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Blood-based biomarkers for hepatocellular carcinoma screening: Approaching the end of the ultrasound era?

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Summary

Hepatocellular carcinoma (HCC) is a leading cause of cancer-related death worldwide, in part because of inadequate early detection strategies. Current recommendations for screening consist of semi-annual abdominal ultrasound with or without serum alpha-fetoprotein in patients with cirrhosis and in demographic subgroups with chronic hepatitis B infection. However, this screening strategy has several deficiencies, including suboptimal early-stage sensitivity, false positives with subsequent harms, inter-operator variability in ultrasound performance, and poor adherence. A blood-based biomarker with sufficient performance characteristics for early-stage disease could overcome several of these barriers to improving early-stage detection. However, prior to use of a biomarker for screening in clinical practice, a multistep validation is required in order to understand test performance characteristics. These steps include case-control validation, followed by validation in prospective cohorts of at-risk patients. Until recently, we lacked adequate longitudinal validation cohorts for early HCC detection; however, several validation cohorts are maturing, including the Hepatocellular Carcinoma Early Detection Study and the Texas Hepatocellular Carcinoma Consortium, which will allow for rigorous validation of candidate biomarkers. While there are several promising biomarkers awaiting validation, in order to supplant abdominal ultrasound, a candidate biomarker must show adequate test performance and overcome practical hurdles to ensure adoption in clinical practice. The promise of blood-based biomarkers is significant, especially given the limitations of ultrasound-based screening; however, they require adequate validation and several logistical obstacles must be overcome prior to clinical implementation.

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Authors' contributions

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Keywords

HCC; liver cancer; surveillance; biospecimen

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related mortality. While mortality for most cancers is decreasing, HCC has remained one of the fastest growing causes of cancer-related death worldwide.^{1,2} High mortality in patients with HCC is due to several factors including inadequate early detection strategies, lack of curative treatments for those detected beyond an early stage, inconsistent application of curative therapies in clinical practice, and competing risks of mortality from comorbid liver disease. Tumour stage at diagnosis is associated with curative treatment receipt and overall survival, including 5-year survival below 5% in patients with advanced stage disease compared to >70% for those with early-stage HCC.³ Current recommendations for HCC screening, endorsed by professional society guidelines, include semi-annual abdominal ultrasound, with or without serum alpha-fetoprotein (AFP), in patients with cirrhosis and subgroups with chronic hepatitis B virus infection.^{4,5} HCC screening is supported by limited randomised clinical trial data from Asia among patients with chronic hepatitis B virus infection and numerous cohort studies among patients with cirrhosis.^{6,7} These studies consistently demonstrate that screening is significantly associated with early HCC detection, increased curative treatment receipt, and improved survival.⁷

Limitations of ultrasound-based screening

A meta-analysis of cohort studies reported the sensitivity of ultrasound for early-stage HCC detection is only 45%, which increases to 63% with the addition of AFP.⁸ While an ultrasound-based strategy has been effective in some settings, *e.g.* in Japan, significant national public health resources have been required to promote HCC screening uptake.^{9,10} Further, ultrasound exams are often performed and undergo real-time interpretation by hepatologists, optimising exam quality, and are coupled with widespread use of several biomarkers, such as PIVKA-II (protein-induced by vitamin K absence or antagonist-II), AFP, and the *Lens culinaris* agglutinin-reactive fragment of AFP (AFP-L3).¹⁰

However, the effectiveness of ultrasound-based screening in many other countries worldwide is inadequate for three main reasons.

First, there is significant variation in ultrasound performance, ranging from 21% to 89% across studies, due to both patient and provider factors.⁸ Ultrasound has moderate to severe visualisation limitations in approximately 20% of patients, with increased odds of suboptimal visualisation in obese individuals and those with non-viral liver disease aetiologies or increased liver echotexture.¹¹ In one study including 941 individuals with cirrhosis, visualisation was inadequate in up to one-third of those with decompensated cirrhosis and a body mass index >35.¹² Poor visualisation significantly decreases sensitivity for early-stage HCC detection.¹³ Considering the changing epidemiology of cirrhosis, with increasing proportions of individuals with non-viral cirrhosis, such limitations will likely

become increasingly problematic in clinical practice.¹⁴ Ultrasound-based visualisation and test performance varies. Ultrasound requires capture of specific windows for adequate visualisation of the whole liver, which partly depends on the experience of the ultrasound operator.¹⁵

Second, ultrasound-based screening is associated with potential harms due to false positive or indeterminate results that can lead to cross-sectional imaging with CT or MRI, percutaneous liver biopsy, and psychological distress.¹⁶ In one study including 999 individuals with cirrhosis, up to 25% of those undergoing ultrasound screening had a false positive result over a median of 1.5 years.¹⁷ Similarly, in another cohort of 680 individuals with cirrhosis, 27.5% experienced a screening-related harm over a 3-year period.¹⁸ To date, most studies have suggested physical harms of HCC screening are mild in severity, although larger studies with longer follow-up are still needed.¹⁹ Further, most studies have solely focused on physical harms, with no data on potential psychological or financial harms.^{7,20} While psychological harms have yet to be formally characterised, anxiety and worry caused by false positives are apparent in other cancer screening paradigms.^{21,22}

Third, ultrasound screening suffers from poor adherence. A meta-analysis showed that the average published rate of adherence to screening ultrasound in at-risk individuals was 24%.²³ There are several patient and provider barriers to ultrasound completion. From the patient perspective, there are knowledge gaps about the risk of HCC and reasons for ultrasound attainment.^{24,25} In addition, barriers including need for separate radiology appointment, costs, and travel time can be unique to ultrasound and contribute to diminished adherence, especially in the context of longitudinal semi-annual testing.^{24–26} Provider barriers include lack of up-to-date knowledge about screening recommendations and limited time in clinic to address screening.²⁷ While certain interventions targeting patients, such as mailed outreach, and providers, such as electronic reminders, modestly improve screening rates, we lack widely applicable methods to attain consistent longitudinal screening rates in practice.^{28,29} Finally, the lack of broad acceptance of ultrasound screening, in part due to the lack of robust data supporting its use, has limited widespread provider uptake, and fuelled controversy about HCC screening as a quality measure in clinical care.^{30,31} The lack of randomised data supporting HCC screening in patients with cirrhosis has led to inadequate acknowledgements of competing risks, harms, and overall effectiveness in this population. With the introduction of novel strategies for HCC screening, there is an opportunity to generate better data supporting the use of screening as an effective cancer control strategy in individuals with cirrhosis.

The limitations of an ultrasound-based screening strategy for early HCC detection are manifold and thus more sensitive tests that overcome these current barriers to adherence are needed. When eliciting patient preferences regarding screening modalities, patients strongly prefer more convenient and accurate tests vs. current standard of care ultrasound-based screening.³² The promise of blood-based biomarkers becoming the new standard of care for HCC screening has existed for years; however, we have yet to validate a biomarker with sufficient performance to replace ultrasound. There are now several maturing validation cohorts available for biomarker testing, in addition to novel biomarker validation designs that could allow for the more rapid adoption of a biomarker-based strategy for early HCC

detection. Herein, we will review recent developments in biomarker validation for HCC and the challenges and opportunities of moving beyond an ultrasound-based strategy for HCC screening.

Phases of biomarker validation

The progression of a biomarker from the discovery phase to full clinical validation is a multistep process that can take years to complete and additionally requires appropriate samples to conduct full validation.³³ There are several distinct phases of biomarker validation that provide a roadmap to clinical implementation (Fig. 1A–E).

