HHS Public Access

Author manuscript

Clin Gastroenterol Hepatol. Author manuscript; available in PMC 2016 February 01.

Published in final edited form as:

Clin Gastroenterol Hepatol. 2015 February ; 13(2): 237–245. doi:10.1016/j.cgh.2013.10.038.

Update on biomarkers for hepatocellular carcinoma

Roongruedee Chaiteerakij[1,2], **Benyam D. Addissie**[1], and **Lewis R. Roberts**[1]

[1]Division of Gastroenterology and Hepatology, College of Medicine, Mayo Clinic and Mayo Clinic Cancer Center, Rochester, MN [2]Department of Medicine, Faculty of Medicine, Chulalongkorn University and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Bangkok, Thailand

Abstract

A number of novel biomarkers for hepatocellular carcinoma (HCC) have been recently identified by advanced genomic, proteomic and metabolomic technologies. New biomarkers are in development for HCC diagnosis, for prediction of patient and treatment outcomes, and for individualizing the use of targeted therapies. Nonetheless, the major current use of HCC biomarkers remains surveillance for early HCC detection, with the goal of reducing mortality from HCC. Most new HCC biomarkers are in phase 1 or 2 biomarker studies, and further investigation is needed to determine whether they have utility in clinical practice. The diagnostic or predictive performance of individual biomarkers is limited by the highly heterogeneous nature of HCC tumors. Consequently, there is no single perfect biomarker for HCC. To maximize biomarker performance, future trends in the use of biomarkers will therefore include the combination of multiple biomarkers or the combination of biomarkers with imaging, clinical parameters or other laboratory tests in diagnostic, predictive or prognostic panels. This review provides a brief update on the known and novel promising biomarkers for HCC. The challenges and key considerations in the phases of biomarker development and the application of biomarkers in clinical practice are also discussed.

Correspondence: Lewis R. Roberts, M.B. Ch.B., Ph.D. (Corresponding Author), Division of Gastroenterology and Hepatology, College of Medicine, Mayo Clinic, 200, First Street SW, Rochester, MN 55905, Roberts.lewis@mayo.edu; Phone: 507-538-4877; Fax: 507-284-0762.

Author contribution

Roongruedee Chaiteerakij: study concept and design; acquisition of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content

Benyam D. Addissie: study concept and design; acquisition of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content

Lewis R. Roberts: study concept and design; acquisition of data; drafting of the manuscript; critical revision of the manuscript for important intellectual content; study supervision

Disclosure: None

Keywords

Alpha-fetoprotein; Cancer biomarker; Predictive biomarker; Prognostic biomarker; Hepatocellular carcinoma

Definition of cancer biomarkers

Cancer biomarkers are molecules or substances that are objectively measurable in cells, tissues, or body fluids and that indicate the presence of cancer or predict the risk of cancer development. Traditionally, cancer biomarkers are proteins detected in serum or plasma. Recent technologic advances in genomics, proteomics and metabolomics are enabling better elucidation of cancer biology and characterization of key molecular events during carcinogenesis. This is allowing the discovery of novel HCC biomarkers, including DNA – fusion genes, genetic mutations and epigenetic changes, messenger RNA (mRNA), non-coding RNA – including microRNAs (miRNA), long non-coding RNAs (lncRNA) and other species, proteins and post-translational protein modifications (e.g. phosphorylation), metabolites and antibodies. These different analytes may be detectable in whole blood, white blood cells, serum, plasma, urine, normal tissue (e.g. buccal mucosa), tumor tissue or adjacent benign liver tissue.

Application of HCC biomarkers for clinical management

The serum alpha-fetoprotein (AFP) has been the most commonly used HCC biomarker, functioning as a risk assessment tool in patients with cirrhosis, as a screening tool for early HCC detection, as a diagnostic tool, and as a prognostic tool for tumor recurrence or patient survival. Screening is the performance of a diagnostic test in individuals at risk of HCC who have no symptoms or other *a priori* reason to suspect the presence of HCC. Surveillance is the repeated application of a screening test. More recently, the scope of applications for HCC biomarkers has expanded beyond diagnostic and surveillance/screening purposes. HCC biomarkers can be used to identify at-risk populations, stratify patients for clinical trials, tailor therapy, and predict treatment response (Figure 1).

