum protein biomarkers

Several serum protein biomarkers, e.g., Golgi protein 73, osteopontin, glypican-3, midkine, and aldo-keto reductase family 1 member 10, have been evaluated as HCC early detection biomarkers in phase 2 studies and their meta-analysis.[211–220] Their performance is generally limited at least as single biomarkers, and the previous studies have failed to demonstrate superiority and/or additive benefit to the current standard-care tumor marker, AFP. HCC-associated autoantibodies represent an alternative serum protein-based approach to identify early-stage HCC. In a phase 1/2 comprehensive seromic survey, 7-autoantibody panel was developed and validated in a large multi-center cohort, showing sensitivity, specificity, and AUROC of 70%, 91%, and 0.88, respectively, for detection of early-stage (BCLC stage 0/A) HCC.[221]

Urine-based HCC early detection biomarkers

Urine is another type of biospecimen even more accessible, i.e., less invasively obtainable, than blood. In a phase 1/2 multi-center study, a urine ctDNA panel of *TP53* mutation and two methylation markers, *mRASSF1A* and *mGSTP1* was tested in 279 chronic hepatitis B, 144 cirrhosis, and 186 HCC patients.[222] This ctDNA panel alone did not outperform AFP, showing AUROC of 0.74 and 0.85, respectively. However, when two-step strategy was applied, i.e., AFP was first applied and then the ctDNA panel was used in patients with AFP < 20 ng/mL, AUROC was improved to 0.91. Sensitivities of detecting BCLC stage 0 and A HCC tumors with this two-step strategy were 92% and 77%, respectively, at fixed specificity of 90%. Elevated levels of urine *miR-93-5p* showed AUROC of 0.90 in detecting early-stage HBV-related HCC, although it is likely over-estimated performance given that the controls were heathy subjects.[223] Surface-enhanced Raman spectroscopy (SERS) is a highly sensitive technique to detect low-abundant biomolecules.[224] SERS combined with support vector machine algorithm applied to urine samples yielded sensitivity of 80% and specificity of 76% for detection of all-stage HCC in 55 HCC and 49 cirrhosis controls.[225] Given the logistical advantage in sample accessibility, urine will remain a promising source of molecular information for HCC early detection.

Imaging-based HCC early detection tests

MRI with multi-phase gadoxetic acid enhancement is a standard-care test for HCC diagnostic (not early detection).[6] This full MRI study shows obviously superior sensitivity (85%) compared to ultrasound (27%) for detection of early-stage HCC, but is too costly and logistically demanding as a test repeatedly applied at regular interval (i.e., 6 months) for HCC screening.[226] To leverage the performance of MRI with limited costs and procedural requirements, abbreviated MRI (AMRI) has been actively explored to develop protocol tailored as an HCC screening test.[227, 228] AMRI protocols can be classified into three types: non-contrast-enhanced, hepatobiliary contrast-enhanced, and dynamic extracellular contrast-enhanced AMRI.[229] The overall patient-level sensitivity and specificity of AMRI for HCC detection were 86% and 94%, respectively, in a meta-analysis regardless of the AMRI type, presence of cirrhosis, and HCC etiology.[227] Of note, sensitivity for BCLC stage 0 HCC significantly dropped to 69%.[227] In a prospective study directly comparing non-contrast-enhanced AMRI and ultrasound for HCC detection in 192 patients with chronic

liver disease, sensitivity of AMRI for detecting six HCC patients was inferior to ultrasound (83% vs. 100%), although the sample size is too small to make a conclusive statement.[230] Another prospective study of 382 cirrhosis patients reported that non-contrast-enhanced AMRI had better patient-level sensitivity and specificity for HCC detection compared to ultrasound (sensitivities of 79% vs. 28% and specificities of 98% vs. 94% for AMRI vs. ultrasound, respectively).[231] These inconsistent findings may be attributable to variations in liver disease severity and/or etiology as well as other clinical confounders that affect baseline HCC risk. In addition, these studies were conducted in patients mostly affected with HBV and HCV infection, and the performance in patients with NAFLD and alcohol-associated liver disease is yet to be determined in ongoing studies.

CT with multi-phase dynamic iodinated contrast enhancement is another standard-care HCC diagnostic test.[6] Given the radiation exposure, CT is not generally considered as an HCC screening test that is applied every 6 months. In a single-center RCT in 163 compensated cirrhosis patients, annual triple-phase CT and biannual ultrasound showed similar sensitivities of 67% and 71%, specificities of 94% and 98%, and early-stage HCC detection rates of 63% vs. 56%, respectively.[232] To mitigate potential harms from radiation exposure, a prospective study compared biannual dual-phase low-dose CT (LDCT) and ultrasound in 137 chronic liver disease patients.[233] In this relatively small study, the dual-phase LDCT had better sensitivity in detecting all-stage HCC and BCLC-stage 0 HCC than ultrasound (83% and 29% for all-stage HCC; 82% and 18% for BCLC stage 0 HCC, respectively), suggesting potential utility of LDCT for HCC screening.

Contrast-enhanced ultrasound (CEUS) using microbubble-based agents enables assessment of vascularity for focal liver lesions for improved early-stage HCC detection.[234] A prospective intra-individual comparison was conducted to evaluate added value of CEUS to conventional B-mode ultrasound for HCC detection in 524 patients with predominantly HBV-related cirrhosis.[235] There was no significant improvement in detecting any stage or early-stage HCC with CEUS, whereas the false referral rate for definite diagnosis was significantly lower in the CEUS group. On the other hand, a multi-center RCT enrolling 622 HCV- or HBV-infected cirrhosis patients found that CEUS-based screening had a higher sensitivity for HCC detection than conventional B-mode ultrasound (100% and 65%, respectively).[236] In addition, observed HCC size detected by CEUS was significantly smaller compared to conventional ultrasound in all patients and HCV-infected subgroup (all patients: 13.0 mm vs. 16.7 mm; HCV subgroup: 12.7 mm vs. 17.6 mm). Further studies will be needed to determine utility/role of CEUS in HCC in early HCC detection.

FUTURE DIRECTIONS AND CONCLUSIONS

The evolving landscape of HCC etiology, particularly the global rise of NAFLD/MAFLD, continues to hamper the development of effective HCC screening strategy. HCC risk post HCV cure remains high for nearly a decade when cirrhosis is present, and therefore requires HCC screening.[75] Alcohol-associated liver disease stays as a major HCC etiology with notable inter-individual heterogeneity.[237] With the etiological landscape, precision and practical feasibility will need to be carefully balanced to ensure clinically acceptable costs and complexity for actual clinical translation and implementation of the risk-stratified

HCC screening strategy. Prospective biorepositories and clinical databases representing global liver disease patient population will enhance and facilitate evaluation of clinical utility for promising biomarkers across geographic regions under the PRoBE principle. Innovative clinical trial design will also expedite the sequence of validations and help timely translation of the biomarkers. Predefined framework will be needed to measure net benefit of the screening intervention in controlling HCC burden and mortality at population level. Future research will also explore use of the biomarkers and assay technologies beyond the scope of HCC screening, including assessment of therapeutic effect, monitoring of residual disease after treatment, and prediction of recurrence or progression following surgical or medical therapies. Collectively, these developments are expected to lead to a transformative improvement of HCC mortality over the next decade.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

HCC	hepatocellular carcinoma
HBV	hepatitis B virus
HCV	hepatitis C virus
NAFLD	non-alcoholic fatty liver disease
MAFLD	metabolic dysregulation-associated fatty liver disease
AFP	alpha-fetoprotein
CT	computed tomography
MRI	magnetic resonance imaging
SVR	sustained virologic response
DCP	des-gamma-carboxy prothrombin
PLSec	Prognostic Liver Secretome Signature
NIS	Non-invasive score
NIT	non-invasive test

FIB-4	fibrosis-4
SNP	single nucleotide polymorphism
AUROC	area under receiver operating characteristic
PLS	Prognostic Liver Signature
HVPG	hepatic venous pressure gradient
GALAD	Gender, Age, AFP-L3%, AFP, and DCP
BCLC	Barcelona Clinic Liver Cancer
cfDNA	cell-free DNA
ctDNA	circulating tumor DNA
CTC	circulating tumor cell
ncRNA	noncoding RNA
AMRI	abbreviated MRI
CEUS	contrast-enhanced ultrasound

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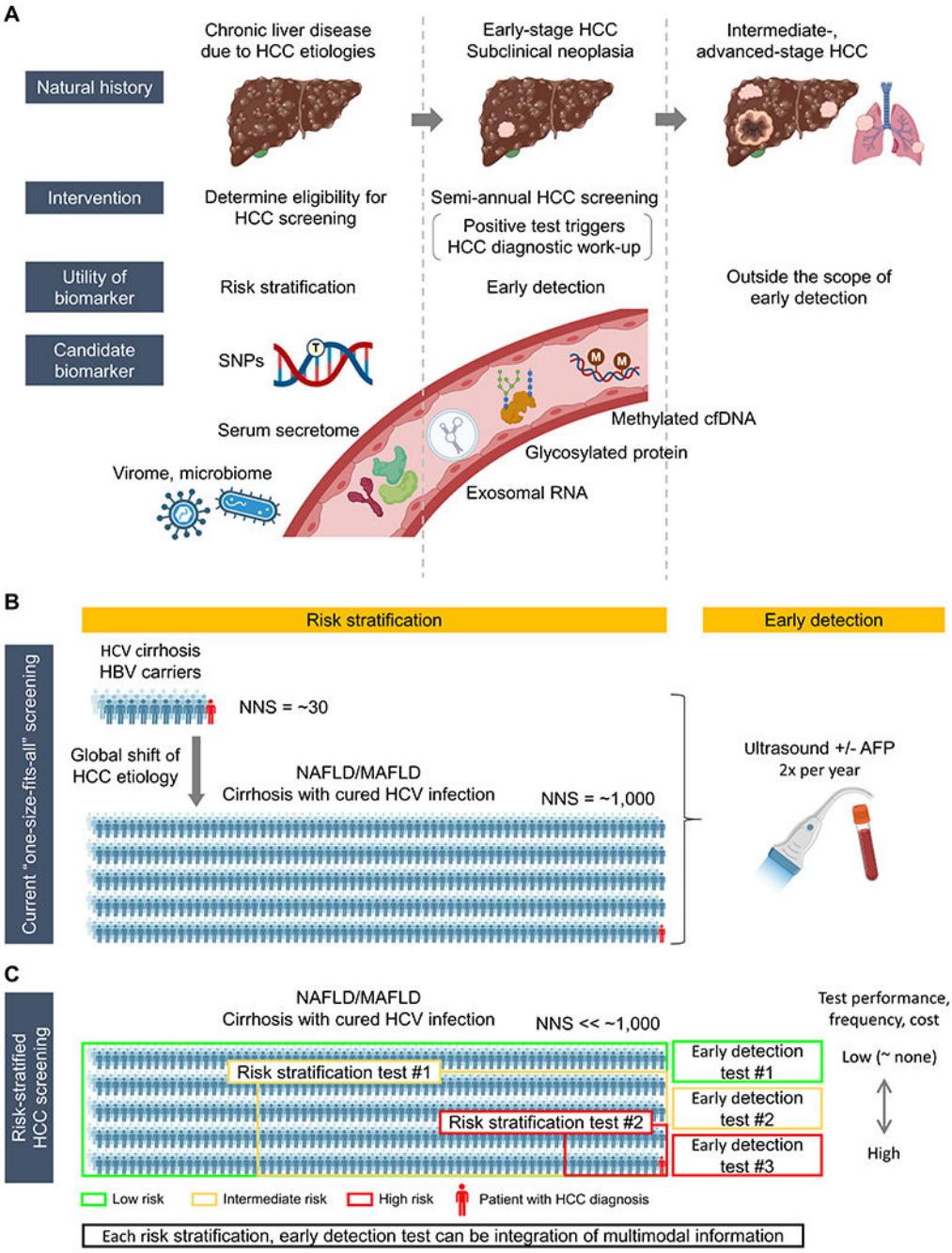