Phase I: Initial discovery occurs in preclinical models, which is followed by clinical assay validation, where the assay for biomarker measurement is developed. The process for discovery varies depending on the biomarker type. For example, proteomic discovery typically uses mass-spectroscopy to analyse blood proteins. The scale of these discovery platforms has largely been underpowered due to the limited availability and costs associated with mass-spectroscopy analyses. On the other hand, transcriptomic analysis allows for high throughput analysis of biomarkers; however, interpretation of the data and identification of candidate markers can be difficult. Regardless of the approach, an assay must be developed that is reproducible and able to distinguish between cases and controls accurately.

Phase II: Validation with samples from retrospective case-control studies, comparing HCC cases (preferably early-stage) and non-HCC controls. Cases and controls should ideally be derived from the recommended screening population (*i.e.* individuals with cirrhosis). This early phase is critical in determining biomarker performance in distinguishing cases and controls; however, phase II studies may overestimate the performance of a biomarker compared to cohort studies given biomarker performance is dependent on cancer incidence, which is artificially inflated in case-control studies.

Phase III: Testing of longitudinal samples of the biomarker to determine its performance in detecting preclinical disease, also termed a PRoBE (prospective-specimen collection, retrospective-blinded-evaluation) design. Testing in this phase will help validate performance using pre-specified cut-offs for a positive screening test. This requires serial samples from a screening-eligible population over time, where some individuals will develop cancer and others will not.

Phase IV: Testing in a prospective cohort where the biomarker is acted upon in real time with diagnostic work-up for positive results. Ideally, validation occurs against a gold standard (via randomisation or parallel design) to minimise the risk of ascertainment bias. A key strength of this phase is the ability to determine detection rate and false positive rate (FPR) in a representative population.

Phase V: This late stage of validation addresses whether screening reduces the burden of cancer on the population in real-world settings. The screening strategy using the biomarker is evaluated in the context of treatment effectiveness for early-stage cancer, compliance with screening, and potential for cancer over-diagnosis. Although HCC is typically considered

a highly lethal cancer, recent data have suggested variation in tumour volume doubling times and potential for over-diagnosis.^{34,35} Ultimately the goal is to gain estimates for the reduction in cancer mortality afforded by the screening test.

While rigorous validation is challenging and resource intensive, the iterative validation of biomarkers ensures adequate performance, assessment of accuracy, development of false positive algorithms, and ultimately that biomarker-based screening results in reductions in mortality in the screening population. A recent white paper from the International Liver Cancer Association provides details of how these phases can be applied to HCC screening, incorporating the singularities of HCC and cirrhosis.³⁶ Given these challenges, AFP remains the only biomarker that has been validated beyond phase III.

Current and emerging biomarkers for HCC

While some early validation data exists for several candidate biomarkers (Table 1), we will focus on commonly used and emerging biomarkers for early HCC detection.

AFP—AFP is the only widely used biomarker for HCC detection and disease monitoring; however, AFP is not considered to have adequate performance characteristics as a standalone test for screening. A meta-analysis showed that AFP can increase sensitivity for early-stage HCC detection when used in combination with abdominal ultrasound (63% vs. 45% with ultrasound alone.), and a modelling study showed that ultrasound combined with AFP was the most cost-effective screening strategy across a majority of simulations.⁸ AFP elevations can occur in other conditions, which can lead to false positive results, particularly in patients with active chronic hepatitis C and B infections.³⁷ Published cohort studies estimate that, at its traditional cut-off of 20 ng/ml, AFP has a wide range of sensitivities for early-stage HCC detection, ranging from 39–64%, with specificities ranging from 76–97%.^{38–43} Recent data suggest that AFP levels observed in practice are decreasing in parallel with increased use of antiviral treatment, suggesting the optimal threshold of AFP for screening may now be lower.⁴⁴ Beyond single-threshold assessments, the change in AFP value across serial measurements has been shown to be superior to single AFP values for the detection of early-stage HCC.^{45,46} Further, delta AFP has been integrated into the hepatocellular carcinoma early detection screening (HES) algorithm (discussed below).^{47,48} Overall, AFP likely has a role in conjunction with other tests for the early detection of HCC; however, it is insufficient as a standalone test for screening.

AFP L3—AFP-L3, or *Lens culinaris* agglutinin-reactive AFP, is a fucosylated glycoform of AFP that has been proposed as a biomarker for early HCC detection.⁴⁹ AFP-L3 has exhibited a wide range of sensitivities for the detection of early-stage HCC in the literature (49–60%), depending on cohort characteristics.^{38,50,51} Recent data from a small phase III cohort (n = 397) in the US showed AFP-L3, at a cut-off of 11.9%, had a sensitivity of 46.2%, at a 10% FPR, within 6 months prior to HCC diagnosis.⁵² In a separate phase III cohort of 534 patients in the US, AFP-L3 at a cut-off of 8.3% had a sensitivity of 40% for early-stage HCC, with FPR fixed at 10%.⁴⁸ These data suggest that AFP-L3 is inadequate as a biomarker for HCC, but it has been integrated into other panels of biomarkers and thus may play a role in a biomarker panel-based strategy for screening.

DCP—Des-gamma carboxyprothrombin (DCP) is another serum biomarker that has undergone phase II and early phase III validation. In a phase II study of 131 individuals with early HCC, DCP alone had an AUROC of 0.72.³⁸ However, limited phase III evaluation has demonstrated poor sensitivity in detecting preclinical HCC (26.3%) with a fixed FPR of 10%.⁵³ A phase II study comparing the performance of multiple published biomarkers identified AFP and DCP as having the best clinical performance.⁵⁴ However, other data suggest DCP may not significantly increase the discriminatory power of the combination of AFP and AFP-L3 for early HCC detection.⁵⁵

DNA methylation/cell-free DNA—DNA methylation is an early step in hepatocarcinogenesis and has been postulated to be a potential circulating marker for the early detection of HCC.⁵⁶ To date, there have been limited data beyond phase II to support clinical use of DNA methylation, although several different methylation panels are currently under investigation. An algorithm called the multi-target HCC blood test, which includes three methylated markers, in combination with AFP and sex, showed an 82% sensitivity for early-stage HCC with a specificity of 87% and an AUROC of 0.91 in a phase II validation case-control study.⁵⁷ While these initial results are promising, this panel is still undergoing larger prospective validation in direct comparison to ultrasound with or without AFP ([NCT05064553](#)). Another multi-analyte cell-free DNA test for HCC (HelioLiver) showed early-stage detection of 76% of cases, with a specificity of 91% in a phase II study, including 122 individuals with HCC and 125 with chronic liver disease.⁵⁸ This test is undergoing further validation in a larger phase II cohort ([NCT05199259](#)). Finally, several companies (e.g. GRAIL, Freenome) are launching studies to examine the utility of multi-cancer detection platforms, including for liver cancer, based on cell-free DNA.

EVs—Another form of liquid biopsy includes analysis of extracellular vesicles (EVs), which are enclosed structures excreted by cells and which can be detected in plasma. They can contain various biochemical signals, including genetic material, and have been investigated as a biomarker for the early detection of HCC. To improve EV purification, various groups have developed EV detection chips with immunoaffinity assays for efficient isolation. In one study, comparing plasma from 36 individuals with early-stage HCC to 26 controls with cirrhosis, the EV chip had a sensitivity of 94.4% and specificity of 88.5%.⁵⁹ These and other EV-based platforms are undergoing larger scale validation.

Algorithms—Given the heterogeneity in HCC, combination biomarkers that incorporate patient-specific risk factors, such as gender and age, have been explored.