Challenges to the use of biomarkers in clinical practice

The difficulties with developing highly sensitive and specific diagnostic, predictive and prognostic cancer biomarkers stem from two fundamental issues: the molecular heterogeneity of individual persons, and the molecular heterogeneity of cancers. There is therefore first a difficulty with establishing a baseline, “normal”, value of any biomarker, and second, an appreciation that no unique marker is present in all cancers of a particular tissue type. Thus, from a philosophical perspective, two things are necessary to develop the perfect biomarker for any disease. First, each person has to serve as their own control - in other words, ideally, we would collect a blood, urine, stool, tissue, expired air or other sample from each person multiple times during their lifetime and use these to ascertain the changes in individual biomarkers over time. Second, we need to develop highly sensitive and specific assays for a large selection of disease-related biomarkers, including genes, mRNAs, non-coding RNAs, proteins, post-translational protein modifications, and

biochemical metabolites. This will allow us to prospectively acquire multiple molecular and physiologic data points for each individual. With the anticipated advances in computing capacity it should be feasible to analyze the large amounts of data generated in a timely fashion and use it to optimize health and minimize illness for each individual.[1] Currently, given the absence of the first two requirements, a key strategy to optimize the information acquired from currently available biomarkers is to develop methods for using combinations of biomarkers to achieve acceptable test performance. One typical example is the fluorescent in situ hybridization (FISH) test for the diagnosis of cancer in suspicious biliary strictures; no one marker provides acceptable sensitivity and specificity, but the assessment of polysomy using a combination of four markers has markedly improved sensitivity and specificity for the diagnosis of cholangiocarcinoma.[2]

Phases of biomarker development for early HCC detection[3]

Although the scope of uses of HCC biomarkers has been broadened, the major purpose of HCC biomarkers is early HCC detection within a surveillance program, with the goal of reducing mortality from HCC. To achieve this goal, biomarkers need to be established through the following phases:

Phase 1 (Preclinical exploratory studies)

The aim is to identify potential markers by (1) comparing the differences in expression of genes, proteins or other analytes between cancer vs. normal tissue, or (2) detecting differences in the spectrum of circulating antibodies in patients with cancer compared to control individuals.

Phase 2 (Clinical assay development and validation, Case-control studies)

A clinical assay is developed to measure the biomarkers in biospecimens that can be obtained by less invasive methods (e.g. blood, urine, stool, or exhaled air). Biospecimens are obtained from established HCC cases and non-HCC control subjects representative of the target screening population. A receiver operating characteristic (ROC) curve is generated to assess the diagnostic performance of the assay. The reproducibility of the assay is also evaluated within and between laboratories.

Phase 3 (Retrospective longitudinal repositories studies)

The ability of an assay to detect preclinical HCC is assessed by obtaining biospecimens at regular intervals from cohorts of individuals at risk for cancer, e.g. those with established cirrhosis, and following the cohort for development of cancer over time. New biomarkers can then be assessed for their ability to predict the subsequent development of cancer. If the assay can distinguish those who will subsequently develop cancer from controls who do not develop cancer months or years before clinical presentation, the criteria for a positive screening test are then defined for phase 4 studies.

Phase 4 (Prospective screening studies)

The aim is to determine the detection rate and false-referral rate (or false positive rate) of a biomarker assay. The assay is applied for screening of a large cohort of the target population

for HCC screening. Subjects testing positive are referred for further investigation to establish the diagnosis of HCC. The detection rate is the proportion of screened subjects who test positive and have HCC, while the false-referral rate is the proportion of screened subjects who test positive but do not have HCC. Since patients in the false-referral group may be referred for downstream investigations that may expose them to physical or psychological harm, this provides a measure of the negative consequences of the assay.

Phase 5 (Cancer control randomized studies)

Finally, in the most rigorous proof of the clinical utility of a biomarker, a prospective randomized study is performed to determine whether use of the screening test can reduce HCC mortality in the target population. At-risk HCC subjects are randomly assigned into 2 groups, those who undergo and those who do not undergo the screening test, and will be followed up to compare their survival outcomes.

Considerations for applying HCC biomarkers in clinical practice

New biomarkers should ideally be evaluated through all 5 phases of biomarker development before being used as a screening tool in practice. However, it may be impractical to conduct a phase 5 randomized study to prove assay efficacy.

