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Biomarkers for the Early Detection of Hepatocellular Carcinoma

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Abstract

Hepatocellular carcinoma (HCC) is a leading cause of cancer death worldwide, and the cancer with the fastest increase in mortality in the USA, with more than 39,000 cases and 29,000 deaths in 2018. As with many cancers, survival is significantly improved by early detection. The median survival of patients with early HCC is >60 months but <15 months when detected at an advanced stage. Surveillance of at risk patients improves outcome but fewer than 20% of those at risk for HCC receive surveillance, and current surveillance strategies have limited sensitivity and specificity. Ideally, blood based biomarkers with adequate sensitivity or specificity would be available for early detection of HCC; however, the most commonly used biomarker for HCC, alpha fetoprotein, has inadequate performance characteristics. There are several candidate serum proteomic, glycomic, and genetic markers that have gone through early stages of biomarker validation and have shown promise for the early detection of HCC, but these markers require validation in well curated cohorts. Ongoing prospective cohort studies will permit retrospective longitudinal (phase III biomarker study) validation of biomarkers. In this review, we highlight promising candidate biomarkers and biomarker panels that have completed phase II evaluation but require further validation prior to clinical use.

Introduction

Hepatocellular carcinoma (HCC) is the seventh most common cancer diagnosis worldwide with high associated mortality.(1) HCC is a unique malignancy, as it typically arises in the setting of chronic liver disease in particular cirrhosis, with competing risks of liver failure, contributing to its low 5-year survival rates of 18%-20%.(2) More than half of worldwide

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HCC deaths occur in Asia, due to endemic hepatitis B (HBV) infection. HCC incidence is rising in many Western countries due to the rising prevalence of nonalcoholic fatty liver disease (NAFLD), alcohol-related liver disease, and hepatitis C (HCV) related complications despite the availability of direct acting anti-viral therapies.(3-5)

Stage of HCC diagnosis is highly predictive of overall mortality. Early stage patients are eligible for curative therapies, including resection, ablative therapies, and liver transplantation, whereas late stage patients are generally only eligible for palliative systemic therapies with suboptimal response rates. As a result, 5-year survival exceeds 70% in patients with early stage HCC while it is less than 5% at advanced stages.(6,7) Unfortunately, due to poor utilization of surveillance, inadequate surveillance methods, and lack of risk-based strategies most patients are diagnosed at late stages.(8)

Surveillance for HCC is recommended in at risk patients, including those with cirrhosis of all etiologies, and certain populations with chronic HBV infection (Table 1).(9) Guidelines recommend HCC surveillance with abdominal ultrasound (US) with or without serum alpha-fetoprotein (AFP) measurement every 6 months.(9,10)

A recent meta-analysis showed that sensitivity of US based surveillance for HCC early detection is 45%.(11) Notably there was significant heterogeneity in sensitivity of US-based surveillance (21%-89%) across studies included in the meta-analysis, which highlights a limitation of imaging-based surveillance.(11) Other studies have also highlighted the harms of US surveillance due to suboptimal specificity leading to unnecessary further testing that may carry risks of complications.(12,13) Furthermore, the presence of cirrhosis and obesity, both more prevalent in Western patients when compared to Asians, have been shown to decrease sensitivity of abdominal US.(14) The limitations of US-based surveillance have been well documented and highlights the need for more objective and sensitive methods to conduct surveillance for HCC. In addition, US surveillance is intensive and requires a separate patient encounter (i.e. every 6 months in radiology) which can represent significant logistical barriers to attainment for both patients and providers.(15)

Ideally, validated blood based biomarkers with sufficient sensitivity and specificity for the early detection of HCC would be available. There are several candidate biomarkers that have been studied for HCC early detection, and herein we will review the current landscape of these biomarkers.