GALAD score—The GALAD (gender, age, AFP-L3, AFP, DCP) score includes a panel of serum-based markers (AFP, AFP-L3 and DCP), combined with demographic factors (gender and age).⁶⁰ It was derived in a cohort of 833 individuals (394 with HCC and 439 with chronic liver disease) from the United Kingdom and validated in case-control populations of 6,834 individuals (2,430 with HCC [1,038 early stage] and 4,404 with chronic liver disease) from Japan, Germany and Hong Kong. The GALAD score's sensitivity for early-stage HCC ranged from 71.7% to 82.1%, while the specificity ranged from 81.3% to 89.7% across the populations.⁶¹ In a phase II validation study in patients with NASH-related cirrhosis with

and without early-stage HCC, from a multicentre German cohort, it was shown to have a sensitivity of 68% and a specificity of 95%.⁶² However, its performance in small phase III cohorts has been less good, with one study reporting a sensitivity of 53.8% and another a sensitivity of 30.8% at an FPR of 10%.^{48,52} The initially reported results from the phase III HEDS (Hepatocellular Early Detection Study), including 1,550 people, indicated that GALAD had a sensitivity of 50% within 6 months of HCC diagnosis at an FPR of 10%.⁶³ While we await the final results of this analysis, the presented data indicate that GALAD may not have sufficient performance characteristics as a standalone biomarker.

Doylestown algorithm—The Doylestown algorithm is a panel with consists of laboratory (log AFP, alkaline phosphatase, and alanine aminotransferase) and demographic factors (age and gender). In a phase II study of 69 individuals with early-stage HCC (stage T1 or T2 disease) and 93 controls with cirrhosis, the addition of fucosylated kininogen to the algorithm led to a higher AUROC than for either the Doylestown algorithm or AFP alone (0.97 vs. 0.93 and 0.80, respectively).⁶⁴ In a nested case-control study of 29 individuals with HCC (17 early stage) compared to 58 controls, the Doylestown plus algorithm had a sensitivity of 63.2%.⁶⁵ Testing of a modified version of the Doylestown plus algorithm in larger phase II cohorts is underway ([NCT03878550](#)).

HES algorithm—The HES algorithm includes demographic (age) and laboratory parameters (AFP, rate of AFP change, alanine aminotransferase, and platelet count) and has been validated in phase II and phase III cohorts.⁶⁶ In a validation cohort comprising 7,432 people, the HES algorithm outperformed AFP alone for HCC detection in the 6 months prior to a clinical diagnosis of HCC, with a sensitivity of 53% vs. 48% at an FPR of 10%.⁶⁶ In a small phase III validation study, HES had a sensitivity of 36.7% at an FPR of 10% for early-stage HCC, which was similar to GALAD, AFP, and AFP-L3.⁴⁸ Based on the published cohorts, HES does not appear to have sufficient performance as a standalone test for HCC screening.

Moving beyond ultrasound-based screening

With numerous emerging biomarkers for early HCC detection and the limitations of ultrasound-based surveillance, the question of how we might transition from imaging-based to biomarker-based surveillance arises. Numerous challenges from both a scientific and logistical perspective must be addressed prior to implementation of a biomarker-based strategy.

Biomarker-based screening in other cancers—While biomarker-based cancer screening is currently a relatively rare paradigm, this has been adopted in colorectal cancer with both the faecal immunohistochemical test (FIT) and the multi-target stool DNA test. FIT testing was incorporated into United States Preventative Task Force (USPSTF) colorectal cancer screening guidelines based on several randomised trials.⁶⁷ The pivotal study that led to US Food and Drug Administration approval of the multi-target stool DNA test, compared its performance to the gold standards of colonoscopy and FIT testing in asymptomatic patients undergoing routine screening.⁶⁸ The performance of the test, which showed adequate sensitivity and acceptable specificity, when compared to accepted

modalities for screening, allowed for its approval as an option for colorectal cancer screening and it is currently included in the USPSTF guidelines.⁶⁹ While these large-scale validation studies are costly and require large numbers of participants due to the relatively low prevalence of cancer in a cross-sectional population, this design has the advantage of rapid cohort maturation when compared to a phase IV and V biomarker validation design. There are important limitations to this study design, including lack of understanding of the longitudinal nature of the biomarker or the implications of a false positive result in clinical practice; however, similarly designed trials are currently being conducted for a methylated DNA marker panel for the early detection of HCC.

Considerations when moving to biomarker-based surveillance

There are several potential barriers and factors to consider prior to the adoption of a biomarker-based screening strategy for HCC (Table 2). First, performance characteristics should at least be comparable to ultra sound and AFP for early-stage HCC detection. The published threshold is 63%, based on the sensitivity of ultrasound combined with AFP for early-stage HCC; however, the performance may be worse in modern cohorts with metabolic causes of cirrhosis, where ultrasound has lower sensitivity.⁷⁰ If a biomarker shows non-inferiority to ultrasound and AFP within an acceptable margin, the biomarker may still become an acceptable modality given the potential for increased adherence due to lower barriers associated with biomarker-based surveillance (Fig. 2). A prior modelling study by Mourad and colleagues demonstrated the relationship between test utilisation and sensitivity, with lower sensitivity achieving the same benefits if adherence is increased.⁷¹ Biomarker-based strategies could enable increased adherence to screening through avoidance of logistical hurdles present with ultrasound-based screening (*e.g.* need for separate radiology appointments). Biomarker-based strategies may also reduce the time it takes to report results, facilitating earlier diagnostic evaluation, and helping to address other downstream failures in the screening process.⁷² Nevertheless, sensitivity will need to be sufficient for reliable detection across patient subgroups, as well as specificity to minimise false positives. Specifically, understanding whether a false positive may indicate a precancerous state is a critical delineation for patient follow-up pathways. Reflecting differences in pathways to hepatocarcinogenesis, biomarkers can have differential performance depending on the underlying liver disease.⁷³ However, while contemporary biomarker validation cohorts include the breadth of aetiologies of liver disease, the comorbid presence of metabolic liver disease in individuals with other aetiologies may make a liver disease-specific approach challenging.⁷⁴

Costs for commercialised testing products and payor coverage is another important consideration, especially given the serial nature of HCC surveillance testing. Additionally, with the shift in aetiologies of cirrhosis to metabolic causes (NAFLD/ALD) that are associated with a lower annual incidence of HCC,⁷⁵ the costs of any surveillance test will be an important consideration, given the high number needed to screen to detect a cancer with lower incidence rates.⁷⁶ Clinical and/or biomarker-based risk stratification schema may become increasingly important to guide the modality and intensity of screening in low incidence populations.^{77,78}

The logistical aspects of blood processing and reporting will also have to be well delineated. For proprietary assays, shipping blood to centralised labs may lead to reporting delays, sample mishandling, and incomplete linkage to follow-up testing in the setting of a positive result. For biomarkers that can be run in local laboratories, assays will require standardisation across sites to ensure accurate interpretability at pre-specified cut-offs. Screening test results can be both dichotomous (positive/negative) or continuous. There are advantages and disadvantages to both – while dichotomous testing is simple and easily interpretable, a numerical test level may allow for a more nuanced analysis of indeterminate results and longitudinal interpretation of biomarker trends which can improve test performance, as seen with AFP.

While performance of biomarkers is important, the transition from a paradigm of direct liver visualisation to biomarker-based screening may introduce unique challenges. Imaging for screening does have several limitations as previously outlined, including operator dependency; however, visualisation does have the advantages of detecting findings besides cancer, such as precancerous nodules, subclinical ascites, portal vein thrombosis, or clinically significant extrahepatic findings, which may not be detectable with a purely biomarker-based strategy. Shifting this paradigm may be uncomfortable for providers or patients, and thus some may adopt hybrid screening methods, combining imaging and biomarkers for screening, however the cost-effectiveness of such an approach will need to be assessed.