An assay should be validated before being routinely used in a particular population because the positive and negative predictive value of the assay varies depending upon the disease prevalence in each population.

The key measure of the overall assay performance is the area under the ROC curve (AUROC), which is a plot of the sensitivity vs. 1-specificity. The quoted sensitivity and specificity of an assay generally varies depending on the designated cut-off. An optimal cut-off may be estimated that maximizes the assay sensitivity and specificity, however, ultimately, the selection of the best cut-off depends on the purpose for which the biomarker will be used, i.e. a cut-off that provides a high sensitivity may be most appropriate for HCC screening while a cut-off that provides high specificity is most appropriate for confirming the HCC diagnosis.

Dynamic changes in biomarker levels over time may be more useful than a single measurement in isolation.[4] However, most publications report biomarker performance calculated from a single measurement, thus there is limited scientific proof of the utility of changes in biomarker levels over time. A recent U.K. study reported that elevation of AFP over time was the trigger for further investigation to confirm the diagnosis of HCC in 10% of HCC patients.[5] In real-life practice, experts frequently use the dynamic changes of biomarker values rather than a single measurement. The assay performance in clinical practice may therefore be better than that reported in the literature.

Biomarker performance may vary depending on the etiology of liver disease. For example, the AUROC (95% confidence interval – 95% CI) of AFP in differentiating cirrhosis vs. HCC in HCV-infected patients was 0.64 (0.49–0.80) whereas the AUROC (95%CI) in

differentiating HBV-related cirrhosis or chronic HBV infection vs. HBV-induced HCC was 0.90 (0.84–0.97).[6]

KNOWN HCC BIOMARKERS

1. Biomarkers for prediction of HCC risk

The HCC-4 risk score for predicting HCC risk in HCV-infected patients with all stages of liver fibrosis was developed by combining AFP with other patient and laboratory factors, including age, gamma globulin and platelet count. The score predicted the annual risk for HCC development with an AUROC of 0.80.[7] For HBV-infected patients, an HCC risk predictive model was developed using viral factors, including HBV DNA, HBsAg level, HBeAg status, and HBV genotype, and host factors, including age, gender, family history of HCC and ALT.[8] This model achieved AUROCs of 0.86, 0.86 and 0.83 for predicting 5, 10 and 15 year-risk, respectively.[8] The HCC-4 risk score and the HBV risk predictive model require external validation. External validation is critically important for predictive models as the models can show overfitting to the cohort used to develop the model. Consequently, the model performance may drop substantially when the model is applied to other populations.

2. Biomarkers for surveillance for early HCC detection

To date, AFP is the only HCC biomarker that has been studied through to phase 5 of biomarker development.[9] A prospective cluster randomized trial conducted in Shanghai, China showed that a surveillance program using AFP and liver ultrasound performed every 6 months resulted in a 37% reduction in HCC mortality.[9] Another population-based cohort study of HBV-infected Alaska natives showed that surveillance using AFP alone every 6 months improved survival.[10] As compared to historical controls who were not screened, over 16 years follow up, prospectively-screened patients had significantly longer survival.[10] Because both studies were conducted in chronically-infected HBV patients, the benefit of AFP may not be directly extrapolated to patients with other chronic liver diseases. Despite these findings, imaging currently remains the backbone of HCC surveillance and the AFP is used to complement ultrasound. Current evidence is conflicting as to whether AFP provides additional value when added to ultrasound.[11, 12]

3. Biomarkers for HCC diagnosis

There is variation in the performance of AFP and AFP-L3 in early HCC diagnosis in different at-risk populations. Generally, AFP is more sensitive than AFP-L3%, which measures the AFP isoform that binds to *Lens culinaris* agglutinin. The sensitivity and specificity of AFP at a cutoff of 10.9 ng/mL and AFP-L3% at a cutoff of 1.7% were 65% and 82% for AFP and 37% and 94% for AFP-L3%, respectively.[13] The combination of AFP with AFP-L3% or DCP only slightly improved the AFP performance for early HCC diagnosis (Figure 2).