Biomarker Validation

The definition of a biomarker is broad, and includes any measurable substance, structure, or process that can detect or predict the outcome of a disease. Originally proposed by Pepe et al in conjunction with the Early Detection Research network (EDRN) of the National Cancer Institute (NCI), the discovery and validation of biomarkers require several phases prior to routine clinical usage.(16) (Figure 1) Initial discovery occurs in a preclinical exploratory phase followed by clinical assay validation which involves developing the assay for its measurement (Phase I). Clinical validation begins with conducting retrospective case control studies, comparing, preferably early stage cases and controls without cancer from a relevant

screening population (Phase II). Phase III validation involves longitudinal assessment of the biomarker to determine its performance in detecting preclinical disease. Late stage validation studies assess the biomarker performance in clinical practice and determine its impact in reducing the burden of a cancer in a population. Very few biomarkers in HCC have undergone adequate validation due to the lack of large, representative prospective cohorts with adequate duration of follow-up and outcomes (early stage HCC), which is the reason behind their limited clinical use. The NCI EDRN Hepatocellular carcinoma Early Detection Strategy study and ongoing prospective cohort studies will overcome this barrier.(17)

Of the biomarkers described below, AFP has been most extensively studied, and despite its limitations have been in use clinically for several decades. By contrast, most of the other biomarkers except for AFP-L3 and des-gamma carboxy prothrombin (DCP) have only been evaluated in few studies and their performance for early HCC detection in clinical settings, particularly among patients with non-viral liver disease, is unknown. (Table 2)

Biomarker Completed 5 Phases of Validation

Alpha fetoprotein (AFP)—AFP is the most commonly used biomarker in the early detection of HCC and the only biomarker which has been validated for clinical use. AFP alone is not currently included in societal guidelines for HCC surveillance due to concerns about specificity and limited sensitivity in the detection of early stage HCC.(18) However, a recent meta-analysis has shown that AFP can increase the sensitivity of HCC early detection when used in combination with abdominal US (63% vs 45% of US alone.)(11) False positive AFP elevations can occur with elevated serum alanine aminotransferase (ALT) level, seen in chronic hepatitis C and B infections.(19) Up to 40-50% of HCCs do not have elevated levels of AFP, limiting the sensitivity of AFP alone for HCC detection. Published cohort studies, including a large multi-center study funded by the NCI EDRN,(20) show the sensitivity of AFP for detecting early HCC ranges from 39-64% and specificity ranges from 76-97%. (21-25) Cut-off values for serum AFP vary widely across studies, however a value of 20 ng/mL is accepted as a valid threshold in the early detection of HCC. For patients undergoing surveillance, the change in AFP value over time is superior to single AFP values, in the detection of early stage HCC (receiver operating curve 0.81 vs 0.76).(26,27) The change in AFP has also been integrated into the recently validated Hepatocellular Carcinoma Early Detection Screening (HES) algorithm.(28) Thus, while AFP has been through the 5 phases of biomarker development, it's routine use as a part of the surveillance strategy for HCC early detection is controversial.

Biomarkers with limited Phase III validation data

AFP L3—AFP-L3, or lens culinaris agglutinin-reactive AFP, is a fucosylated glycoform of AFP that has been studied for the detection of early stage HCC.(29) While traditional AFP-L3 assay requires an AFP level above 10 ng/mL for detection, the use of a highly sensitive assay for AFP-L3 (hs-AFP-L3) makes measurements possible in patients with AFP levels as low as 2 ng/mL.(30) Unfortunately, while AFP-L3 has a better specificity for early HCC detection than AFP (~90%), its sensitivity is inferior (49-60%).(20,31,32) A biomarker validation study from Korea, including 42 patients with HCC, showed that AFP-L3 has an area under the receiver operating characteristic curve (AUROC) of 0.73 at the time of HCC

diagnosis, compared to 0.77 for AFP. For the 38 Barcelona Clinic Liver Cancer (BCLC) stage 0/A patients (i.e. early stage HCC) in this cohort, the AUROC of AFP was similar at 0.76 which improved to 0.81 when combined with AFP-L3. Additionally, AFP-L3 was significantly higher in patients with HCC 6 months prior to clinical diagnosis compared to controls.(33) In the EDRN HEDS Phase II validation including 131 patients with early stage HCC, the AUROC of AFP-L3 was 0.66 versus 0.80 for AFP alone.(20) One major limitation with AFP-L3 is that AFP itself has low sensitivity, and examination of any isoform will not improve sensitivity. Further phase III validation of AFP-L3 is needed, to confirm whether it has incremental value compared to or in combination with AFP alone.