Generating the evidence

Adoption of biomarker-based strategies has been hampered by the historical lack of appropriate validation cohorts. Several prospective phase III cohorts including HEDS,⁷⁹ with over 1,500 individuals with cirrhosis, and the Texas Hepatocellular Carcinoma Consortium, with over 3,000 individuals with cirrhosis, are maturing, allowing for validation of various candidate biomarkers.⁸⁰ One limitation of these cohorts is that their collection techniques may not allow for validation of several novel biomarkers, such as methylated DNA markers or EVs, which often require specialised processing or tubes. Nevertheless, these cohorts will provide a valuable data resource given their size, the presence of serial longitudinal sample collection and their diversity.⁸¹ Validation using large cross-sectional cohorts of at-risk patients has the advantage of faster cohort maturation compared to longitudinal cohorts, which can take years to mature. However, the implications of false positive results and subsequent care pathways may be challenging to delineate using such a validation design.

Once a marker has demonstrated sufficient performance in a phase III validation set, a clinical utility study is necessary prior to clinical utilisation. While these can be challenging and costly, such a trial is important to provide the evidence needed to understand test characteristics, define clinical pathways, and provide estimates on the population-based impact of clinical application. Importantly in HCC screening, we lack high levels of evidence for screening benefits, so such a trial would be particularly valuable and could potentially lead to broadly increased uptake of screening for HCC. An adequately powered trial could account for harms associated with screening, competing risks of mortality and overdiagnosis, and the overall effectiveness of a screening programme. Various trial designs

have advantages and disadvantages. For example, pragmatic trials can be limited by loss of follow-up for enrolled patients; however, such a design may be able to give estimates of real-world adherence in addition to test effectiveness. Additionally, a pragmatic approach could allow for estimates of the impact of HCC screening vs. no screening on HCC-related and overall mortality. Inclusion of various practice types (*e.g.*, community-based and academic centres) in any trial design will be critical as well, to both increase the external validity of trial results, but also to reflect the “real-world” effectiveness of current and novel screening programmes. Fig. 3 shows a schematic of a proposed clinical utility trial for HCC screening, using a novel combination of phase IV and phase V validation by incorporation of a randomised phase IV design and an interim analysis.

Conclusions

Biomarker-based HCC screening holds significant promise given the emergence of several novel biomarkers. Blood-based screening allows for point of care testing and objective results, which would overcome major barriers to both the completion and interpretation of current screening modalities. Given the failings of the current ultrasound-based screening strategy and the emergence of several cohorts for validation, the time is right for robust biomarker validation. In parallel with assessment of clinical utility in validation studies, additional practical considerations must be worked out prior to clinical implementation of a biomarker-based paradigm. With proper validation and pre-implementation considerations, the transition beyond an ultrasound-based paradigm for HCC screening is feasible and will be welcomed by both patients and providers, as it holds the potential to significantly reduce the burden of HCC in at-risk populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Conflicts of interest

Dr. Parikh has served as a consultant for Bristol Myers-Squibb, Exact Sciences, Eli Lilly, and Freenome; has served on advisory boards of Genentech, Eisai, Bayer, Exelixis, Wako/Fujifilm; and has received research funding from Bayer-supportTarget RWE, Exact Sciences, Genentech and Glycotest. Dr. Tayob has no conflicts to declare. Dr. Singal has served on advisory boards or as a consultant for Bayer, FujiFilm Medical Sciences, Exact Sciences, Glycotest, and GRAIL.

Please refer to the accompanying ICMJE disclosure forms for further details.

Abbreviations

AFP	alpha-fetoprotein
EVs	extracellular vesicles

FIT	faecal immunohistochemical test
FPR	false positive rate
HCC	hepatocellular carcinoma
HES	hepatocellular carcinoma early detection screening
USPSTF	United States Preventative Task Force

References

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- [1]. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a Cancer J Clinicians* 2021;71:209–249.
- [2]. Tapper EB, Parikh ND. Mortality due to cirrhosis and liver cancer in the United States, 1999–2016: observational study. *BMJ* 2018;362:k2817.
- [3]. Zhang X, El-Serag HB, Thrift AP. Predictors of five-year survival among patients with hepatocellular carcinoma in the United States: an analysis of SEER-Medicare. *Cancer Causes & Control* 2021;32:317–325. [PubMed: 33394207]
- [4]. Marrero JA, Kulik LM, Sirlin CB, Zhu AX, Finn RS, Abecassis MM, et al. Diagnosis, staging, and management of hepatocellular carcinoma: 2018 practice guidance by the American association for the study of liver diseases. *Hepatology* 2018;68:723–750. [PubMed: 29624699]
- [5]. Liver EAFTSOT. EASL clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2018;69:182–236. [PubMed: 29628281]
- [6]. Zhang B-H, Yang B-H, Tang Z-Y. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417–422. [PubMed: 15042359]
- [7]. Singal AG, Zhang E, Narasimman M, Rich NE, Waljee AK, Hoshida Y, et al. HCC surveillance improves early detection, curative treatment receipt, and survival in patients with cirrhosis: a systematic review and meta-analysis. *J Hepatol* 2022;77:128–139. [PubMed: 35139400]
- [8]. Tzartzeva K, Obi J, Rich NE, Parikh ND, Marrero JA, Yopp A, et al. Surveillance imaging and alpha fetoprotein for early detection of hepatocellular carcinoma in patients with cirrhosis: a meta-analysis. *Gastroenterology* 2018;154:1706–1718. e1701.
- [9]. Toyoda H, Hiraoka A, Olivares J, Al-Jarrah T, Devlin P, Kaneoka Y, et al. Outcome of hepatocellular carcinoma detected during surveillance: comparing USA and Japan. *Clin Gastroenterol Hepatol* 2021;19:2379–2388. e2376.
- [10]. Johnson P, Berhane S, Kagebayashi C, Satomura S, Teng M, Fox R, et al. Impact of disease stage and aetiology on survival in hepatocellular carcinoma: implications for surveillance. *Br J Cancer* 2017;116:441–447. [PubMed: 28081537]
- [11]. Schoenberger H, Chong N, Fetzer DT, Rich NE, Yokoo T, Khatri G, et al. Dynamic changes in ultrasound quality for hepatocellular carcinoma screening in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2021;20(7):1561–1569. [PubMed: 34119640]
- [12]. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. *Aliment Pharmacol Ther* 2017;45:169–177. [PubMed: 27862091]
- [13]. Chong N, Schoenberger H, Yekkaluri S, Fetzer DT, Rich NE, Yokoo T, et al. Association between ultrasound quality and test performance for HCC surveillance in patients with cirrhosis: a retrospective cohort study. *Aliment Pharmacol Ther* 2022;55:683–690. [PubMed: 35170052]
- [14]. Moon AM, Singal AG, Tapper EB. Contemporary epidemiology of chronic liver disease and cirrhosis. *Clin Gastroenterol Hepatol* 2020;18:2650–2666. [PubMed: 31401364]