DCP, or protein induced by vitamin K absence/antagonist-II (PIVKA-II), is an abnormal prothrombin resulting from defective post-translational carboxylation of the prothrombin precursor. The serum DCP performance for HCC diagnosis varies among studies. Marrero *et*

al. reported DCP at a cut-off of 125 mAU/mL better distinguished HCC from chronic liver diseases and cirrhosis than AFP at a cut-off of 11 ng/mL (sensitivity of 89% vs. 77% and specificity of 95% vs. 73%) whereas Nakamura *et al.* reported AFP outperformed DCP for the diagnosis of HCC <3 cm but DCP had better performance than AFP for the diagnosis of HCC >5 cm.[14,15] These differences may be due to population differences in patient and tumor characteristics. DCP is more likely to be elevated in patients with more advanced HCCs (e.g. larger tumors, vascular invasion or metastasis).[15, 16] Additionally, non-specific elevation of DCP can occur in vitamin K deficiency from impaired liver function or administration of vitamin K antagonists.

Although the diagnostic performance of AFP-L3 and DCP has been studied in the U.S., it is important to note that the FDA-approved indications for AFP-L3 and DCP are for risk-stratification of chronic liver disease patients, rather than in screening for HCC. ***GPC3***, a plasma membrane bound heparan sulfate proteoglycan, regulates cell growth by modulating activities of several tyrosine kinases and the Wnt signaling pathway. Serum GPC3 had comparable performance to AFP for HCC diagnosis with AUROC of 0.81.[17] When used in combination with AFP, the sensitivity increased from 52% (AFP) or 57% (GPC3) to 77% (AFP plus GPC3) without a significant decrease in specificity.[17]

4. Prognostic biomarkers for predicting patient survival

Single or serial AFP measurements can be used as predictors of survival and outcome of HCC patients after therapy. A preoperative AFP >100 ng/mL was associated with a higher risk of recurrence after surgical resection.[18] However, the AFP level was not prognostic for survival of Child A cirrhotic patients with a single HCC ≤3 cm treated with curative intent.[19] Patients who had HCCs within the Milan criteria with an AFP >15 ng/mL at the time of transplantation had worse survival than patients who underwent transplantation for non-HCC indications.[20] Radiologic response and survival after locoregional therapy or systemic chemotherapy can also be predicted by the change of AFP after treatment. Three months after chemoembolization or radioembolization a >50% decrease in AFP from a baseline value of >200 ng/mL was associated with tumor response; similarly a decrease in AFP >20% from a baseline value of >20 ng/mL was significantly associated with better survival after 2 cycles of systemic chemotherapy.[21, 22]

The performance of AFP in predicting survival of HCC patients improved when the AFP was combined with patient and tumor characteristics, including the Model For End-Stage Liver Disease (MELD) score, albumin, tumor size and number, vascular invasion and metastasis in the Model for Estimating Survival in Ambulatory Hepatocellular Carcinoma patients (MESIAH; score calculator available at http://www.mayoclinic.org/meld/mayomodel10.html).[23] The MESIAH model outperforms BCLC, CLIP and JIS scores in predicting patient survival and has been already validated externally.[23]

5. Predictive biomarkers for response to treatment

It is controversial whether the AFP is useful for predicting response to sorafenib treatment.[24,25] Nakazawa *et al.* reported that an increase in AFP >20% within 4 weeks after sorafenib treatment was associated with shorter survival, whereas Llovet *et al.* found a

change AFP from baseline to week 12 after sorafenib initiation did not predict survival or time to progression. It is important to note that AFP is not considered a standard means of assessing sorafenib response; imaging is the currently accepted standard for assessing sorafenib response.

Maximizing the utility of currently available HCC biomarkers

Recent efforts have focused on strategies for maximizing the utility of AFP, AFP-L3%, DCP, and GPC3, which have been investigated for years but have not reached the stage of widespread clinical utility in Europe or North America. The AFP has been used for many years worldwide as a HCC biomarker, and the AFP-L3% and DCP have been used for several years in Asia, particularly in Japan, as an adjunct to ultrasound and AFP in HCC surveillance. However, studies in Western countries have demonstrated that the AFP, AFP-L3%, and DCP have relatively low sensitivity for the early HCC detection. Consequently, their use has become controversial, with current U.S. and European guidelines recommending against their use in HCC surveillance. Since few clinicians will act on the result of a single biomarker test in isolation, recent efforts have focused on integrating the time trends in biomarker levels into clinical decision-making. For example, the variability of AFP over time, determined by the standard deviation of the AFP, has been shown to be more useful than the value of AFP itself for early HCC detection.[4]