Des-gamma carboxyprothrombin—Des-gamma carboxyprothrombin (DCP) is an abnormal prothrombin produced because of vitamin K insufficiency caused by dysfunctional intracellular transport mechanisms; defects in gamma-carboxylase enzyme; and cytoskeletal changes that impair vitamin K uptake as hepatocytes undergo malignant transformation.(34) Sensitivity and specificity of DCP in detecting early stage HCC ranges from 34-62% and 81-98%, respectively.(35) In the EDRN phase II study of 131 early HCC patients, DCP had an AUROC of 0.72.(20) Limited Phase III evaluation has demonstrated poor sensitivity in detecting pre-clinical HCC (12.1%).(36) Combining DCP and AFP levels can increase the sensitivity of DCP to 80% for large tumors (>3cm) and 70% for small tumors (2-3 cm). Recent data suggest DCP does not increase discriminatory power when combined with AFP and AFP-L3 for early HCC detection.(33) Despite lack of formal phase III or IV validation, DCP is used in many countries worldwide for HCC early detection. Based on the validation studies thus far, DCP alone does not appear to have sufficient performance characteristics for early stage HCC detection but may still have value as part of a biomarker panel.

Biomarkers with Phase II validation data

All of the below biomarkers have promising results in Phase II evaluation but still require Phase III validation given biomarker performance can be overestimated in Phase II studies.

Osteopontin—Osteopontin is an integrin-binding phosphoprotein that can mediate cell signaling involved in regulating tumor progression.(37-39) Osteopontin for HCC early detection, defined by BCLC stage 0/A, was evaluated in a meta-analysis of 4 studies which showed a sensitivity of 49% (95% CI: 42–56) and specificity of 72% (95% CI: 68–76) both comparable to the performance of AFP alone. (40,41) When osteopontin and AFP were combined the sensitivity improved to 73% (95% CI: 67–79) with little change in the specificity 68% (95% CI: 64–72). One of the studies included in the meta-analysis was a Phase II validation study of 78 patients with early stage HCC and 76 patients with cirrhosis. Osteopontin outperformed AFP alone for early detection (AUROC: 0.73 [95% CI: 0.62-0.85] vs AUROC: 0.68 [95% CI: 0.54-0.82])(38) and performance of osteopontin was further improved when combined with AFP, with AUROC for early stage HCC of 0.81 (95% CI: 0.70-0.91).

Midkine—Midkine (MDK) is a heparin binding growth factor involved in cell growth, invasion and angiogenesis during cancer progression.(42) MDK levels have been shown to be elevated in patients with very early stage HCC and decline following curative surgery.

MDK levels rise or remain elevated in patients with incompletely treated or recurrent HCC. (43) A phase II validation study included 119 BCLC 0/A patients, found that the sensitivity of MDK was 87% and specificity 90%, while AFP's sensitivity was 52% and specificity 35%. (44) The AUROC of MDK in this study was 0.92 for patients with BCLC 0/A HCC compared to patients with cirrhosis. The combination of MDK and AFP further improved the early detection rate of HCC to 96%. (44) In a small phase 3 study of patients with cirrhosis related to nonalcoholic steatohepatitis, MDK was not superior to AFP for the early detection of AFP, but was elevated in roughly half of the patients who did not have an elevation of AFP. (43)

Dikkopf-1—Dikkopf-1 (DKK1) is a glycoprotein that functions as a secretory antagonist of the Wnt/B-catenin signaling pathway. In one study of 1,284 patients (831 in the test cohort and 453 in the validation cohort), DKK1 concentrations were significantly higher in patients with HCC than controls in the test cohort, and values did not differ significantly between the control (cirrhosis patients and healthy controls) groups ($p < 0.001$). (45) The sensitivity for detection of early-stage HCC was 70-72% and specificity was 87-90% in the validation cohort (AUROC: 0.88). Combining DKK1 levels with AFP enhanced the detection rate of early-stage HCC (AUROC: 0.89). (45) In a separate Phase II study of predominantly hepatitis B infected patients in South Korea ($n=208$), the combination of AFP and DKK1 was similar to AFP alone for the detection of early stage HCC (AUROC: 0.63 vs 0.69). (46) These preliminary data suggest etiology of liver disease may be an important factor in DKK1 performance as an early detection biomarker; thus, its utility remains to be determined.