Figure 1.

Conceptual framework and clinical implementation strategies of biomarker-guided precision HCC screening. (**A**) HCC risk stratification and early detection along the natural history of HCC development and progression. Risk stratification is the first step to identify specific patient population with elevated HCC risk (left). Subsequently, to the high-risk population, repeated HCC detection tests are applied at regular interval for diagnosis of early-stage HCC (middle). Intermediate- to advanced-stage HCC is theoretically outside the concept of HCC screening for early detection (right). New early detection biomarkers should achieve higher

sensitivity compared to the current modalities, while maintaining a high specificity, ideally in less-invasively accessible biospecimens. Anticipated high sensitivity of the early detection biomarkers may lead to detection of subclinical neoplasia which is not recognizable with the current diagnostic tools such as contrast-enhanced dynamic MRI (i.e., false negative biomarker test based on MRI as goldstandard). Specific recall policies need to be developed according to confirmed association of the detection with subsequent HCC diagnosis. (**B**) Global shift of HCC etiology from viral to metabolic liver diseases over the past decade and accompanying drastic increase of the number needed to screen (NNS) for the current "one-size-fits-all" HCC screening. (**C**) Risk stratification by stepwise application of integrative HCC risk biomarkers to identify high-risk patients to focus the effort and resource of HCC screening. Tailored HCC detection tests are regularly applied according to predicted HCC risk by altering intensity of screening. Both HCC risk stratification biomarkers and early detection biomarkers can be integration of multimodal information, e.g., clinical, molecular and/or imaging features.

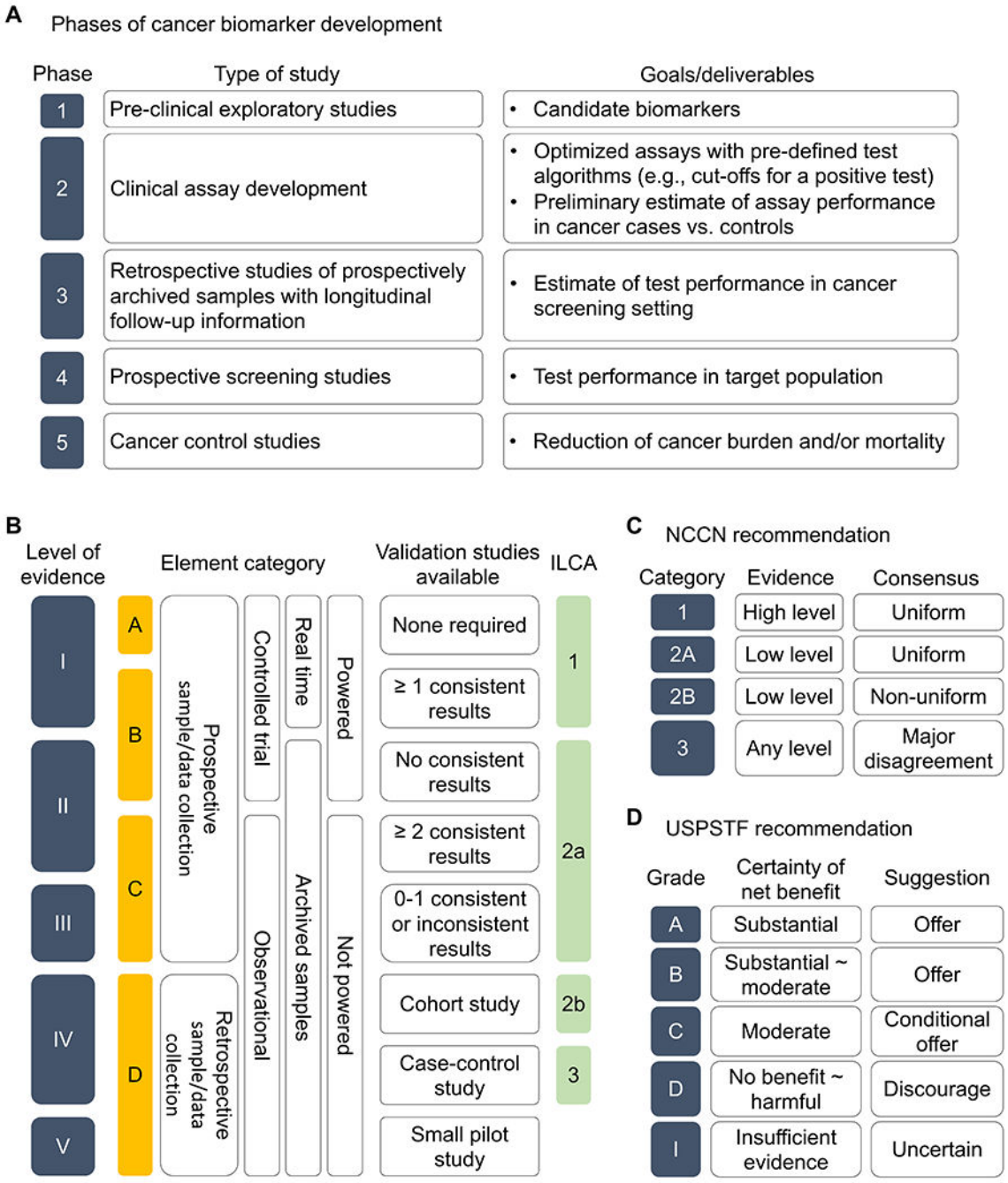

Figure 2.

(**A**) Phases of cancer screening biomarker development.[54] (**B**) Levels of evidence (LOE) for cancer screening biomarkers, defined based on the element category and status of validation studies are determined according to the study design elements.[55, 238] Correspondence to the LOE defined in the International Liver Cancer Association (ILCA) white paper[239] is shown. (**C**) Categories of recommendation for clinical implementation by the National Comprehensive Cancer Network (NCCN) according to the levels of scientific evidence and consensus among the NCCN expert panel. (**D**) Grades of recommendation for

clinical implementation by the U.S. Preventive Services Task Force (USPSTF) according to certainty of net benefit for preventive intervention.

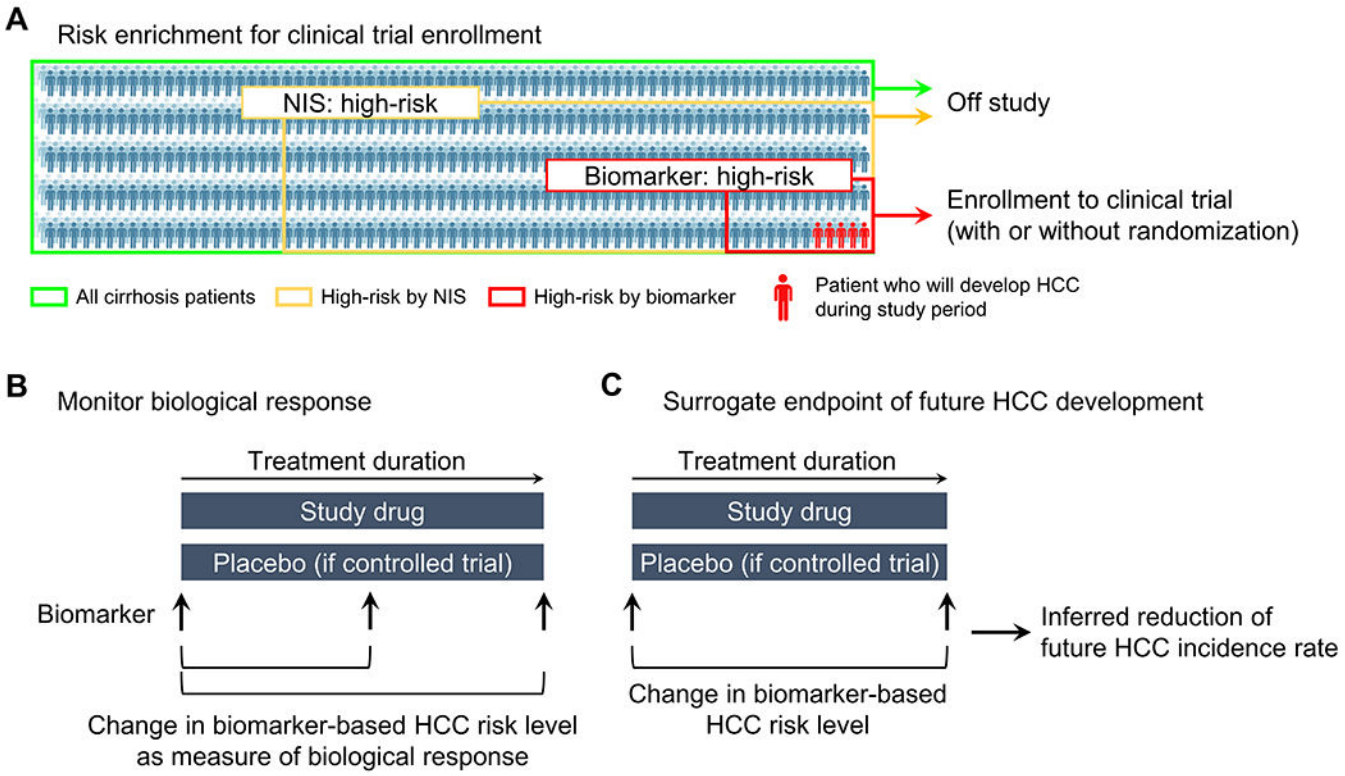

Figure 3.