- [15]. Fetzer DT, Browning T, Xi Y, Yokoo T, Singal AG. Associations of ultrasound LI-RADS visualization score with examination-, sonographer-, and radiologist-factors: retrospective assessment in over 10,000 examinations. *Am J Roentgenology* 2021;218(6):1010–1020.
- [16]. Hanouneh IA, Alkhouri N, Singal AG. Hepatocellular carcinoma surveillance in the 21st century: saving lives or causing harm? *Clin Mol Hepatol* 2019;25:264. [PubMed: 30827081]
- [17]. Konerman MA, Verma A, Zhao B, Singal AG, Lok AS, Parikh ND. Frequency and outcomes of abnormal imaging in patients with cirrhosis enrolled in a hepatocellular carcinoma surveillance program. *Liver Transplant* 2019;25:369–379.
- [18]. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, Parikh ND, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. *Hepatology* 2017;65:1196–1205. [PubMed: 27775821]
- [19]. Singal AG, Patibandla S, Obi J, Fullington H, Parikh ND, Yopp AC, et al. Benefits and harms of hepatocellular carcinoma surveillance in a prospective cohort of patients with cirrhosis. *Clin Gastroenterol Hepatol* 2021;19:1925–1932. e1921.
- [20]. Harris RP, Sheridan SL, Lewis CL, Barclay C, Vu MB, Kistler CE, et al. The harms of screening: a proposed taxonomy and application to lung cancer screening. *JAMA Intern Med* 2014;174:281–286. [PubMed: 24322781]
- [21]. Lin K, Lipsitz R, Miller T, Janakiraman S. Benefits and harms of prostate-specific antigen screening for prostate cancer: an evidence update for the US Preventive Services Task Force. *Ann Intern Med* 2008;149:192–199. [PubMed: 18678846]
- [22]. Brewer NT, Salz T, Lillie SE. Systematic review: the long-term effects of false-positive mammograms. *Ann Intern Med* 2007;146:502–510. [PubMed: 17404352]
- [23]. Wolf E, Rich NE, Marrero JA, Parikh ND, Singal AG. Use of hepatocellular carcinoma surveillance in patients with cirrhosis: a systematic review and meta-analysis. *Hepatology* 2021;73:713–725. [PubMed: 32383272]
- [24]. Farvardin S, Patel J, Khambaty M, Yerokun OA, Mok H, Tiro JA, et al. Patient-reported barriers are associated with lower hepatocellular carcinoma surveillance rates in patients with cirrhosis. *Hepatology* 2017;65: 875–884. [PubMed: 27531684]
- [25]. Goldberg DS, Taddei TH, Serper M, Mehta R, Dieperink E, Aytaman A, et al. Identifying barriers to hepatocellular carcinoma surveillance in a national sample of patients with cirrhosis. *Hepatology* 2017;65:864–874. [PubMed: 27531119]
- [26]. Singal AG, Tiro JA, Murphy CC, Blackwell J-M, Kramer JR, Khan A, et al. Patient-reported barriers are associated with receipt of hepatocellular carcinoma surveillance in a multicenter cohort of patients with cirrhosis. *Clin Gastroenterol Hepatol* 2021;19:987–995. e981.
- [27]. Simmons OL, Feng Y, Parikh ND, Singal AG. Primary care provider practice patterns and barriers to hepatocellular carcinoma surveillance. *Clin Gastroenterol Hepatol* 2019;17:766–773. [PubMed: 30056183]
- [28]. Beste LA, Ioannou GN, Yang Y, Chang MF, Ross D, Dominitz JA. Improved surveillance for hepatocellular carcinoma with a primary care-oriented clinical reminder. *Clin Gastroenterol Hepatol* 2015;13:172–179. [PubMed: 24813175]
- [29]. Singal AG, Reddy S, aka Patel HR, Villarreal D, Khan A, Liu Y, et al. Multicenter randomized clinical trial of a mailed outreach strategy for hepatocellular carcinoma surveillance. *Clin Gastroenterol Hepatol* 2021. In press.
- [30]. Kansagara D, Papak J, Pasha AS, O’Neil M, Freeman M, Relevo R, et al. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. *Ann Intern Med* 2014;161:261–269. [PubMed: 24934699]
- [31]. Sherman M, Bruix J. Screening for liver cancer: the rush to judgment. *Ann Intern Med* 2012;157:300–301.
- [32]. Woolen SA, Singal AG, Davenport MS, Troost JP, Khalatbari S, Mittal S, et al. Patient preferences for hepatocellular carcinoma surveillance parameters. *Clin Gastroenterol Hepatol* 2022;20:204–215. e206.
- [33]. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, et al. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054–1061. [PubMed: 11459866]

- [34]. Rich NE, John BV, Parikh ND, Rowe I, Mehta N, Khatri G, et al. Hepatocellular carcinoma demonstrates heterogeneous growth patterns in a multicenter cohort of patients with cirrhosis. *Hepatology* 2020;72:1654–1665. [PubMed: 32017165]
- [35]. Rich NE, Singal AG. Overdiagnosis of hepatocellular carcinoma: prevented by guidelines? *Hepatology* 2022;75:740–753. [PubMed: 34923659]
- [36]. Singal AG, Hoshida Y, Pinato DJ, Marrero J, Nault J-C, Paradis V, et al. International liver cancer association (ILCA) white paper on biomarker development for hepatocellular carcinoma. *Gastroenterology* 2021;160:2572–2584. [PubMed: 33705745]
- [37]. Yang JD, Dai J, Singal AG, Gopal P, Addissie BD, Nguyen MH, et al. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. *Cancer Epidemiol Biomarkers Prev* 2017;26(7):1085–1092. [PubMed: 28258053]
- [38]. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology* 2009;137:110–118. [PubMed: 19362088]
- [39]. Oka H, Tamori A, Kuroki T, Kobayashi K, Yamamoto S. Prospective study of a-fetoprotein in cirrhotic patients monitored for development of hepatocellular carcinoma. *Hepatology* 1994;19:61–66. [PubMed: 7506227]
- [40]. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-c-carboxy prothrombin and a-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. *Gastroenterology* 2010;138:493–502. [PubMed: 19852963]
- [41]. Chang TS, Wu YC, Tung SY, Wei KL, Hsieh YY, Huang HC, et al. Alpha-fetoprotein measurement benefits hepatocellular carcinoma surveillance in patients with cirrhosis. *Am J Gastroenterol* 2015;110:836–844. quiz 845. [PubMed: 25869392]
- [42]. Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995;22:432–438. [PubMed: 7543434]
- [43]. Chalasani N, Horlander JC Sr, Said A, Hoen H, Kopecky KK, et al. Screening for hepatocellular carcinoma in patients with advanced cirrhosis. *Am J Gastroenterol* 1999;94:2988–2993. [PubMed: 10520857]
- [44]. Vipani A, Lauzon M, Luu M, Roberts LR, Singal AG, Yang JD. Decreasing trend of serum a-fetoprotein level in hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2021;20(5):1177–1179. [PubMed: 34391921]
- [45]. Lee E, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of alpha-fetoprotein, over time. *Clin Gastroenterol Hepatol* 2013;11:437–440. [PubMed: 23247324]
- [46]. Tayob N, Stingo F, Do KA, Lok ASF, Feng Z. A Bayesian screening approach for hepatocellular carcinoma using multiple longitudinal biomarkers. *Biometrics* 2018;74:249–259. [PubMed: 28482112]
- [47]. Tayob N, Christie I, Richardson P, Feng Z, White DL, Davila J, et al. Validation of the hepatocellular carcinoma early detection screening (HES) algorithm in a cohort of veterans with cirrhosis. *Clin Gastroenterol Hepatol* 2019;17:1886–1893 e1885.
- [48]. Tayob N, Kanwal F, Alsarraj A, Hernaez R, El-Serag HB. The performance of AFP, AFP-3, DCP as biomarkers for detection of hepatocellular carcinoma (HCC): a phase 3 biomarker study in the United States. *Clin Gastroenterol Hepatol* 2022. In press.
- [49]. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive a-fetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993;53:5419–5423. [PubMed: 7693340]
- [50]. Li D, Mallory T, Satomura S. AFP-L3: a new generation of tumor marker for hepatocellular carcinoma. *Clinica Chim Acta* 2001;313:15–19.
- [51]. Sassa T, Kumada T, Nakano S, Uematsu T. Clinical utility of simultaneous measurement of serum high-sensitivity des-[gamma]-carboxy prothrombin and Lens culinaris agglutinin A-reactive a-fetoprotein in patients with small hepatocellular carcinoma. *Eur J Gastroenterol Hepatol* 1999;11:1387–1392. [PubMed: 10654799]