Glypican-3 (GPC-3)—Glypican 3, (GPC-3) is a cell-surface heparan sulfate proteoglycan that regulates cell proliferation and tumor suppression. (47-49) Meta-analysis of 19 Phase II biomarker studies found the sensitivity of GPC-3 for early detection of HCC is suboptimal when used alone (~55%), and increases to 76% when combined with AFP. However, the available data on early stage HCC detection was limited. (50) The specificity of GPC-3, is >95%, (50-52) suggesting its potential utility as a complementary biomarker to increase sensitivity of AFP or other serum biomarkers.

Alpha-1 fucosidase—Alpha-1 fucosidase (AFU) is a lysosomal enzyme that has been shown to be elevated in patients with HCC. In one Phase II biomarker study of 57 patients with early stage HCC, the sensitivity and specificity of AFU in the early detection of HCC was 56% and 69%, respectively. Combining AFU and AFP did not raise the sensitivity or AUROC to an acceptable level as AFP alone outperformed the combination. (53) The specificity of AFU is poor as it is also over-expressed in diabetes, pancreatitis, and hypothyroidism, and varies across patient race/ethnicities. (54) However, a small Phase III study of 27 patients found that AFU activity was elevated in 85% of patients at least 6 months before the detection of HCC indicating additional studies may be warranted. (55)

Golgi protein-73—Golgi protein-73 (GP-73) is a transmembrane protein expressed in epithelial cells and can be elevated in patients with HCC and advanced fibrosis secondary to hepatitis B or hepatitis C infection. It was first identified as a potential biomarker of HCC

through glycoproteomics.(56,57) In a Phase II study, GP-73 was found to have a sensitivity and specificity of 69% and 86%, respectively, for distinguishing between HCC and cirrhosis. (58) The sensitivity and specificity for detecting early stage HCC (BCLC 0/A) in this cohort was similar at 62% and 88%, respectively. Combining GP-73 and AFP increased sensitivity and specificity to 98% and 85% for differentiating all stages of HCC from cirrhosis, however performance of the combination in early stage HCC was not reported.(58) In one meta-analysis, the sensitivity of GP-73 was 79%, while the specificity was 62%, similar to the performance of AFP.(59) One major issue with GP-73 is the reliance on western-blot analysis for the accurate measurement of the isoform associated with HCC. ELISA based assays, which are inherently better suited for clinical use, have proven difficult to develop for the specific GP-73 isoforms associated with HCC.

Squamous cell carcinoma antigen—Squamous cell carcinoma antigen (SCCA) is a serine protease inhibitor that is present in squamous epithelium. SCCA is expressed by neoplastic epithelial cells and hepatocytes in which it promotes tumor growth through the inhibition of apoptosis. In addition to SCCA, the SCCA-immune complex (SCCA-IgM) have been investigated for the detection of HCC. SCCA has high sensitivity for HCC (89%), however it suffers from poor specificity (50%) in differentiating HCC from cirrhosis.(60,61) In a meta-analysis of 11 studies, SCCA had an AUROC of 0.80, while SCCA-IgM had an AUROC of 0.77. Unfortunately, performance in the detection of early stage HCC was not separately reported.(62,63)

Glycosylation variants

Fucosylated Glycoproteins: Based upon the knowledge that changes in biomarkers can occur at the cellular level and not the protein level, others have attempted to identify proteins with glycan changes that could be used as biomarkers of HCC (56,64-72). Increased levels of fucosylated proteins such as hemopexin (66,73-76), fetuin A (74,77,78), alpha1-antitrypsin (65,77,79-83), ceruloplasmin (64,81,84), haptoglobin (80,85-88), serum paraoxonase 1 (89,90), and histidine-rich glycoprotein (78,84) have been observed in the serum of patients with HCC, either by direct glycan sequencing or by lectin based methods. Fucosylation has also been observed directly in the tumor itself (91) and together these results strongly suggest that increased fucosylation, both core and outer arm, occurs on a large number of proteins.