Potential use of HCC risk biomarkers in chemoprevention clinical trials. (**A**) Risk enrichment to select participants to be enrolled in chemoprevention clinical trials. Stepwise approach can be employed to identify super high-risk subgroup to increase HCC incidence rate for detection of chemopreventive effect in shorter time period with smaller sample size compared to conventional all-comer enrollment.[92] (**B**) Use of therapeutically modifiable HCC risk biomarker to monitor effect of experimental intervention on quantitative molecular HCC risk level. (**C**) Use of therapeutic modulation of HCC risk biomarker as a surrogate endpoint to estimate reduction of future HCC incidence.

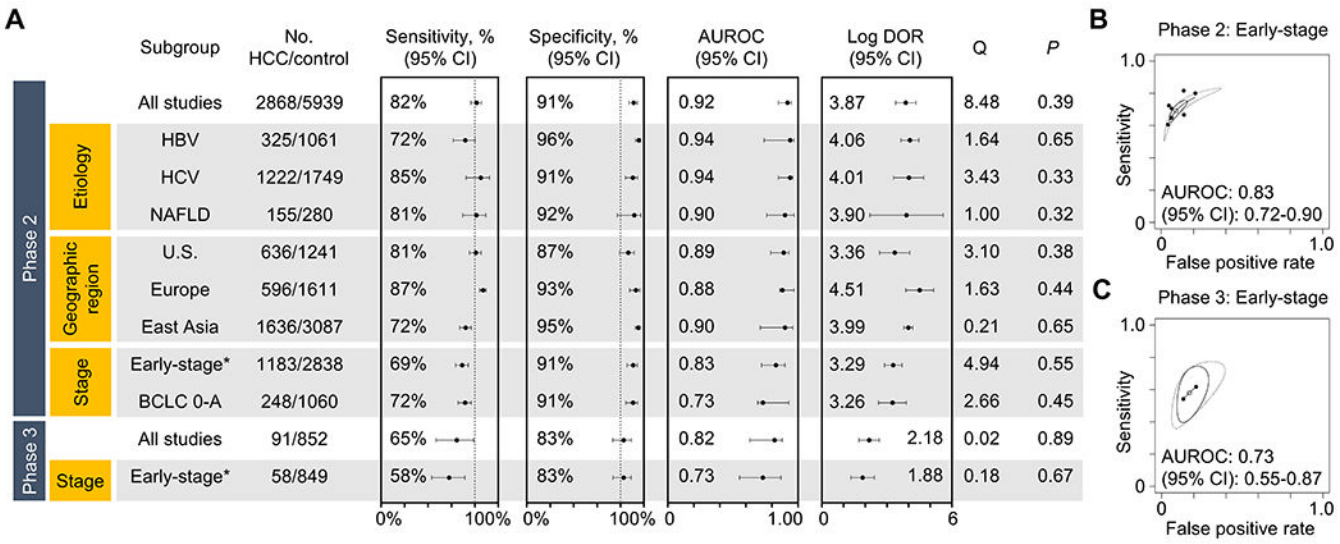

Figure 4.

Performance of the GALAD score according to clinical subgroups and the phase of cancer screening biomarker study (meta-analysis). (**A**) Sensitivity, specificity, AUROC, and log diagnostic odds ratio (DOR) by clinical subgroups defined by HCC etiology, geographic region, and HCC stage. (**B**) Summary ROC curves for early-stage HCC in phase 2 (upper panel) and 3 (lower panel) studies are separately presented. DerSimonian and Laird random-effect method was used for the meta-analysis, and heterogeneity was assessed by Cochrane's Q statistic. See Table 2 and Supplementary table 3 for details of the individual studies used for the meta-analysis.

Table 1.

C risk stratification scores and biomarkers (with independent validation).