- [52]. Singal AG, Tayob N, Mehta A, Marrero JA, El-Serag H, Jin Q, et al. GALAD demonstrates high sensitivity for HCC surveillance in a cohort of patients with cirrhosis. *Hepatology* 2022;75:541–549. [PubMed: 34618932]
- [53]. Kumada T, Toyoda H, Tada T, Kiriya S, Tanikawa M, Hisanaga Y, et al. High-sensitivity Lens culinaris agglutinin-reactive alpha-fetoprotein assay predicts early detection of hepatocellular carcinoma. *J Gastroenterol* 2014;49:555–563. [PubMed: 24057163]
- [54]. Piratvisuth T, Tanwandee T, Thongsawat S, Sukeepaisarnjaroen W, Esteban JI, Bes M, et al. Multimarker panels for detection of early stage hepatocellular carcinoma: a prospective, multicenter, case-control study. *HepatoL Commun* 2022;6:679–691. [PubMed: 34796691]
- [55]. Choi J, Kim GA, Han S, Lee W, Chun S, Lim YS. Longitudinal assessment of three serum biomarkers to detect very early-stage hepatocellular carcinoma. *Hepatology* 2019;69:1983–1994. [PubMed: 30153338]
- [56]. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. *Nat Mater* 2017;16:1155–1161. [PubMed: 29035356]
- [57]. Chalasani NP, Porter K, Bhattacharya A, Book AJ, Neis BM, Xiong KM, et al. Validation of a novel multi-target blood test shows high sensitivity to detect early stage hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2022;20:173–182. e177.
- [58]. Lin N, Lin Y, Xu J, Liu D, Li D, Meng H, et al. A multi-analyte cell-free DNA-based blood test for early detection of hepatocellular carcinoma. *HepatoL Commun* 2022. In press.
- [59]. Sun N, Lee Y-T, Zhang RY, Kao R, Teng P-C, Yang Y, et al. Purification of HCC-specific extracellular vesicles on nanosubstrates for early HCC detection by digital scoring. *Nat Commun* 2020;11:1–12. [PubMed: 31911652]
- [60]. Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. *Cancer Epidemiol Biomarkers Prev* 2014;23:144–153. [PubMed: 24220911]
- [61]. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. *Clin Gastroenterol Hepatol* 2016;14:875–886. e876.
- [62]. Best J, Bechmann LP, Sowa JP, Sydor S, Dechene A, Pflanz K, et al. GALAD score detects early hepatocellular carcinoma in an international cohort of patients with nonalcoholic steatohepatitis. *Clin Gastroenterol Hepatol* 2019;18(3):728–735. [PubMed: 31712073]
- [63]. Marrero JA, Marsh TL, Parikh ND, Roberts LR, Schwartz ME, Nguyen MH, et al. Galad score improves early detection OF HCC prior to the diagnosis of HCC: a phase 3 biomarker validation study. *NJ USA: WILEY 111 RIVER ST, HOBOKEN 07030–5774; 2021. p. 92A–93A. HEPATOLOGY; 2021.*
- [64]. Wang M, Devarajan K, Singal AG, Marrero JA, Dai J, Feng Z, et al. The Doylestown algorithm: a test to improve the performance of AFP in the detection of hepatocellular carcinoma. *Cancer Prev Res* 2016;9: 172–179.
- [65]. Singal AG, Tayob N, Mehta A, Marrero JA, Jin Q, Lau J, et al. Doylestown plus and GALAD demonstrate high sensitivity for HCC detection in patients with cirrhosis. *Clin Gastroenterol Hepatol* 2022;20:953–955. e952.
- [66]. Tayob N, Christie I, Richardson P, Feng Z, White DL, Davila J, et al. Validation of the hepatocellular carcinoma early detection screening (HES) algorithm in a cohort of veterans with cirrhosis. *Clin Gastroenterol Hepatol* 2019;17:1886–1893. e1885.
- [67]. Hewitson P, Glasziou P, Watson E, Towler B, Irwig L. Cochrane systematic review of colorectal cancer screening using the fecal occult blood test (hemoccult): an update. *Official J Am Coll Gastroenterol ACG* 2008;103:1541–1549.
- [68]. Imperiale TF, Ransohoff DF, Itzkowitz SH, Levin TR, Lavin P, Lidgard GP, et al. Multi-target stool DNA testing for colorectal-cancer screening. *New Engl J Med* 2014;370:1287–1297. [PubMed: 24645800]

- [69]. Davidson KW, Barry MJ, Mangione CM, Cabana M, Caughey AB, Davis EM, et al. Screening for colorectal cancer: US preventive services Task Force recommendation statement. *Jama* 2021;325:1965–1977. [PubMed: 34003218]
- [70]. Samoylova ML, Mehta N, Roberts JP, Yao FY. Predictors of ultrasound failure to detect hepatocellular carcinoma. *Liver Transplant* 2018;24:1171–1177.
- [71]. Mourad A, Deuffic-Burban S, Ganne-Carrié N, Renaut-Vantroys T, Rosa I, Bouvier AM, et al. Hepatocellular carcinoma screening in patients with compensated hepatitis C virus (HCV)-related cirrhosis aware of their HCV status improves survival: a modeling approach. *Hepatology* 2014;59:1471–1481. [PubMed: 24677195]
- [72]. Rao A, Rich NE, Marrero JA, Yopp AC, Singal AG. Diagnostic and therapeutic delays in patients with hepatocellular carcinoma. *J Natl Compr Cancer Netw* 2021;19:1063–1071.
- [73]. Schlaeger C, Longerich T, Schiller C, Bewerunge P, Mehrabi A, Toedt G, et al. Etiology-dependent molecular mechanisms in human hepatocarcinogenesis. *Hepatology* 2008;47:511–520. [PubMed: 18161050]
- [74]. Mak L-Y, Hui RW-H, Fung J, Liu F, Wong DK-H, Cheung K-S, et al. Diverse effects of hepatic steatosis on fibrosis progression and functional cure in virologically quiescent chronic hepatitis B. *J Hepatol* 2020;73:800–806. [PubMed: 32504663]
- [75]. El-Serag HB, Kanwal F, Feng Z, Marrero JA, Khaderi S, Singal AG. Risk factors for cirrhosis in contemporary Hepatology practices—findings from Texas hepatocellular carcinoma Consortium cohort. *Gastroenterology* 2020;159:376. [PubMed: 32234536]
- [76]. Kanwal F, Kramer JR, Mapakshi S, Natarajan Y, Chayanupatkul M, Richardson PA, et al. Risk of hepatocellular cancer in patients with nonalcoholic fatty liver disease. *Gastroenterology* 2018;155:1828–1837. e1822.
- [77]. Fujiwara N, Kobayashi M, Fobar AJ, Hoshida A, Marquez CA, Koneru B, et al. A blood-based prognostic liver secretome signature and long-term hepatocellular carcinoma risk in advanced liver fibrosis. *Med* 2021;2:836–850. e810.
- [78]. Goossens N, Singal AG, King LY, Andersson KL, Fuchs BC, Besa C, et al. Cost-effectiveness of risk score–stratified hepatocellular carcinoma screening in patients with cirrhosis. *Clin translational Gastroenterol* 2017;8:e101.
- [79]. Borges KA, Dai J, Parikh ND, Schwartz M, Nguyen MH, Roberts LR, et al. Rationale and design of the hepatocellular carcinoma early detection strategy study: a multi-center longitudinal initiative of the national cancer institute’s early detection research network. *Contemp Clin trials* 2019;76:49–54. [PubMed: 30439517]
- [80]. Feng Z, Marrero JA, Khaderi S, Singal AG, Kanwal F, Loo N, et al. Design of the Texas hepatocellular carcinoma Consortium cohort study. *Am J Gastroenterol* 2019;114:530–532. [PubMed: 30699099]
- [81]. Younossi Z, Stepanova M, Ong JP, Jacobson IM, Bugianesi E, Duseja A, et al. Nonalcoholic steatohepatitis is the fastest growing cause of hepatocellular carcinoma in liver transplant candidates. *Clin Gastroenterol Hepatol* 2019;17:748–755 e743.
- [82]. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajang S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* 2012;55:483–490. [PubMed: 21953299]
- [83]. Wan H-G, Xu H, Gu Y-M, Wang H, Xu W, Zu M-H. Comparison osteopontin vs AFP for the diagnosis of HCC: a meta-analysis. *Clin Res Hepatol Gastroenterol* 2014;38:706–714. [PubMed: 25034355]
- [84]. Sun T, Tang Y, Sun D, Bu Q, Li P. Osteopontin versus alpha-fetoprotein as a diagnostic marker for hepatocellular carcinoma: a meta-analysis. *Onco Targets Ther* 2018;11:8925–8935. [PubMed: 30573979]
- [85]. Zhu W-W, Guo J-J, Guo L, Jia H-L, Zhu M, Zhang J-B, et al. Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. *Clin Cancer Res* 2013;19:3944–3954. [PubMed: 23719264]
- [86]. Shen Q, Fan J, Yang X-R, Tan Y, Zhao W, Xu Y, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a largescale, multicentre study. *Lancet Oncol* 2012;13:817–826. [PubMed: 22738799]