Only a limited number of Phase I and Phase II studies have been conducted for the fucosylated glycoproteins. The most notable is fucosylated kininogen, which has been examined in several independent phase II cohorts (internal and external validation). (71,92,93). On its own, fucosylated kininogen is not an adequate biomarker, but when combined with AFP and other clinical factors appears to have excellent biomarker performance with an AUROC of 0.97 in one Phase II study which included 69 patients with early stage HCC.(94)

Glycosylated Haptoglobin—Another glycoprotein that has been identified as having altered glycosylation in HCC is haptoglobin. Initial work identified alterations in fucosylation and sialylation on this molecule along with other changes.(95) Subsequent work

has identified increased levels of branching on this molecule as well.(96-98) Similar to the other glycoprotein markers, individual performance is limited but these markers in combination with AFP and other clinical factors achieve sensitivities close to 80% at 95% specificity for early detection in Phase II studies.(95)

Notably, the analysis of protein glycoforms in plate based assays utilizing lectins are dramatically impacted by the presence of heterophilic antibodies in patients with liver fibrosis.(93,99-102) As most patients with HCC have advanced liver fibrosis, these antibodies have to be accounted for prior to analysis. Direct mass spectrometry based approaches have shown promise but will require some level of refinement and simplification before routine clinical use.(103-105)

Genomic Markers

MicroRNA—The aberrant expression of MicroRNAs (miRNA), which are circulating non-coding RNAs, can contribute to oncogenesis and cancer progression. Due to their inherent stability and their role in tumor proliferation, several studies have evaluated circulating miRNA as a biomarker for the diagnosis of HCC. Two specific miRNA, miRNA-21 and miRNA-199a, have been proposed as potential biomarkers for the early diagnosis of HCC. Serum levels of miRNA-21 have been found to be elevated in patients with HCC, and have shown promise in differentiating between cirrhosis and HCC in small Phase II studies, although the results of these studies can be difficult to interpret due to inadequate control patients and small numbers of early stage HCC patients.(106) There are several additional candidate miRNAs under investigation for the early detection and prognostication of HCC, and many are being studied individually or as components of miRNA panels combined with other biomarkers in Phase I and Phase II studies.(107,108) There are challenges with miRNA analyses due to variable annotation; however, efforts are underway to ensure uniformity in characterization of miRNA molecules.(109,110)

DNA Mutations—Cells derived from HCC tissue harbor genetic mutations and epigenetic modifications that can be involved in the oncogenesis of HCC (111,112), while others may be “passengers” and not of biological consequence, in themselves. In large scale analysis of HCC-specific mutations, deletions or epigenetic modifications occur in at least one of 31 different genes.(111) The most frequent mutation associated with HCC is the TERT promoter, with approximately 60% of all HCC containing these mutations.(113) The next most commonly mutated genes are in p53 and CTNNB1, which are mutated in 25-35% of the HCC tissues.(114) Cell free DNA in plasma and/or urine derived from HCC tissue, containing these abnormalities, has been detected and proposed for use in risk stratification and cancer detection.(115) In one study, detection of TERT promoter mutated DNA in the plasma was 47% sensitive for HCC (all stages), overall, but reached 87% when restricted to males with chronic HCV.(116) Sensitivities and specificities vary greatly with different genes tested and populations studied, complicating implementation of these assays, and practical use awaits further development.

Epigenetic Modifications/DNA Methylation—Methylation of DNA is often involved in the carcinogenesis of HCC and thus studies have been conducted investigating circulating

cell free methylated DNA for the early detection of HCC.(117) Several panels of DNA methylation signature exist; however to date, there has been limited validation and adequate comparison to controls for clinical use. One panel of 4 methylated markers, in combination with AFP and AFP-L3 showed a 71% sensitivity for early stage HCC (BCLC 0/A) with a specificity of 90% and an AUROC of 0.91. This panel compared well to AFP alone (sensitivity 21, specificity 98%; AUROC 0.81).(118) While these initial results are promising, these data are awaiting further validation.