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
Clinical NIS/NIT	aMAP risk score	3	II/2a	Age, sex, albumin-bilirubin, platelets	Cohort	Prospective-retrospective	Development (3/5y)	HBV, HBV on NA, HCV, HCV post-SVR, alcohol	International; UK; Egypt; Japan; China; Egypt; Australia, UK	3,688 + 13,686; 2,139 + 606; 2.085; 1,113; 1,042; 3,075; 269	Asian, Caucasian, Black	11% + 27%; 100% + 100%; 100% (F3-4); 100%; 66%; 100% (F3-4); 100%	In independent studies	[73, 74, 240–243]
	ADRESS-HCC	3	II/2a	Age, sex, diabetes, race, etiology, Child-Pugh score	Cohort	Prospective-retrospective	Development (1y)	HCV, alcohol, NASH, HBV, other	U.S., China	17,124 + 17,808 + 1,050	Caucasian, Hispatnic/ Latino, Black, Asian	100% + 100% + 100%	Within the study	[244]
	LCR1-LCR2	3	II/2a	Age, sex, apolipoprotein A1, haptoglobin, GGT, alpha2-macroglobulin	Cohort	Prospective-retrospective	Development	HCV, HBV	France; Europe, Asia, Africa; Europe, Asia, Africa	4,944 + 4,948; 4,903; 3,520	Caucasian, Asian, Black	15% + 14%; 22%; 9%	In independent studies	[43, 245, 246]
	CU-HCC	3	II/2a	Age, albumin, bilirubin, HBV-DNA, cirrhosis	Cohort	Prospective-retrospective	Development (5y)	HBV	Hong Kong; Korea; Korea; Canada.; Hong Kong; Korea; U.S.	1,005 + 424; 1,308; 1,330; 2,105; 1,531; 1,092; 3,101	Asian, Caucasian, Black	38% + 16%; 18%; 25%; 22%; 18%; 37%; 32%	In independent studies	[247–253]
	REACH-B	3	II/2a	Age, sex, ALT, HBeAg, HBV-DNA	Cohort	Prospective-retrospective	Development (3/5/10y)	HBV	Taiwan; Korea; Korea; Canada; Hong Kong; Korea	3,584 + 1,505; 1,308; 1,241; 2,105; 1,531; 1,092	Asian, Caucasian	0% + 18%, 18%, 25%, 22%, 37%	In independent studies	[248–252, 254]
	GES score	3	II/2a	Age, sex, fibrosis stage, albumin, AFP	Cohort	Prospective-retrospective	Development (1/2/3y)	HCV post-SVR with DAA	Egypt; Egypt; International	2,372 + 687 + 1,341; 3,075; 12,038	n.a.	100% + 100% + 100% (all F3-4); 100%	In independent studies	[242, 255–257]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
												(F3-4); 44%		
	REACH-B2	3	III/2a	Age, sex, ALT, family history of HCC, HBeAg, HBV-DNA, HBsAg, Genotype	Cohort	Prospective-retrospective	Development (5/10/15y)	HBV	Taiwan	3,340 (2 : 1 for training and validation)	Asian	0%	Within the study	[258]
	UM regression model	3	III/2a	Machine-learning (23 clinical variables)	Cohort	Prospective-retrospective	Development (3/5y)	HCV, cryptogenic, alcohol, other	U.S.	442 + 1,050	Caucasian, Black, Hispanic	100% + 41%	Within the study	[259]
	Hung *et al*	3	III/2a	Age, sex, ALT, previous liver disease, history of HCC, smoking, HBV/HCV infection	Cohort	Prospective-retrospective	Development (3/5/10y)	HBV, HCV	Taiwan	8,252 + 4,125	n.a.	n.a.	Within the study	[260]
	LSM-HCC	3	III/2a	Age, LSM, albumin, HBV-DNA	Cohort	Prospective-retrospective	Development (3/5y)	HBV	Hong Kong; Korea; Korea	1,035 + 520; 1,308; 1,241	Asian	32% + 31%; 18%; 24%	In independent studies	[248, 261, 262]
	NGM1/HCC	3	III/2a	Age, sex, family history of HCC, alcohol, ALT, HBeAg	Cohort	Prospective-retrospective	Development (5/10y)	HBV	Taiwan; Canada	2,435 + 1,218; 2,105	Asian, Caucasian	n.a.; 25%	In independent study	[250, 263]
	RWS-HCC	3	III/2a	Age, sex, cirrhosis, AFP	Cohort	Prospective-retrospective	Development	HBV	Singapore; U.S.	538 + 3,353; 3,101	Asian, Caucasian, Black	15% + n.a.; 32%	In independent study	[253, 264]
	GAG-HCC	3	III/2a	Age, sex, HBV-DNA, core promoter mutations, cirrhosis	Cohort	Prospective-retrospective	Development (5/10y)	HBV, HBV on NA	Taiwan; Korea; Korea; Taiwan; Hong Kong; Korea; Japan; Korea; Canada	820; 1,330; 3,001; 1,325; 1,531; 1,308; 225; 1,092; 2,105	Asian, Caucasian	15%; 46%; 19%; 36%; 22%; 18%; 26%; 37%; 25%	In independent studies	[248–252, 265–268]
	REVEAL-HCV	3	III/2a	Age, ALT, AST/ALT ratio, HCV-RNA, cirrhosis, HCV genotype	Cohort	Prospective-retrospective	Development (5/10/15y)	HCV	Taiwan	1,095 + 572	n.a.	1% + 7%	Within the study	[269]
	Ganne-Carri *et al*	3	III/2a	Age, alcohol, platelets, GGT, SVR	Cohort	Prospective-retrospective	Development (1/3y)	HCV, HCV post-SVR	France; Switzerland, Belgium	720 + 360; 192	Caucasian	100% + 100%; 100%	In independent study	[270, 271]
	Semmler *et al.*	3	III/2a	Age, albumin, LSM, AFP, alcohol consumption	Cohort	Prospective-retrospective	Development (4y)	HCV post-SVR with DAA	Austria, Spain	475 + 1,500	Caucasian	100% +100% (F3-4/	Within the study	[77]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
												HVPG≥6 mmHg/LSM ≥10 kPa)		
	Pons *et al.*	3	III/2a	albumin, LSM	Cohort	Prospective-retrospective	Development (1y)	HCV post-SVR with DAA	Spain	290 + 282	Caucasian	100% + 100% (LSM ≥10 kPa)	Within the study	[272]
	FIB-4	2	IV/2b	FIB-4 (AST, ALT, platelets, age)	Cohort	Retrospective	Development	HBV, HCV, alcohol, NAFLD	Korea; Italy; Korea; Germany; Japan	986; 4,492; 6,661; 29,999; 3,823	Asian, Caucasian	9%; n.a.; n.a.; n.a.; n.a.	In independent studies	[273–277]
	THRI	2	IV/2b	Age, sex, etiology, platelets	Cohort	Retrospective	Development (5/10y)	HCV, HBV, steatohepatitis, PBC, AIH	Canada, Netherlands; China; Turkey; Sweden	2,079 + 1,144; 2,836; 1,287; 2,491	Asian, Caucasian	100% + 100%; 100%; 100%; 100%	In independent studies	[72, 278–280]
	Hughes *et al*	2	IV/2b	AFP	Cohort	Retrospective	Development	HCV, HBV	Japan, Scotland	3,450 + 4,754	Asian, Caucasian	n.a.	Within the study	[35]
	AGED	2	IV/2b	Age, sex, HBeAg, HBV-DNA	Cohort	Retrospective	Development	HBV	China	628 + 1,663	Asian	0% + 0%	Within the study	[281]
	D^2AS risk score	2	IV/2b	Age, sex, HBV-DNA	Cohort	Retrospective	Development (3/5y)	HBV	Korea	971 + 507	Asian	0% + 0%	Within the study	[282]
	PAGE-B	2	IV/2b	Age, sex, platelets	Cohort	Retrospective	Development (5y)	HBV, HBV under NA	Europe; Korea; Hong Kong; Turkey; U.S.	1,325 + 490; 1,330; 32,150; 647; 3,101	Caucasian, Asian, Black	20% + 48%; 46%; 14%; 9%; 32%	In independent studies	[249, 253, 283–285]
	Modified PAGE-B	2	IV/2b	Age, sex, platelets, albumin	Cohort	Retrospective	Development (5y)	HBV on NA	Korea; Korea; Turkey; U.S.	2,001 + 1,000; 3,171; 647; 3,101	Asian, Caucasian, Black	19% + 20%; 33%; 9%; 32%	In independent studies	[253, 266, 285, 286]
	CAGE-B	2	IV/2b	Age, cirrhosis	Cohort	Retrospective	Development	HBV on NA	Europe; Korea; Korea; Korea	1,427; 1,763; 1,557; 734	Caucasian, Asian	26%; 37%; 28%; 47%	In independent studies	[287–290]
	SAGE-B	2	IV/2b	Age, LSM	Cohort	Retrospective	Development	HBV on NA	Europe; Korea; Korea; Korea	1,427; 1,763; 1,557; 734	Caucasian, Asian	26%; 37%; 28%; 47%	In independent studies	[287–290]
	Modified REACH-B	2	IV/2b	Age, LSM, sex, ALT, HBeAg	Cohort	Retrospective	Development	HBV on NA	Korea; Korea	192; 1,308	Asian	40%; 18%	In independent study	[248, 291]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
	HCC-RESCUE	2	IV/2b	Age, sex, cirrhosis	Cohort	Retrospective	Development	HBV on NA	Korea; Korea; Turkey; U.S.	990 + 1,071; 3,171; 647; 3,101	Asian, Caucasian, Black	61% + 65%; 33%; 9%; 32%	In independent studies	[253, 285, 286, 292]
	CAMPAS model score	2	IV/2b	Age, sex, cirrhosis, platelets, albumin, LSM	Cohort	Retrospective	Development (7y)	HBV on NA	Korea	1,511 + 252	Asian	40% + n.a.	Within the study	[293]
	GBM-based model	2	IV/2b	Age, sex, cirrhosis, platelets, ETV or TDF, ALT, HBV-DNA, albumin, bilirubin, HBeAg	Cohort	Retrospective	Development	HBV on NA	Korea, Greece, Italy, German	6,051 + 5,817 + 1,640	Asian, Caucasian	50% + 35% + 27%	Within the study	[294]
	ALT flare	2	IV/2b	ALT	Cohort	Retrospective	Development	HBV on NA	China, U.S.	8,152 + 4,893	Asian, Caucasian	18% + 17%	Within the study	[294]
	REAL-B	2	IV/2b	Age, sex, alcohol, diabetes, cirrhosis, platelets, AFP	Cohort	Retrospective	Development (3/5/10y)	HBV on NA	U.S., Asia-Pacific; U.S.	5,365 + 2,683; 3,101	Asian, Caucasian, Black	20% + 22%; 32%	In independent study	[253, 295]
	AASL-HCC score	2	IV/2b	Age, sex, albumin, cirrhosis	Cohort	Retrospective	Development (3/5y)	HBV on NA	Korea; U.S.	944 + 298; 3,101	Asian, Caucasian, Black	39% + 39%; 32%	In independent study	[253, 296]
	CAMD score	2	IV/2b	Age, sex, cirrhosis, diabetes	Cohort	Retrospective	Development (1/2/3y)	HBV on NA	Taiwan, Hong Kong; Korea; U.S.	23,851 + 19,321; 3,277; 3,101	Asian, Caucasian, Black	26% + 7%; 32%; 32%	In independent studies	[253, 297, 298]
	APA-B	2	IV/2b	Age, platelets, AFP	Cohort	Retrospective	Development	HBV on NA	Taiwan; U.S.	883 + 442; 3,101	Asian, Caucasian, Black	36% + 37%; 32%	In independent study	[253, 267]
	HCC-SVR score	2	IV/2b	Sex, FIB-4, AFP	Cohort	Retrospective	Development	HCV post-SVR	Korea; Egypt	669 + 524; 3,075	Asian	17% + 21%; 66%	In independent study	[242]
	ADRES score	2	IV/2b	Sex, SVR24, FIB-4, AFP	Cohort	Retrospective	Development (1/2y)	HCV post-SVR with DAA	Japan; Egypt	484 + 585; 3,075	Asian	n.a.; 66%	In independent study	[242, 299]
	HEPATHER HCC score	2	IV/2b	Age, sex, HCV Genotype, hypercholesterolemia, albumin, bilirubin, esophageal varices, FIB-4	Cohort	Retrospective	Development	HCV post-SVR with DAA	France, Egypt	3,531 + 3,075	n.a.	69% + 100% (all F3-4)	Within the study	[300]
	Watanabe *et al.*	2	IV/2b	Sex, FIB-4, albumin	Cohort	Retrospective	Development (1/2y)	HCV post-SVR with DAA	Japan; Egypt	1,174; 3,075	Asian	n..a.; 100% (F3-4)	In independent study	[242, 301]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
	Alonso López *et al.*	2	IV/2b	2 models: albumin, LSM, 1y-ΔLSM; albumin, FIB-4, 1y-FIB-4, 1y-GGT	Cohort	Retrospective	Development	HCV post-SVR with DAA	Spain; Egypt	993; 3,075	Caucasian	100% (F3-4/LSM >9.5 kPa) + 100% (F3-4)	In independent study	[242, 302]
	Tani *et al.*	2	IV/2b	Age, AFP	Cohort	Retrospective	Development	HCV post-SVR with DAA	Japan; Egypt	1,088; 3,075	Asian	18%; 100% (F3-4)	In independent study	[242, 303]
	Abe *et al.*	2	IV/2b	ALBI score, platelets, diabetes	Cohort	Retrospective	Development (1/2/3/4y)	HCV post-SVR with DAA	Japan; Egypt	188; 3,075	Asian	100%; 100% (F3-4)	In independent study	[242, 304]
	Hu *et al.*	2	IV/2b	Age, bilirubin, AFP, SVR, cirrhosis	Cohort	Retrospective	Development	HCV, HCV post-SVR	Taiwan; Egypt	665 + 78; 3,075	Asian	28% + 29%; 100% (F3-4)	In independent study	[242, 305]
	Sinn *et al.*	2	IV/2b	Age, sex, smoking, diabetes, total cholesterol, ALT	Cohort	Retrospective	Development (10y)	non-HCV, non-HBV, non-alcohol	Korea	467,206 + 91,357	Asian	n.a., general population	Within the study	[306]
SNP	Genetic risk score	3	III/2a	SNPs of *PNPLA3, TM6SF2, HSD17B13*	Cohort	Prospective-retrospective	Developement	General population	Denmark, UK	110,761 + 334,691	Caucasian	0.4% + 0.1%	Within the study	[88]
	Genetic and Metabolic Staging (GEMS) scoring	3	III/2a	SNPs of P*NPLA3, TM6SF2, HSD17B13*, age, diabetes, platelets, HDL, albumin	Cohort	Prospective-retrospective	Liver related event (HCC + liver decompensation)	NAFLD	Germany, UK	546 + 303,075	Caucasian	100% + n.a.	Within the study	[307]
	EGF	2	n.a.	*EGF* 61AG (rs4444903, A>G)	Meta-analysis of 16 case-control studies	Retrospective	Presence	HBV, HCV	France, Italy, China, Egypt, Japan, U.S.	2,475 : 5,381	Asian, European, Black	n.a.	In independent studies	[81]
	IFNL3	2	n.a.	IFNL3 (rs12979860: C>T, rs8099917: T>G)	Meta-analysis of 24 case-control studies	Retrospective	Presence	HBV, HCV, HCV post-SVR	China, Japan	4,212 : 5,489	Asian, European	n.a.	In independent studies	[82]
	MICA	2	n.a.	MICA (rs2596542, C>T)	Meta-analysis of 11 case-control studies	Retrospective	Presence	HCV	Japan, China, Switzerland, Italy, Egypt, Taiwan, Vietnam	4,678 : 16,867	Asian, European	n.a.	In independent studies	[308]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
	KIF1B or 1p36.22	2	n.a.	KIF1B or 1p36.22 (rs17401966, A>G)	Meta-analysis of 19 case-control studies	Retrospective	Presence	HBV	China, Japan, Thailand	8,741 : 10,812	Asian	n.a.	In independent studies	[309]
	STAT4	2	n.a.	STAT4 (rs7574865, G>T)	Meta-analysis of 7 case-control studies	Retrospective	Presence	HBV	China, Vietnam, Korea, Thailand	2,028 : 9,388	Asian	n.a.	In independent studies	[310]
	PNPLA3	2	n.a.	PNPLA3 (rs738409: C>G)	Meta-analysis of 6 case-control studies	Retrospective	Presence	NAFLD, alcohol, HCV	Europe, Japan	544 : 1,543	European	n.a.	In independent studies	[311]
	TM6SF2	2	n.a.	TM6SF2 (rs58542926: C>T)	Meta-analysis of 5 case-control studies	Retrospective	Presence	NAFLD, alcohol	Europe, Thailand	2,594 : 4,279	European	n.a.	In independent studies	[85]
	HSD17B13	2	IV/3	HSD17B13 (rs72613567: TA)	Case-control	Retrospective	Presence	NAFLD, alcohol	Europe	1,109 : 2,206	European	49% : 79%	Within the study	[312]
	WNT3A-WNT9A	2	IV/3	WNT3A-WNT9A (rs708113: T>A)	Case-control	Retrospective	Presence	Alcohol	Europe	775 : 1,332 + 874 : 1,059	European	80% : 94% + 83% : 96% (all F3-4)	Within the study	[87]
	Polygenic risk scores (PRS-HFC, PRS-5)	2	IV/3	SNPs of *PNPLA3, TM6SF2, MBOAT7, GCKR, HSD17B13* + hepatic fat	Case-control	Retrospective	Presence	NAFLD	Italy, UK, Germany	226 : 2,340 + 84 : 343 + 202 : 363,846	Caucasian	n.a. : 13% + n.a. : 21% + n.a. : 0.4%	Within the study	[41]
sue nscriptome	Prognostic liver signature (PLS)	3	II/2a	186 mRNAs	Cohort	Prospective-retrospective	Development, recurrence	HCV, HBV, alcohol, NAFLD	Italy; U.S.; Japan	216; 145; 263	Caucasian, Asian	100%; 100%; n.a.	In independent studies	[90–92]
	PLS-NAFLD	3	II/2a	133 mRNAs	Cohort	Prospective-retrospective	Development, recurrence	NAFLD	Japan	48 + 106 + 59	Asian	90% + 25% + 41% (all F3-4)	Within the study	[37]