Advances in techniques for detecting and measuring biomarkers have been used to improve the initial limited performance of conventional DCP and GPC3 assays. A major problem of the conventional DCP assay is the low specificity; this has been addressed by the use of a novel DCP assay. The new DCP assay uses a sandwich electrochemiluminescence immunoassay with 2 monoclonal antibodies, the P-11 and P-16 antibody (designated NX-DCP), to detect non-specifically elevations of DCP due to vitamin K deficiency, in contrast, the conventional assay uses the MU-3 antibody which detects both DCP elevations induced by HCC and DCP elevations induced by vitamin K deficiency. Because NX-DCP is not elevated in HCC, patients with HCC have a higher DCP/NX-DCP ratio than non-HCC individuals. The new DCP/NX-DCP ratio therefore has increased the specificity for HCC diagnosis from 62% for DCP only to 92% for the DCP/NX-DCP ratio, albeit with a slight decrease in sensitivity.[26] There are also ongoing attempts to improve the serum GPC3 assay; a new sandwich ELISA assay improved the specificities for HCC diagnosis to 87–99%, however, the sensitivity remained low at 40%.[27]

Although several potential HCC biomarkers have been reported, AFP will likely continue to be the most commonly used HCC biomarker in practice over the next few years, despite its limited sensitivity and specificity. Additional approaches that are being explored to improve the AFP performance in diagnosis and prognostic prediction include the use of AFP in combination with other biomarkers, laboratory or clinical parameters. For example, as compared to AFP alone at a cut-off of 19.8 ng/mL, when AFP was used in combination with vascular endothelial growth factor, the sensitivity for HCC detection increased from 68% to 96% and the specificity increased from 75% to 85%.[28]

NEWER BIOMARKERS FOR HCC

1. Biomarkers of HCC risk prediction

Genome wide association studies (GWAS) have identified a number of single nucleotide polymorphisms (SNPs) associated with HCC susceptibility (Table 1).[29–34] Although these results are encouraging, there are practical challenges to the use of SNPs for predicting HCC risk due to several reasons. First, the results of GWAS studies vary across different ethnic populations. More importantly, the odds ratio (OR) of each susceptibility SNP identified thus far has been less than 1.5, the cut-off OR considered to be clinically meaningful. This reflects the immense genetic heterogeneity of populations and suggests that using a single SNP as a genetic test for HCC risk prediction may not be feasible. The development of a panel of genetic variants would likely be more informative and potentially more useful for predicting HCC risk. Additionally, risk prediction models including both genetic and non-genetic risk factors for HCC could address the complexity of the interaction between host genetic risk factors, viral factors and other environmental risk factors in contributing to HCC development. This approach was taken by Guyot *et al.* who developed a risk predictive model for HCC development in alcoholic cirrhosis patients.[35] The model including the rs738409 GG genotype in the *PNPLA3* gene, age, gender and BMI predicted HCC risk at 3 and 6 years.

2. Biomarkers for early HCC detection

Osteopontin—Osteopontin is a glycoprotein produced by a number of different cell types, particularly bone and epithelial cells, and highly expressed in various cancers, including HCC. For differentiating early HCC from cirrhosis, the plasma osteopontin at an optimal cut-off of 91 ng/mL was found to be superior to AFP at a cut-off of 20 ng/mL, with an AUROC (95%CI) of 0.73 (0.62–0.85) vs. 0.68 (0.54–0.82), a sensitivity of 75% vs. 46% and a specificity of 62% vs. 93%, respectively.[6] The combination of osteopontin and AFP at these cutoffs had better performance than either test alone, with an AUROC (95%CI) of 0.81 (0.70–0.91), a sensitivity of 83% and a specificity of 63%.[6] Further studies are needed to validate the benefit of using osteopontin as a complementary test to AFP for early HCC diagnosis.