Algorithms

HCC tumor biology is heterogeneous, with carcinogenesis involving several genetic alterations even within a single patient.(119) This in part explains the limited performance of any single biomarker. Algorithms/panels comprising multiple biomarkers encompassing heterogeneous pathways in carcinogenesis and tumor biology, and clinical factors associated with risk of HCC such as sex, age and etiology of liver disease have been developed and undergone validation in order to improve the sensitivity and specificity of HCC early detection. (Table 3)

Gender, Age, AFP-L3, AFP, and Des-carboxy-prothrombin (GALAD) Score—

The GALAD score includes gender, age, AFP, AFP-L3 and DCP.(120) The ability of the GALAD score to discern between HCC, cirrhosis, and other hepatobiliary malignancies (e.g. cholangiocarcinoma) has been examined. Derivation of this model was based on data from 833 patients (394 with HCC and 439 with chronic liver disease) in two centers in the United Kingdom, and the model was validated in independent cohorts of 6834 patients in Japan, Germany and Hong Kong (2430 with HCC and 4404 with chronic liver disease). A total of 1038 patients across all centers had early stage HCC, defined as tumor size < 3cm. Overall sensitivity ranged from 80-91%, while the specificity ranged from 81-90% across the cohorts with an AUROC of 0.85-0.95.(121) A Phase 2 validation study in patients with nonalcoholic steatohepatitis with vs. without HCC from 8 centers in Germany had an AUROC of 0.91 for early detection with 68% sensitivity and 95% specificity.(122) There are ongoing studies to provide Phase III validation of this panel in prospective cohorts.

Doylestown Algorithm—The Doylestown algorithm comprised log AFP, age, gender, alkaline phosphatase, and alanine aminotransferase. In a Phase II study of 69 patients with early stage HCC (stage T1 or T2 disease) and 93 cirrhosis controls, the addition of fucosylated kininogen to the algorithm had a higher AUROC than both the Doylestown algorithm and AFP alone (0.97 vs 0.93 and 0.80, respectively). Notably, in 29 patients with early HCC and an AFP<20, the Doylestown algorithm with fucosylated kinnogen had a 89% detection and maintained an AUROC of 0.97.(92,123) This level of performance should be confirmed in larger Phase II studies in addition to Phase 3 validation studies, which are in progress.

Hepatocellular Carcinoma Early Detection Screening (HES) Algorithm—The HES algorithm including age, AFP, rate of AFP change, alanine aminotransferase, and platelet count has been validated in a Phase 2 study using data from the Veterans Administration.(124) In a cohort comprising 4,804 patients with predominantly HCV-related

HCC, the HES algorithm outperformed AFP alone in HCC detection in the 6 months prior to clinical diagnosis with a sensitivity of 53% vs 48% at a 10% false positive rate. The validation was limited, however because it did not include the performance in patients with early stage HCC and its performance in other non-viral etiologies of liver disease requires further validation.(124)

Future Directions

While several candidate biomarkers for HCC early detection exist, there has been little translation to clinical practice largely because of lack of well-annotated cohorts for validation studies. There are, however, several prospective studies including the EDRN HEDS study(17) and the Texas Hepatocellular Carcinoma Consortium, that may provide the opportunity to perform large scale Phase III validation studies.(125) These cohorts will allow for validation of individual biomarkers as well as algorithms combining multiple biomarkers and clinical data, in the detection of early HCC for viral as well as non-viral etiologies of liver disease, which is important given the changing epidemiology of HCC in the USA and worldwide.(126) Efforts are underway to better risk stratify patients with regards to their risk of HCC. Patient level factors, such as age and gender, are currently included in many of the existing biomarker algorithms. Further understanding of individual risk based on genetic profile or other biomarkers, may allow for personalized surveillance strategies of patients at risk for HCC(127,128) such that high risk groups may be identified for more intense surveillance while low risk groups may forego surveillance. Finally, studies are underway to develop and validate imaging techniques (e.g. abbreviated MRI) and technologies, such as digital extraction of high dimensional quantitative data from imaging (e.g. radiomics), in order to improve the sensitivity of HCC early detection and for patient risk stratification that may complement serum biomarkers.(129,130)

Conclusions

There are several candidate biomarkers that have the potential to dramatically improve the early detection of HCC. Approaches that combine patient risk stratification and multiple candidate biomarkers will likely yield the best performance characteristics. Support for the establishment and long-term follow-up of well annotated cohorts of diverse race/ethnicity and etiologies of liver disease, and proper collection and storage of biospecimens is crucial for validation of new biomarkers and algorithms in Phase 3 studies, with the goal that some will qualify for progression to Phase 4/5 studies and ultimately contribute to improving outcomes in patients with HCC.