- [87]. Jang ES, Jeong SH, Kim JW, Choi YS, Leissner P, Brechot C. Diagnostic performance of alpha-fetoprotein, protein induced by vitamin K absence, osteopontin, dickkopf-1 and its combinations for hepatocellular carcinoma. *PLoS One* 2016;11:e0151069.
- [88]. Jia X, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. *Arch Med Res* 2014;45:580–588. [PubMed: 25446613]
- [89]. Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol* 2008;21:1011–1018. [PubMed: 18536657]
- [90]. Qiao S-S, Cui Z, Gong L, Han H, Chen P-C, Guo L-M, et al. Simultaneous measurements of serum AFP, GPC-3 and HCCR for diagnosing hepatocellular carcinoma. *Hepato-gastroenterology* 2010;58:1718–1724.
- [91]. Xing H, Qiu H, Ding X, Han J, Li Z, Wu H, et al. Clinical performance of alphaL-fucosidase for early detection of hepatocellular carcinoma. *Biomark Med* 2019;13:545–555. [PubMed: 31140827]
- [92]. Zhou Y, Yin X, Ying J, Zhang B. Golgi protein 73 versus alpha-fetoprotein as a biomarker for hepatocellular carcinoma: a diagnostic meta-analysis. *BMC Cancer* 2012;12:17. [PubMed: 22244200]
- [93]. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. *J Hepatol* 2005;43:1007–1012. [PubMed: 16137783]
- [94]. Giannelli G, Marinosci F, Trerotoli P, Volpe A, Quaranta M, Dentico P, et al. SCCA antigen combined with alpha-fetoprotein as serologic markers of HCC. *Int J Cancer* 2005;117:506–509. [PubMed: 15906357]
- [95]. Pozzan C, Cardin R, Picocchi M, Cazzagon N, Maddalo G, Vanin V, et al. Diagnostic and prognostic role of SCCA-IgM serum levels in hepatocellular carcinoma (HCC). *J Gastroenterol Hepatol* 2014;29:1637–1644. [PubMed: 24635038]
- [96]. Witjes CD, van Aalten SM, Steyerberg EW, Borsboom GJ, de Man RA, Verhoef C, et al. Recently introduced biomarkers for screening of hepatocellular carcinoma: a systematic review and meta-analysis. *Hepatol Int* 2013:1–6.
- [97]. Liu CH, Gil-Gomez A, Ampuero J, Romero-Gomez M. Diagnostic accuracy of SCCA and SCCA-IgM for hepatocellular carcinoma: a meta-analysis. *Liver Int* 2018;38:1820–1831. [PubMed: 29704434]

Keypoints

- HCC is a leading cause of cancer-related mortality and early detection has been associated with improved survival.
- Semi-annual ultrasound-based screening has several limitations which limits its effectiveness as an early detection strategy.
- Prior to clinical utilisation, biomarkers require rigorous evaluation to determine performance parameters *vs.* a comparable gold standard.
- Several existing biomarkers have undergone early-stage validation, with limited data in small phase III cohorts. Further reporting of phase III results in larger cohorts will enable selection of candidate biomarkers for further testing.
- Maturing well-powered phase III cohorts will facilitate validation of candidate biomarkers. Promising biomarkers can go on to larger phase IV/V validation studies for clinical implementation.