Golgi Protein 73 (GP73)—GP73 is a transmembrane protein localized in the Golgi complex. Its expression is upregulated in chronic liver diseases, and substantially elevated in HCC. Whether GP73 is a better biomarker than AFP for HCC diagnosis remains controversial, with the available results varying with the GP73 assay method. Serum GP73 measured by immunoblot was superior to AFP for differentiating early HCC from cirrhosis.[36, 37] However, serum GP73 measured by ELISA was not better than AFP for early HCC diagnosis.[38]

Circulating miRNAs—Given that a large number of miRNA are dysregulated in HCC, miRNA levels in serum, plasma or urine have been explored for their utility as HCC biomarkers. Of the miRNAs assessed thus far, increased serum or plasma miR-21 of HCC patients has been the most frequently reported.[39] Compared to AFP at a cut-off of 19.0 ng/ml, plasma miR-21 had an only slightly better performance for distinguishing HCC from

chronic hepatitis, with an AUROC (95%CI) of 0.77 (0.69–0.86) vs. 0.74 (0.66–0.82), resulting in a sensitivity of 61% vs. 60% and a similar specificity of 83%.[40] However, when AFP and miR-21 were combined, the assay performance improved to an AUROC (95%CI) of 0.82 (0.74–0.90), sensitivity of 81%, and specificity of 77%.[40]

Blood and urine metabolomics—Advanced chromatography and mass spectrometry technologies are enabling the detection of small-molecule metabolites produced by dysregulated metabolic pathways during hepatocarcinogenesis. Canavaninosuccinate (CSA), an organic acid metabolite produced in the liver, is a promising serum biomarker for early HCC diagnosis in cirrhotic patients. Compared to the AFP at a cut-off of 20 ng/mL, the serum CSA outperformed AFP, with an AUROC of 0.90 vs. 0.61, a sensitivity of 79% vs. 74% and a specificity of 100% vs. 38%. Combining AFP with CSA increased the sensitivity to 96% while maintaining a specificity of 100%.[41] Urine metabolomics in HCC is under active investigation. Recently, glycocholic acid, a secondary bile acid, was identified in urine of HCC patients.[42] Further studies are needed to determine the performance of glycocholic acid in distinguishing between HCC and cirrhosis.

3. Biomarkers for prognostication and stratification for therapy

Five-gene score—A score for predicting survival and outcome of HCC patients treated with curative surgical resection was created from a panel of 5 genes involved in different dysregulated pathways in hepatocarcinogenesis (*TAF9, RAMP3, HN1, KRT19,* and *RAN*).[43] The score has been shown to accurately predict disease-specific survival and early recurrence. Patients with a score ≥0 had worse survival than those with the score <0 with a hazard ratio (HR) (95%CI) of 3.5 (1.9–6.6). The 5-gene score was more accurate than the previously described G3 transcriptome classification, which primarily reflected cell cycle activation.[44, 45] The HR (95%CI) of the 5-genes score vs. the G3 signature was 4.7 (2.7–8.2) vs. 1.0 (0.5–1.7) with Wald test P value of 7.7×10^{-8} vs. 0.9. The five-gene score has also been validated in different ethnic populations in Europe, U.S. and Asia.[43] Despite the impressive performance, the generalizability of the score may be limited because it was developed using surgically resected specimens and has only been proven to reliably predict the outcomes of patients treated with curative resection. Whether it can be applied to patients with unresectable disease is currently unknown.

Fibroblast growth factors 3 and 4 (FGF3/FGF4) amplification—*FGF3* and *FGF4*, ligands for the fibroblast growth factor receptors, serve as proto-oncogenes that are amplified in HCCs. *FGF3* and *FGF4* amplification has been shown to predict response to sorafenib (Figure 3).[46] Of a group of 48 patients studied, 30% (3/10) of the partial or complete responders to sorafenib, vs. 0% (0/38) of non-responders, had *FGF3/FGF4* amplification. Although this result suggests that *FGF3/FGF4* amplification can be used as a biomarker to guide targeted therapy for HCC, prospective validation in a larger cohort is required. No other biomarkers have been proven to predict response to sorafenib.[24]

High Met expression—The expression level of *c-Met*, a proto-oncogene encoding the hepatocyte growth factor receptor, can predict patient outcome and guide treatment. High expression of *c-Met* in resected HCC tumors was associated with a higher rate of recurrence

after surgery.[47] Further, in In a randomized phase 2 study of the Met inhibitor tivantinib, patients with high Met expression in the HCC tumor tissue had a significantly better response to tivantinib than those with low Met expression (median time to progression 2.7 months vs. 1.4 months; HR (95%CI): 0.43 (0.19–0.97); p=0.03).[48] This result suggests that Met expression can be used as a biomarker for selecting patients for treatment with Met inhibitors.