omarker type	Score/ biomarker	Biomarker development phase	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Endpoint (HCC)	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis	Independent validation	Reference
culating teins/ leic acids	Prognostic Liver Secretome signature (PLSec)-AFP	3	II/2a	8 proteins + AFP	Cohort	Prospective-retrospective	Developement	HCV, HCV post-SVR, non-viral	U.S., Japan	331 + 164 + 146	Caucasian, Asian	100% + 74% + 80%	Within the study	[36]
	PLSec-NAFLD	3	II/2a	4 proteins	Cohort	Prospective-retrospective	Developement	NAFLD	U.S.	59	Caucasian	100%	Within the study	[37]
	miRNA	3	III/2a	5 miRNAs	Cohort	Prospective-retrospective	Developement	HBV, HCV	Taiwan	220 + 110	Asian	100% + 100%	Within the study	[313]
ging-ed	MEFIB	2	n.a.	MRE, FIB-4	Cohort	Meta-analysis of 4 cohort studies	Developement	NAFLD	U.S., Japan, Turkey	2,018	Caucasian, Asian, Hispanic	n.a.	In independent studies	[40]
nogen-ed	Serum virome	3	III/2a	Viral exposure signature	Case-control + Cohort	Retrospective + Prospective-retrospective	Development	HCV	U.S.	150 : 337 + 173	Caucasian, Black	n.a. + 25%	Within the study	[137]
	Gut microbiome	1	n.a.	Stool microbiome signature	Case-control	Prospective-retrospective	Presence	HBV	China	75 : 40 + 30 : 56	Asian	n.a.	Within the study	[132]
	Serum microbiome	1	n.a.	5-microbiome signature	Case-control	Retrospective	Presence	HBV	Korea	79 : 83 + 79 + 83	Asian	n.a. : 100% + n.a. : 100%	Within the study	[314]

pective-retrospective enrollment indicates Prospective sample collection–Retrospective-Blinded Evaluation (PRoBE) design.

ubjects for training and validation sets are separately shown with "+" in between. No. subjects of different studies are separately shown with ";" in between. No. subjects for case-control studies are n as HCC case : control.

, hepatocellular carcinoma; ILCA, International Liver Cancer Association; NIS, Non-invasive score; NIT, non-invasive tests; HBV, hepatitis B virus; HCV, hepatitis C virus; NASH, non-alcoholic ohepatitis; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; NA, nucleoside analogue; SVR, sustained virologic response; ADRESS, Age, etes, Race, Etiology of cirrhosis, Sex, and Severity of liver dysfunction; REACH-B, risk estimation for hepatocellular carcinoma in chronic hepatitis B; UM, University of Michigan; LSM, liver ess measurement; NGM, nomogram; GAG-HCC, Guide with Age, Gender, HBV DNA, Core promoter mutations and Cirrhosis-HCC; RWS-HCC, Real-world risk score for HCC; REVEAL-HCV, Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer in HCV; FIB-4, fibrosis-4; THRI, Toronto HCC risk index; AGED, Age, Gender, HBeAg and HBV DNA; D^2AS, HBV , age, and sex; PAGE-B, platelets, age, and gender; CAGE-B, cirrhosis and age; SAGE-B, stiffness and age; HCC-RESCUE, HCC-Risk Estimating Score in CHB patients Under Entecavir; CAMPAS, osis, Age, Male, Platelet, Albumin, liver Stiffness; GBM, gradient-boosting machine; ALT, alanine transaminase; REAL-B, Real-world Effectiveness from the Asia Pacific Rim Liver Consortium BV; AASL-HCC, Age, albumin, sex, liver cirrhosis-HCC; CAMD, cirrhosis, age, male sex, and diabetes mellitus; APA-B, age, platelet count, and AFP; ADRES, After DAAs Recommendation for eillance; PRS, polygenic risk scores; PRS-HFC, PRS of hepatic fat content; PLS, Prognostic Liver Signature; PLSec, Prognostic Liver Secretome signature; MEFIB index, an idex calculated from netic resonance elastography and FIB-4; MRE, magnetic resonance elastography.

Table 2.