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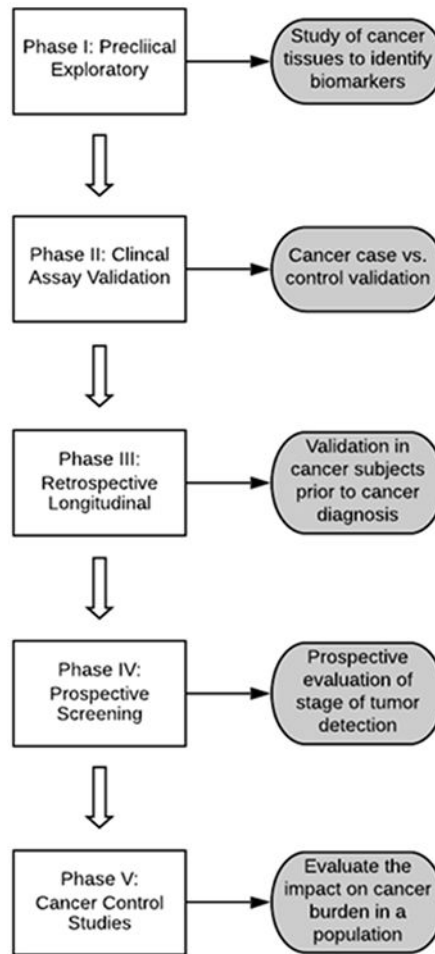


Figure 1.
Phases of clinical biomarker validation

Table 1.

Populations recommended for surveillance for hepatocellular carcinoma

Population	Annual Incidence
Cirrhosis of any etiology	1%-8%
Asian males with chronic hepatitis B 40 years of age	0.4%-0.6%
Asian females with chronic hepatitis B 50 years of age	0.3%-0.6%
African patients and North American blacks with chronic hepatitis B 20 years of age	0.3%-0.6%

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Table 2.

Candidate biomarkers for hepatocellular carcinoma early detection

Biomarker	Phase of Development	Early Detection Performance	AUROC for early detection
Alpha fetoprotein (AFP)(20-25)	5	Sensitivity: 39-64% Specificity: 76%-97%	0.75-0.82
Lens culinaris agglutinin-reactive AFP (AFP-L3) (20,33)	2/3	Sensitivity: 49-62% Specificity: 90%	0.66-0.76
Des-gamma carboxyprothrombin (DCP)(20,33)	2/3	Sensitivity: 34-40% Specificity: 81-98%	0.72
Osteopontin(38,40,41)	2	Sensitivity: 49% Specificity: 72%	0.73
Midikine(44)	2/3	Sensitivity: 87% Specificity: 90%	0.923
Dikkopf-1(45,46)	2	Sensitivity: 41%-74% Specificity: 87%	0.61-0.88
Glypican-3(50-52)	2	Sensitivity: 55% Specificity: >95%	0.793
Alpha-1 fucosidase(53)	2	Sensitivity: 56% Specificity: 69%	0.506
Golgi Protein-73(58,59)	2	Sensitivity: 62%-79% Specificity: 62%-88%	Not available
Squamous cell carcinoma antigen (SCCA)(60-63)	2	Data for early stage HCC not available	Data for early stage HCC not available

Table 3.

Algorithms that have been evaluated for the detection of hepatocellular carcinoma

Algorithms	Components	Phase of Development	Early Detection Performance	AUROC for Early Detection
GALAD score(121)	Gender, age, AFP, AFP-L3 and DCP	2	Sensitivity: 68% Specificity: 95%	0.85-0.95
Doylestown+Fucosylated kininogen(94)	Fucosylated kininogen, log AFP, age, gender, alkaline phosphatase, and alanine aminotransferase	2	7	0.97
Hepatocellular Carcinoma Early Detection Screening (HES) Algorithm(124)	Age, AFP, rate of AFP change, alanine aminotransferase, and platelet count	2/3	Data for early stage HCC not available.	Data for early stage HCC not available.
Methylated DNA Panel(118)	4 Methylated DNA markers, AFP, AFP-L3	2	Sensitivity: 71% Specificity: 90%	0.91

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