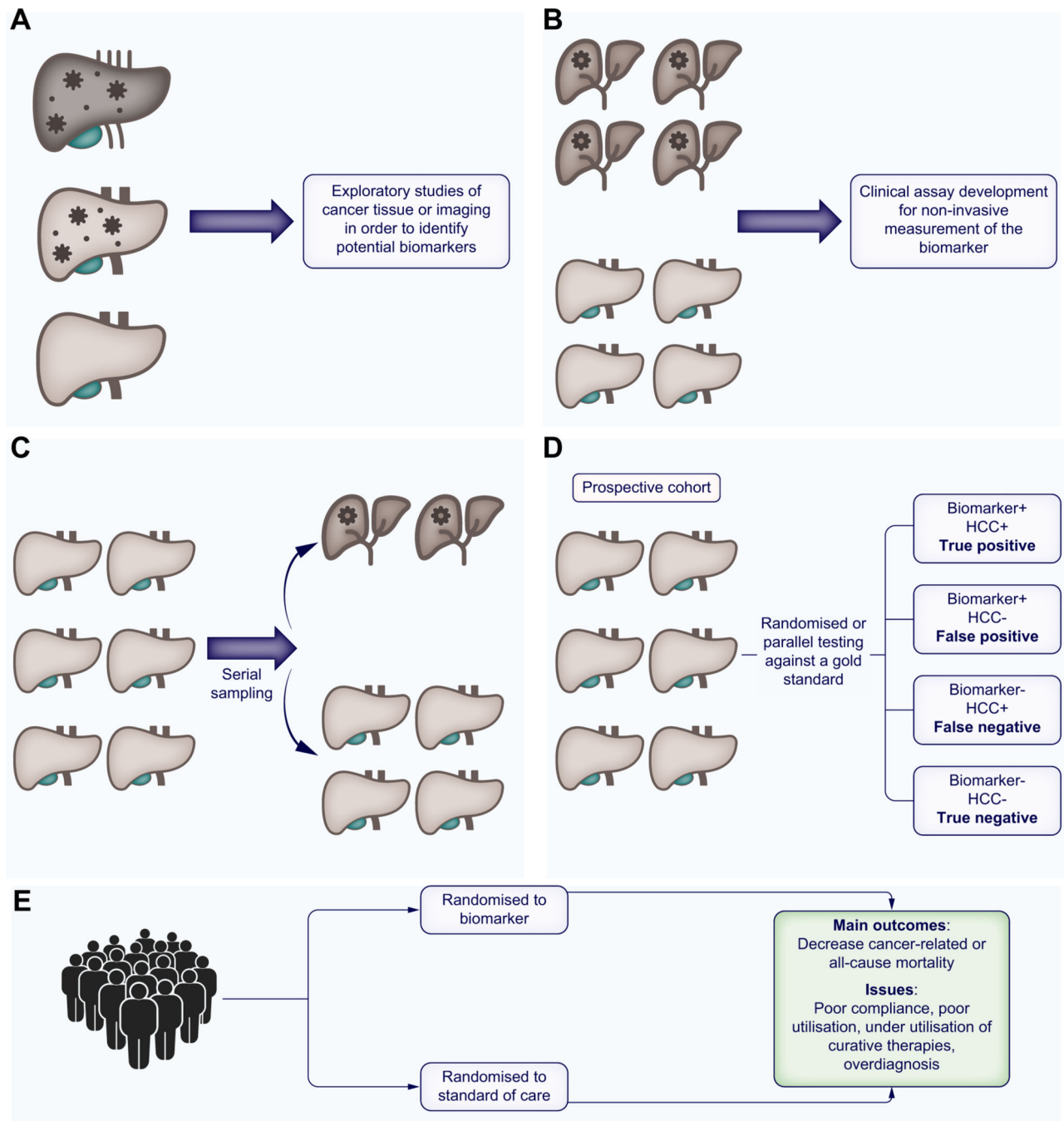


Fig. 1. Phases of biomarker validation.

(A) Phase I - preclinical exploratory to identify candidate biomarkers; (B) Phase II – clinical assay validation using a case-control design; (C) Phase III – longitudinal prospective-specimen collection in at-risk patients, with retrospective blinded-evaluation of biomarker performance; (D) Phase IV - prospective cohort studies or clinical utility trial where the biomarker is tested against a gold standard; (E) Phase V – cancer control studies to determine the impact of biomarker screening on cancer mortality. HCC, hepatocellular carcinoma.

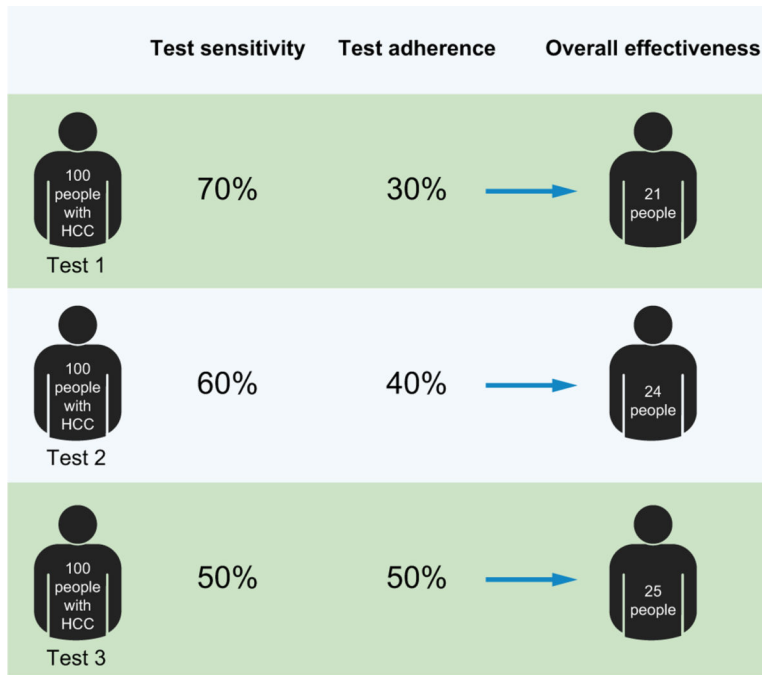


Fig. 2. Schematic of the interplay between test sensitivity and adherence that determines overall effectiveness.
HCC, hepatocellular carcinoma.

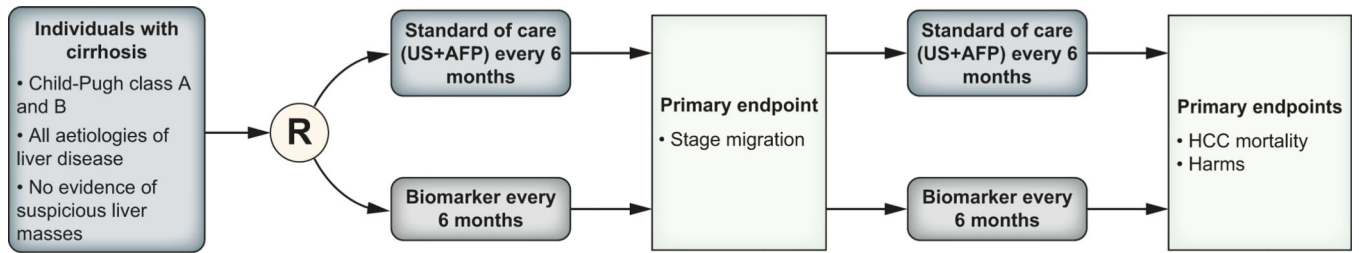


Fig. 3. Proposed schema for a phase IV clinical utility trial for HCC biomarker validation. AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma; US, ultrasound.

Table 1.

Select phase II biomarkers for early-stage hepatocellular carcinoma detection.

Biomarker	Early detection performance
Osteopontin ⁸²⁻⁸⁴	Sensitivity: 49% Specificity: 72%
Midikine ⁸⁵	Sensitivity: 87% Specificity: 90%
Dikkopf-1 ^{86,87}	Sensitivity: 41%–74% Specificity: 87%
Glypican-3 ⁸⁸⁻⁹⁰	Sensitivity: 55% Specificity: >95%
Alpha-1 fucosidase ⁹¹	Sensitivity: 56% Specificity: 69%
Golgi Protein-73 ^{92,93}	Sensitivity: 62%–79% Specificity: 62%–88%
Squamous cell carcinoma antigen ⁹⁴⁻⁹⁷	Data for early-stage HCC not available

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Table 2. Considerations when moving to a biomarker-based screening paradigm for HCC.

Issue	Potential hazards	Solutions
Test performance for early-stage HCC detection	<ul style="list-style-type: none"> Inferior sensitivity to imaging-based screening 	<ul style="list-style-type: none"> Non-inferiority design with acceptable margin Pragmatic trial design to incorporate the impact of adherence
False positive management	<ul style="list-style-type: none"> Lack of care pathways for false positives results Implications of a false positive result on future cancer risk 	<ul style="list-style-type: none"> Longitudinal trial design to understand and delineate the optimal care pathway for false positives and the associated future cancer risk
Costs	<ul style="list-style-type: none"> Lack of payor coverage Low HCC incidence will result in high numbers needed to screen for cancer detection 	<ul style="list-style-type: none"> Rational price setting based on cost-effectiveness analyses with contemporary inputs Calibrate intensity of screening based on patient risk
Blood processing	<ul style="list-style-type: none"> For central processing, errors in local blood collection and shipping Lack of standardisation of results across centres for markers that are processed onsite 	<ul style="list-style-type: none"> Quality control for central shipping of blood samples with adequate site training Calibration of local labs to ensure consistency of results across centres
Test reporting	<ul style="list-style-type: none"> Linkage of test result back to ordering provider/patient Providing a dichotomous or continuous test result 	<ul style="list-style-type: none"> Develop reporting pathways for test results Providing continuous test results depending on assay characteristics with interpretation (positive/negative)

HCC, hepatocellular carcinoma.