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Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
n.a.	AFP	Meta-analysis of 30 cohort & case-control studies	Retrospective, prospective	HBV, HCV, alcohol, NAFLD	Korea, U.S., Taiwan, Japan, Italy, Egypt, Canada, Indonesia, France, Australia, Belgium, Spain	n.a.	n.a.	n.a.	BCLC 0/A or within Milan	49%	88%	n.a.	n.a.	In independent studies	[15]
n.a.	AFP	Meta-analysis of 11 cohort studies	Prospective	HCV, HBV, alcohol, NAFLD	U.S., Japan, Egypt, Italy, Korea, France	n.a.	n.a.	n.a.	BCLC 0/A or within Milan	55%	90%	n.a.	n.a.	In independent studies	[15]
n.a.	AFP	Meta-analysis of 18 cohort studies	Prospective-retrospective	HBV, HCV, alcohol, NAFLD	Korea, Taiwan, U.S., Italy, Japan, Canada, Indonesia, Australia, Belgium, Spain	n.a.	n.a.	n.a.	BCLC 0/A or within Milan	38%	90%	n.a.	n.a.	In independent studies	[15]
n.a.	AFP	Meta-analysis of 6 case-control studies	Retrospective	HBV, HCV	China, Japan, U.S.	1,722	n.a.	n.a.	Resectable	65%	80%	n.a.	n.a.	In independent studies	[315]
IV/2b	AFP	Cohort	Prospective	HBV	U.S. (Alaska)	32 HCC patients from 1,487 AFP-screened patients : 12 historcal HCC patients with no screening	n.a.	n.a.	Single, <6 cm	n.a.	n.a.	n.a.	5y survival = 42% : 0%; 10y survival = 30% : 0%	In independent studies	[316]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
III/2a	AFP	Cohort	Prospective-retrospective	HCV, alcohol, NASH	U.S.; U.S. (VA system)	355 + 484	Caucasian, Black, Latino; Caucasian	100% + 100%	BCLC 0/A; single, ≤5 cm	58%; 19%	92%; 96%	n.a.; 0.71	n.a.	In independent studies	[154, 156]
n.a.	AFP-L3%	Meta-analysis of 6 cohort & case-control studies	Retrospective, prospective	HBV, HCV, alcohol	China, U.S., Germany, Japan, Korea	497 (497) : 1950	n.a.	n.a.	BCLC 0/A, AJCC I	34%	92%	0.76	n.a.	In independent studies	[153]
III/2a	AFP-L3%	Cohort	Prospective-retrospective	HCV, alcohol, NASH	U.S.; U.S. (VA system)	355 + 484	Caucasian, Black, Latino; Caucasian	100% + 100%	BCLC 0/A; single, ≤5 cm	74%; 27%	83%; 95%	n.a.; 0.64	n.a.	In independent studies	[154, 156]
n.a.	DCP	Meta-analysis of 11 cohort & case-control studies	Retrospective, prospective	HCV, HBV	U.S., Japan, China, Germany, France	1,316 (1,316) : 1,892	n.a.	n.a.	Single, <3cm	64%	87%	0.86	n.a.	In independent studies	[155]
III/2a	DCP	Cohort	Prospective-retrospective	HCV, alcohol, NASH	U.S.; U.S. (VA system)	355 + 484	Caucasian, Black, Latino; Caucasian	100% + 100%	BCLC 0/A; single, ≤5 cm	26%; 12%	92%; 99%	n.a.; 0.72	n.a.	In independent studies	[154, 156]
n.a.	Gender, age, AFP, AFP-L3%, DCP	Meta-analysis of 7 case-control studies	Retrospective	HBV, HCV, alcohol, NASH	U.S., Europe, Asia	1,183 (1,183) : 2,838	Caucasian, Asian, Hispanic, Black	n.a.	BCLC 0-A, AJCC I/II, within Milan	69%	91%	0.83	n.a.	In independent studies	-
n.a.	Gender, age, AFP, AFP-L3%, DCP	Meta-analysis of 2 cohort studies	Prospective-retrospective	HCV, alcohol, NASH	U.S.; U.S. (VA system)	849	Caucasian, Black, Latino; Caucasian	100%	BCLC 0/A; single, ≤5 cm	58%	83%	0.73	n.a.	In independent studies	-
III/2a	AFP, change in AFP over the last year, age, platelets, ALT, and interaction terms	Cohort	Prospective-retrospective	HCV, alcohol, NASH	U.S.; U.S. (VA system)	355 + 484	Caucasian, Black, Latino; Caucasian	100% + 100%	BCLC 0/A; single, ≤5 cm	42%; 27%	91%; 95%	n.a.; 0.76	n.a.	In independent studies	[154, 156]
IV/3	Age, gender, logAFP, alkaline phosphatase, ALT	Case-control	Retrospective	HBV, HCV, others	U.S.	165 (101) : 195 + 432 (225) : 438 + 113	n.a.	100% : 100% + 100% : 100% +	BCLC 0/A	43% (validation 1) ; 58% (validation	95% (validation 1) ; 90% (validation	0.81 (validation 1) ; 0.89 (validation	n.a.	In independent studies	[174]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
						(113) : 586 + 425 (140) : 804		100% : 100% + 100% : 100%		2) ; 35% (validation 3)	2) ; 95% (validation 3)	2) ; 0.77 (validation 3)			
IV/3	Gender, age, AFP, DCP	Case-control	Retrospective	HBV	China	908 (318) : 603 + 286 (n.a.) : 211	Asian	n.a. : 52% + n.a. : 46%	BCLC 0/A	74%	90%	n.a.	n.a.	Within the study	[317]
IV/3	AFP, DCP, D-dimer	Case-control	Retrospective	HBV	China	59 (59) : 143	Asian	n.a. : 100%	Single, ≤5 cm	93%	84%	0.96	n.a.	No	[318]
III/2a	Age, logAFP, PEG-precipitated IgG, fucosylated kininogen	Cohort	Prospective-retrospective	HCV, alcohol, NASH	U.S.	29 (17) : 58 (matched)	Caucasian	100% : 100%	BCLC 0/A	80%	90%	n.a.	n.a.	F/u study of Wang et al.	[176]
IV/3	Age, gender, logAFP, alkaline phosphatase, ALT, fucosylated kininogen	Case-control	Retrospective	HCV, HBV, others	U.S.	115 (69) : 93	n.a.	100% : 100%	Within Milan	86%	95%	0.97	n.a.	F/u study of Wang et al.	[175]
IV/3	*N*-glycopeptide N241_A4G4F2S4, AFP, Age	Case-control	Retrospective	NASH	China	32 (32) : 46	Asian	n.a. : 100%	AJCC I/II	72%	90%	0.9	n.a.	Internal (cross-validation)	[319]
III/2a	Mutations in *TP53, CTNNB1, AXIN1,* TERT promoter, HBV integration breakpoint, AFP, DCP	Cohort	Prospective-retrospective	HBV	China	331	Asian	0% : 11%	BCLC 0/A	100%	94%	n.a.	PPV = 17%	Within the study	[188]
IV/3	28 methylation markers, age, sex, AFP, AFP-L3%, DCP	Case-control	Retrospective	HBV, others	China	46 : 236 + 122 (37) : 125	Asian	n.a. + 37% : 37%	AJCC I/II	76%	91%	0.92	n.a.	Within the study	[320]
IV/3	3 cfDNA methylation markers (*HOXA1, TSPYL5, B3GALT6*), sex, AFP	Case-control	Retrospective	HCV, alcohol, NASH, HBV	U.S., France, Germany, Italy, Spain, Taiwan, Thailand	136 (81) : 404 + 156 (78) : 245	Caucasian, Black, Asian	96% : 93% + 97% : 92%	BCLC 0/A	82%	87%	0.92	n.a.	F/u study of Chalasani et al.	[167]
IV/3	4 cfDNA methylation markers (*HOXA1, EMX1, TSPYL5, B3GALT6*), AFP, AFP-L3%	Case-control	Retrospective	HCV, NAFLD, alcohol, HBV	U.S., France, Germany, Italy, Spain, Taiwan, Thailand	135 (76) : 302	Caucasian, Black, Asian	90% : 87%	BCLC 0/A	71%	90%	0.88	n.a.	F/u study of Kiesel et al.	[180]

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Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
IV/3	CtDNA mutations, AFP, DCP	Case-control	Retrospective	HBV	Korea	102 (43) : 41	Asian	59% : 22%	BCLC A	n.a.	n.a.	0.87	n.a.	No	[321]
n.a.	Methylated *SEPT9*	Meta-analysis of 6 case-control studies	Retrospective	NAFLD, HBV, HCV, alcohol, others	China, Japan, U.S., France, Germany, UK	500 : 949	n.a.	n.a.	n.a.	80% (any stage)	90% (any stage)	0.92 (any stage)	n.a.	In independent studies	[186]
IV/3	32 5hmC markers	Case-control	Retrospective	HBV	China	335 (335) : 263 + 220 (220) : 129 + 24 (24) : 180	Asian	70% : 28% + n.a. : 26% + n.a. : 0%	BCLC 0/A	83% (validation 1) ; n.a. (validation 2)	67% (validation 1) ; n.a. (validation 2)	0.85 (validation 1) ; 0.92 (validation 2)	n.a.	Within the study	[187]
IV/3	cfDNA fragmentomics	Case-control	Retrospective	HBV	China	192 (134) : 170 + 189 (140) : 165	Asian	46% : 57% + 29% : 29%	BCLC 0/A	90% (BCLC 0), 97% (BCLC A);	n.a.	n.a.	n.a.	Within the study	[190]
IV/3	5hmC, motif, fragmentation, nucleosome footprint	Case-control	Retrospective	HBV	China	225 (108) : 607 + 95 (35) : 100 + 131 (58) : 1800	Asian	61% : 57% + 64% : 100% + 70% : 100%	n.a.	96%; 95% (any stage)	95%; 98% (any stage)	1.00; 1.00 (any stage)	n.a.	Within the study	[191]
IV/3	*HOXA7, EMX1, AK055957, ECE1, PFKP, CLEC11A, B3GALT6*	Case-control	Retrospective	HCV, alcohol, NAFLD	U.S.	95 (46) : 51	n.a.	98% : 100%	n.a.	95% (any stage)	86% (any stage)	0.93 (any stage)	n.a.	In independent studies	[179]
IV/3	*ASCL2, LDHB, LGALS3, LOXL3, PLXND1, OSR1, RASSF2*	Case-control	Retrospective	NAFLD, alcohol, HCV, others	Germany (training), U.S. (validation)	46 : 41 + 60 (49) : 103	n.a.	100% : 100% + 100% : 100%	n.a.	57% (any stage)	97% (any stage)	0.85 (any stage)	n.a.	Within the study	[183]
IV/3	Somatic copy number aberration	Case-control	Retrospective	HBV	China	108 (73) : 101 + 38 (38) : 38 + 51 (51) : 48	Asian	77% : 42% + 68% : 58% + 63% : 46%	BCLC 0/A	56% (validation 1) ; 53% (validation 2)	90% (validation 1) ; 96% (validation 2)	0.92 (validation 1) ; 0.81 (validation 2)	n.a.	Within the study	[322]
IV/3	*BMPR1A, PSD, ARHGAP25, KLF3, PLAC8, ATXN1, Chr 6 : 170, Chr 6 : 3, ATAD2, Chr 8 : 20*	Case-control	Retrospective	HBV, HCV, NAFLD	China	715 : 560 + 383 : 275	Asian	n.a. : 0%	n.a.	83% (any stage)	91% (any stage)	0.94 (any stage)	n.a.	Within the study	[323]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
IV/3	*EpCAM, CD90, CD133, CK19*	Case-control	Retrospective	HBV	China	200 (131) : 101 + 195 (94) : 200	Asian	78% : n.a. + 80% : n.a.	BCLC 0/A	85%	93%	0.93	n.a.	Within the study	[196]
IV/3	*AFP, ALB, APOH, FABP1, FGB, FGG, AHSG, RBP4,TF*	Case-control	Retrospective	HBV, HCV, alcohol	U.S.	16 (9) : 57	n.a.	n.a. : 25%	n.a.	n.a.	n.a.	0.88 (any stage)	n.a.	Internal (cross-validation)	[195]
IV/3	EpCAM^{mRNA+} CTCs	Case-control	Retrospective	HBV	China	157 (119) : 120	Asian	82% : n.a.	n.a.	43% (any stage)	97% (any stage)	0.70 (any stage)	n.a.	No	[194]
IV/3	*miR-320b, miR-663a, miR-4448, miR-4651, miR-4749-5p, miR-6724-5p, miR-6887-5p, miR-6885-5p*	Case-control	Retrospective	HCV, HBV, others	Japan	172 (108) : 64 + 173 (123) : 75	Asian	n.a. : 69% + n.a. : 65%	AJCC I/II	98%	n.a.	0.99 (any stage)	n.a.	Within the study	[198]
IV/3	*miR-10a, miR-125b*	Case-control	Retrospective	HBV	China	65 : 75	Asian	n.a.	n.a.	99% (any stage)	99% (any stage)	0.99 (any stage)	n.a.	No	[199]
IV/3	*miR-16*	Case-control	Retrospective	n.a.	China	100 (100) : 20	Asian	n.a. : 100%	BCLC 0/A	87%	90%	0.94	n.a.	No	[324]
IV/3	*lncRNA-AF085935*	Case-control	Retrospective	HBV; HBV	China; Egypt	137 : 104 + 70 : 70	Asian; n.a.	n.a.	n.a.; n.a.	n.a.; 56% (any stage)	n.a.; 96% (any stage)	0.86 (any stage); 0.81 (any stage)	n.a.	In independent studies	[200, 201]
IV/3	*lncRNA-uc003wbd*	Case-control	Retrospective	HBV; HBV	China; Egypt	137 : 104 + 70 : 70	Asian; n.a.	n.a.	n.a.; n.a.	n.a.; 87% (any stage)	n.a.; 96% (any stage)	0.70 (any stage); 0.96 (any stage)	n.a.	In independent studies	[200, 201]
IV/3	*MIR4435-2HG, lnc-POLD3-2* in PBMCs	Case-control	Retrospective	HBV	Thailand	100 (35) : 200	Asian	80% : 6%	BCLC 0/A	85%	n.a.	n.a.	n.a.	No	[325]
IV/3	*hsa_circ_0000976, hsa_circ_0007750, hsa_circ_0139897*	Case-control	Retrospective	HBV	China	158 (54) : 102 + 152 (59) : 104 + 290 (88) : 160	Asian	70% : 49% + n.a. : 48% + n.a. : 50%	Single, ≤ 3 cm	83% (validation 1) ; 86% (validation 2)	84% (validation 1) ; 86% (validation 2)	0.85 (validation 1) ; 0.85 (validation 2)	n.a.	Within the study	[202]
IV/3	*hTERT* mRNA	Case-control	Retrospective	HBV, HCV	Vietnam	170 (92) : 170	Asian	100% : 100%	BCLC 0/A	88%	96%	0.94	n.a.	No	[326]
IV/3	*smRC_119591, smRC_135709, smRC_48615*	Case-control	Retrospective	n.a.	U.S.	105 (105) : 85	n.a.	67% : 72%	BCLC 0/A	86%	91%	0.87	n.a.	Internal (cross-validation)	[206]
IV/3	*ENSG00000248932.1, ENST00000440688.1,*	Case-control	Retrospective	HBV, HCV	China	20 + 180 : 200	Asian	n.a.	n.a.	n.a.	n.a.	0.87 (any stage)	n.a.	Within the study	[207]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
	ENST00000457302.2, *AFP*														
IV/3	*LINC00853*	Case-control	Retrospective	HBV	Korea	90 (46) : 63	Asian	n.a. : 56%	mUICC I/II	91%	85%	0.95	n.a.	No	[205]
IV/3	*Lnc85*	Case-control	Retrospective	n.a.	China	112 : 43	Asian	n.a. : 100%	n.a.	80% (any stage)	74% (any stage)	0.89 (any stage)	n.a.	No	[327]
IV/3	*miR-10b-5p*, *miR-223-3p*, *miR-22-3p*, *miR-21-5p*	Case-control	Retrospective	HCV, HBV	India	38 (20) : 60	Asian	71% : 42%	n.a.	58% (any stage)	95% (any stage)	0.80 (any stage)	n.a.	No	[328]
IV/3	*miR-10b-5p*	Case-control	Retrospective	HBV	Korea	90 (46) : 60	Asian	n.a. : 55%	mUICC I/II	94%	78%	0.95	n.a.	No	[204]
IV/3	*ENSG00000258332.1*, *LINC02635*, AFP	Case-control	Retrospective	HBV	China	60 (16) : 96 + 55 : 60	Asian	70% : 0% + n.a. : 0%	n.a.	85% (any stage)	85% (any stage)	0.89 (any stage)	n.a.	Within the study	[329]
IV/3	*miR-122*, *miR-148a*, AFP	Case-control	Retrospective	HBV	China	50 (37) : 40	Asian	n.a. : 100%	Within Milan	87%	90%	0.95	n.a.	No	[330]
IV/3	*AFP*, *GPC3*, *ALB*, *APOH*, *FABP1*, *FGB*, *FGG*, *AHSG*, *RBP4*, *TF*	Case-control	Retrospective	HCV, alcohol, NAFLD	U.S.	36 (36) : 26	Caucasian, Asian, Hispanic, Black	100% : 100%	BCLC 0/A	94%	89%	0.93	n.a.	No	[208]
IV/3	EpCAM$^+$ CD63$^+$ EV, CD147$^+$ CD63$^+$ EV, GPC3$^+$ CD63$^+$ EV	Case-control	Retrospective	HCV, alcohol, NAFLD, HBV	U.S.	45 (45) : 61 + 35 (35) : 37	Caucasian, Hispanic, Asian	82% : 100% + 86% : 100%	BCLC 0/A	91%	81%	0.93	n.a.	Within the study	[210]
IV/3	Amount of AnnexinV$^+$ EpCAM$^+$ ASGPR1$^+$ EV	Case-control	Retrospective	n.a.	Germany	86 : 49	n.a.	n.a. : 100%	n.a.	81% (any stage)	47% (any stage)	0.73 (any stage)	n.a.	No	[331]
IV/3	Amount of total EVs	Case-control	Retrospective	HBV, alcohol	China	48 (48) : 40	Asian	n.a. : 100%	AJCC I/II	63%	89%	0.83 (AJCC I), 0.94 (AJCC II)	n.a.	No	[332]
n.a.	Golgi protein 73	Meta-analysis of 3 case-control studies	Retrospective	HCV, HBV, alcohol, others	U.S., China	354 (354) : 581	n.a.	n.a.	AJCC I/II	79%	62%	n.a.	n.a.	In independent studies	[219]
n.a.	Osteopontin	Meta-analysis of 4 case-	Retrospective	HBV	China, Thailand, Australia	511 (511) : 523	n.a.	n.a.	BCLC 0/A	49%	72%	n.a.	n.a.	In independent studies	[214]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
		control studies													
n.a.	Midkine	Meta-analysis of 4 case-control studies	Retrospective	HBV, HCV	China, Australia, Egypt	n.a.	n.a.	n.a.	BCLC 0/A	84%	82%	0.87	n.a.	In independent studies	[220]
III/2a	Midkine	Cohort	Prospective-retrospective	HCV, HBV	Australia	28 : 84 (matched)	n.a.	n.a.	n.a.	67% (any stage)	n.a.	n.a.	n.a.	No	[333]
IV/3	AKR1B10	Case-control	Retrospective	HBV	China	209 (79) : 50 + 204 (75) : 60	Asian	48% : 80% + 50% : 63%	BCLC 0/A	61%	86%	0.76	n.a.	Within the study	[217]
IV/3	CIAPIN1, EGFR, MAS1, SLC44A3, ASAH1, UBL7, ZNF428	Case-control	Retrospective	HBV, alcohol	China	282 (60) : 130 + 279 (59) : 119	Asian	n.a. : 100% + n.a. : 100%	BCLC 0/A	70%	91%	0.88	n.a.	Within the study	[221]
IV/3	17 proteins, AFP, DCP	Case-control	Retrospective	HBV, HCV	Korea	199 (199) : 199 + 85 (85) : 85 + 109 (109) : 50	Asian	62% : 79% + 66% : 75% + 83% : 100%	Within Milan	81% (validation 1) ; 90% (validation 2)	82% (validation 1) ; 98% (validation 2)	0.91 (validation 1) ; 0.97 (validation 2)	n.a.	Within the study	[334]
IV/3	Benzoic acid, Creatine, Citrulline	Case-control	Retrospective	T2DM	China	58 (n.a.) : 96	Asian	n.a.	AJCC I/II	92%	82%	0.94	n.a.	No	[335]
IV/3	AFP, *TP53* mutation and 2 methylation markers (*mRASSF1A, mGSTP1*)	Case-control	Retrospective	HBV, HCV, others	U.S., Taiwan	186 (86) : 423	n.a.	n.a. : 34%	BCLC 0/A	92% (BCLC 0), 77% (BCLC A)	90% (BCLC 0), 90% (BCLC A)	n.a.	n.a.	Internal (cross-validation)	[222]
IV/3	*miR-93-5p*	Case-control	Retrospective	HBV	China	130 (64) : 65	Asian	100% : 0%	AJCC I/II	88%	95%	0.90	n.a.	No	[223]
IV/3	Surface-enhanced Raman spectroscopy	Case-control	Retrospective	n.a.	China	55 : 49	Asian	n.a. : 100%	n.a.	80% (any stage)	76% (any stage)	n.a.	n.a.	Internal (cross-validation)	[225]
n.a.	Ultrasound	Meta-analysis of 34 cohort & case-	Retrospective, prospective	HCV, HBV, alcohol, NAFLD	U.S., Korea, Taiwan, Thailand, France, Japan, Italy, Egypt,	13,544	n.a.	n.a.	BCLC 0/A or within Milan	52%	88%	n.a.	n.a.	In independent studies	[15]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
		control studies			Canada, Argentina, India, Pakistan, Switzerland, Belgium, Australia, Spain										
n.a.	Ultrasound	Meta-analysis of 17 cohort studies	Prospective	HCV, HBV, alcohol, NAFLD	U.S., Korea, Thailand, France, Japan, Italy, Egypt, Canada, India, Pakistan	n.a.	n.a.	n.a.	BCLC 0/A or within Milan	53%	90%	n.a.	n.a.	In independent studies	[15]
n.a.	Ultrasound	Meta-analysis of 15 cohort studies	Prospective-retrospective	HCV, HBV, alcohol, NAFLD	U.S., Korea, Taiwan, Thailand, Japan, Italy, Argentina, Switzerland, Belgium, Australia, Spain	n.a.	n.a.	n.a.	BCLC 0/A or within Milan	46%	90%	n.a.	n.a.	In independent studies	[15]
n.a.	ECA-enhanced MRI, HBA-enhanced MRI, non-contrast AMRI	Meta-analysis of 5 cohort & case-control studies	Retrospective, prospective	HBV, HCV, alcohol	Korea, Turkey, U.S., Australia	107 (107) : 1,237	n.a.	n.a.	BCLC 0/A	83%	95%	n.a.	n.a.	In independent studies	[336]
III/2a	Gadoxetic acid-enhanced MRI	Cohort	Prospective	HBV	Korea	407	Asian	100%	BCLC 0, A	85% (BCLC 0), 86% (BCLC A)	97%	0.90 (BCLC 0)	n.a.	No	[226]
n.a.	Non-contrast, HBA-enhanced, and dynamic ECA-enhanced AMRI	Meta-analysis of 6 cohort & case-control studies	Retrospective, prospective	HBV, HCV, alcohol	Korea, Australia	n.a.	n.a.	n.a.	BCLC 0	69%	n.a.	n.a.	n.a.	In independent studies	[227]
III/2a	Non-contrast AMRI	Cohort	Prospective	HBV, HCV, alcohol	Australia	192	n.a.	n.a.	BCLC 0/A	83%	98%	n.a.	n.a.	In independent studies	[230]

Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
III/2a	Non-contrast AMRI	Cohort	Prospective	HCV	Egypt	41	n.a.	100%	n.a.	100% (any stage)	100% (any stage)	n.a.	n.a.	In independent studies	[337]
III/2a	Non-contrast AMRI	Cohort	Prospective-retrospective	HBV	Korea	382 with high-risk	Asian	100%	n.a.	79% (any stage)	98% (any stage)	n.a.	n.a.	In independent studies	[231]
II/2a	Iodine-enhanced CT	RCT	Prospective	HCV	U.S. (VA system)	CT : US = 80 : 83	White, Black	100%	n.a.	67% (any stage)	94% (any stage)	n.a.	n.a.	No	[232]
III/2a	Iodine-enhanced CT	Cohort	Prospective	HBV	Korea	137 with high-risk	Asian	92%	BCLC 0, A	82% (BCLC 0), 86% (BCLC A)	96%	n.a.	n.a.	No	[233]
III/2a	Sonazoid-enhanced ultrasound	Cohort	Prospective intra-individual comparison design	HBV	Korea	524	Asian	100%	BCLC 0/A	n.a.	n.a.	n.a.	Detection rate = 1.1%; false referral rate = 1.1%	No	[235]
II/2a	Sonazoid-enhanced ultrasound	RCT	Prospective	HCV, HBV	Japan	CEUS : US = 309 : 313	Asian	100%	n.a.	100% (any stage)	96% (any stage)	n.a.	n.a.	No	[236]
n.a.	Ultrasound, AFP	Meta-analysis of 14 cohort & case-control studies	Retrospective, prospective	HBV, HCV, alcohol, NAFLD	Taiwan, Thailand, Egypt, U.S., Canada, Korea, Australia, Belgium, Spain	7,140	n.a.	n.a.	BCLC 0/A or within Milan	74%	84%	n.a.	n.a.	In independent studies	[15]
II/2a	Ultrasound, AFP	Cohort	Prospective	HBV	China (Shanghai)	Screening : control = 9,373 : 9,443	Asian	n.a.	Single, <5 cm	n.a.	n.a.	n.a.	Screening arm vs. control arm : Standardized incidence (per 100,000) = 279 : 267; Early-stage HCC = 39 (45%) : 0 (0%); 5-year survival = 46% : 0%	No	[150]
IV/3	GALAD score, ultrasound	Case-control	Retrospective	HCV, NAFLD,	U.S.	111 (60) : 180	Caucasian, Asian	98% : 86%	BCLC 0/A	88%	94%	0.97	n.a.	No	[165]

	Level of evidence (Simon et al./ ILCA)	Variables	Study design	Enrollment	Major etiology	Region/ country	No. subjects	Race/ ethnicity	Cirrhosis (HCC : control)	Definition of early-stage HCC	Sensitivity	Specificity	AUROC	Other endpoints	Independent validation	Reference
					alcohol, HBV											

pective sample collection–Retrospective-Blinded Evaluation (PRoBE) design.

CC case (early-stage HCC) : control. No. subjects for training and validation sets are separately shown with "+" in between. No. subjects of different

nonths of diagnosis are presented in cohort studies unless indicated otherwise.

l Liver Cancer Association; AFP, alpha-fetoprotein; AUROC, area under receiver operating characteristic curve; HBV, hepatitis B virus; HCV, hepatitis LD, non-alcoholic fatty liver disease; BCLC, Barcelona clinic liver cancer; AJCC, American Joint Committee on Cancer; DCP, des-gamma-carboxy FP, and DCP; HES, Hepatocellular Carcinoma Early Detection Screening; PEG, polyethylene glycol; cfDNA, cell-free DNA; ctDNA, circulating tumor ing RNA; EV, extracellular vesicle; AKR1B10, aldo-keto reductase family 1 member 10; SVM, support vector machine; MRI, magnetic resonance epatobiliary agent; AMRI, abbreviated MRI; CT, computed tomography; US, ultrasound; CEUS, contrast-enhanced ultrasound.

Table 3.

Ongoing clinical trials evaluating risk stratification and early detection biomarkers for HCC.

Biomarker type	Type of test/ biomarker	Trial name	Test/biomarker	Target population	Biomarker development phase	Planned no. subjects	Country	Anticipated completion year	NCT No.
Risk stratification	Imaging, clinical test/ feature	STARHE	Deep learning of clinical, biological, elastography/ultrasound features	Advanced fibrosis/ cirrhosis on HCC screening	1-3	400	France	2023	NCT04802954
	SNP	n.a.	*MMP1*-1607 1G/2G (rs1799750)	Egyptian HCV cirrhosis	1/2	200	Egypt	2022	NCT03722628
Risk stratification/ early detection	Circulating biomarker, microbiome, imaging	ELEGANCE	miRNA panel (early detection), microbiome/MRI/urine, plasma metabolome (risk prediction)	Chronic liver disease from HBV, HCV, or NAFLD	1-3	2,000	Singapore	2025	NCT04965259
Early detection	Circulating biomarker	n.a.	Serum visfatin, vaspin	HCV-related HCC, cirrhosis, healthy controls	1/2	100	Egypt	2022	NCT04763707
	Circulating biomarker, clinical test/feature	n.a.	Glycotest HCC panel	HCC, cirrhosis	2	766	U.S.	2022	NCT03878550
	Circulating biomarker	n.a.	*lncRNAs-WRAP53, UCA1*	HCC, cirrhosis, and healty controls	1/2	80	Egypt	2022	NCT05088811
	Circulating biomarker, clinical test/feature	ALTUS	mt-HBT, Oncoguard Liver (Multi-target HCC blood test)	Cirrhosis, HBV carriers	4	3,000	U.S.	2025	NCT05064553
	Circulating biomarker, clinical test/feature	LIVER-1	HelioLiver Test	HCC, controls undergoing routine imaging surveillance for HCC	4	1,200	U.S.	2024	NCT05199259
	Circulating biomarker, clinical test/feature	HEPATIC	HelioLiver Test	HCC, cirrhosis, and HD controls	2	1,000	China	2022	NCT05053412
	Circulating biomarker	SEPT9-CROSS	Epi proColon 2.0 CE (Plasma *mSEPT9*)	HCC cases and cirrhosis controls	2	530	France	2023	NCT03311152
	Circulating biomarker	n.a.	ctDNA methylation and fragmentation markers, miRNA7, CTC	Esophageal cancer, gastric cancer, colorectal cancer, HCC, healthy controls, pre-cancer	1-3	2,430	China	2022	NCT05431621
	Imaging	FASTRAK	non-contrast AMRI	Compensated cirrhosis	4	944	France	2027	NCT05095714
	Imaging	n.a.	Low-contrast dose CT and deep learning-based reconstruction	Patients undergoing CT for HCC diagnosis or surveillance	1-3	90	Korea	2022	NCT04027556

Biomarker type	Type of test/ biomarker	Trial name	Test/biomarker	Target population	Biomarker development phase	Planned no. subjects	Country	Anticipated completion year	NCT No.
	Imaging	n.a.	Gadolinium-enhanced AMRI	Cirrhosis	4	150	U.S.	2023	NCT04288323
	Imaging	n.a.	non-contrast AMRI, MRI	Cirrhosis AND reduced visualisation on ultrasound	4	476	Australia, New Zealand	2027	NCT04455932
	Imaging	n.a.	CEUS and MRI	Cirrhosis, chronic liver disease from HBV, atypical hyperplasia nodules	4	100	China	2023	NCT05286099
	Imaging	n.a.	Short MRI surveillance (SMS) protocol	High-risk cirrhosis and/or chronic liver disease	4	470	Netherlands	2026	NCT05429190
	Clinical test/score, imaging	n.a.	HBsAg, AFP, ultrasound	Populations in Zhongshan City	5	20,000	China	2023	NCT02501980
	Circulating biomarker, clinical test/feature, imaging	FAST-MRI Study	GALAD score, ctDNA, nc-AMRI	Cirrhosis	4	820	U.S.	2025	NCT04539717
	Clinical test/score, imaging	STOP-HCC	GALAD score, ultrasound	Compensated cirrhosis	4	1,600	Saudi Arabia, Vietnam	2032	NCT05342350
	Clinical test/score, imaging	n.a.	AFP, AFP-L3%, DCP, ultrasound, CT	Cirrhosis	4	1,418	Korea	2026	NCT04414956
	Circulating biomarker, clinical test/feature, imaging	n.a.	Genetron HCC Methylation PCR Kit, AFP, ultrasound, MRI	HCC, cirrhosis, chronic liver disease; cirrhosis, chronic liver disease under surveillance	1-4	4,816	China	2022	NCT05343832
	Circulating biomarker, imaging	n.a.	EV-RNA, imaging	HCC, biliary tract cancer, cirrhosis, chronic liver disease	1/2	1,810	U.S.	2023	NCT02908048
	Circulating biomarker	n.a.	Chiroptical, Raman, infrared spectroscopy	HCC, cirrhosis, healty controls	1/2	250	Czechia	2022	NCT04221347
Monitor change in HCC risk level	Tissue transcriptome, immunostaining biomarker	n.a.	PLS, phospho-EGFR staining	Compensated cirrhosis	1/2	25	U.S.	2022	NCT02273362
	Circulating biomarker	n.a.	PLSec	Compensated cirrhosis	2	60	U.S.	2026	NCT05028829

ClinicalTrials.gov accessed in July 2022.

HCC, hepatocellular carcinoma; SNP, single nucleotide polymorphism; miRNA, micro RNA; MRI, magnetic resonance imaging; HCV, hepatitis C virus; HBV, hepatitis B virus; NAFLD, non-alcoholic fatty liver disease; ctDNA, circulating tumor DNA; CTC, circulating tumor cell; AMRI, abbreviated MRI; CT, computed tomography; CEUS, contrast-enhanced ultrasound; AFP, alpha-fetoprotein; GALAD, Gender, Age, AFP-L3%, AFP, and DCP; nc-AMRI, non-contrast AMRI; DCP, des-gamma-carboxy prothrombin; EV, extracellular vesicle; PLS, Prognostic Liver Signature; PLSec, Prognostic Liver Secretome Signature.