miRNA signature—Analysis of tumor miRNA expression profiles has been used to classify HCC subtype, to assess associations with tumor characteristics or prognostic clinical features and to identify miRNAs that modulate response to systemic chemotherapy or targeted therapy. Jiang *et al*. have reported a 19-miRNA signature that predicts overall survival.[49] In the context of predicting recurrence after curative resection, Budhu *et al.* reported a 20-miRNA signature associated with venous metastasis that predicted survival and recurrence; while Sato *et al*. reported a different set of 13 miRNAs that predicted early recurrence and another set of 20 miRNAs that predicted late recurrence.[50, 51] The differences in these findings may be due to differences in the populations studied, the outcomes assessed, the sources of tissues used for comparison (healthy liver tissue vs. peritumoral tissue), the techniques used for profiling, and tumor heterogeneity.

EpCAM positive circulating tumor cells—Stem cell-like, epithelial adhesion molecule positive circulating tumor cells (EpCAM+ CTC) are a subpopulation of tumor initiating cells. The presence of EpCAM+ CTC in blood reflects more advanced disease and may be used as a biomarker for stratifying patients for curative or systemic therapy. Detection of EpCAM+ CTC was associated with tumor aggressiveness, i.e. higher BCLC stage, vascular invasion, and worse survival (1.3 vs. 2.8 years).[52] Detection of ≥2 EpCAM+ CTC cells in 7.5 mL before hepatectomy was the strongest independent predictor of HCC recurrence post-resection.[53]

Summary

Despite its performance limitations, AFP remains the most widely used HCC biomarker. Many novel HCC biomarkers have been discovered during the past few decades, however, none have achieved broad acceptance in clinical practice as yet. Several biomarkers are currently under development to improve assay performance and to demonstrate proof of efficacy in clinical practice. Due to the heterogeneous nature of both tumors and humans, it is unlikely that a single ideal biomarker with excellent performance will be identified. Future studies should focus on efforts to combine biomarkers to achieve maximum diagnostic and predictive ability.

Acknowledgments

Grant support: NIH grant CA165076 to LRR

Abbreviations

AFP	alpha-fetoprotein

AFP-L3	Lens culinaris agglutinin-reactive glycoform of AFP
AUROC	area under the ROC curve
CSA	canavaninosuccinate
DCP	des-gamma-carboxy prothrombin
ELISA	enzyme-linked immunosorbent assay
EpCAM+ CTC	epithelial adhesion molecule-positive circulating tumor cells
FGF3/FGF4	fibroblast growth factors 3 and 4
GPC3	glypican-3
GP73	golgi protein 73
GWAS	genome wide association study
HBV	viral hepatitis B
HCV	viral hepatitis C
HCC	hepatocellular carcinoma
HR	Hazard ratio
KIF1B	kinesin-like factor 1 B
MELD	Model For End-Stage Liver Disease
MESIAH	Model for Estimating Survival in Ambulatory Hepatocellular Carcinoma patients
MICA	MHC class I polypeptide-related sequence A
miRNA	microRNA
mRNA	messenger RNA
NK	natural killer
NKG2D	NK cell group 2 member D
OR	odds ratio
PIVKA-II	protein induced by vitamin K absence/antagonist-II
ROC	receiver operating characteristic
SCCA	squamous cell carcinoma antigen
SNPs	single nucleotide polymorphisms
95%CI	95% confidence interval

References

1. Tian Q, Price ND, Hood L. Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. J Intern Med. 2012; 271:111–21. [PubMed: 22142401]

2. Kipp BR, Stadheim LM, Halling SA, et al. A comparison of routine cytology and fluorescence in situ hybridization for the detection of malignant bile duct strictures. Am J Gastroenterol. 2004; 99:1675–81. [PubMed: 15330900]
3. Pepe MS, Etzioni R, Feng Z, et al. Phases of biomarker development for early detection of cancer. J Natl Cancer Inst. 2001; 93:1054–61. [PubMed: 11459866]
4. Lee E, Edward S, Singal AG, et al. Improving screening for hepatocellular carcinoma by incorporating data on levels of alpha-fetoprotein, over time. Clin Gastroenterol Hepatol. 2013; 11:437–40. [PubMed: 23247324]
5. 10.1136/gutjnl-2013-304907.403
6. Shang S, Plymoth A, Ge S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. Hepatology. 2012; 55:483–90. [PubMed: 21953299]
7. Gavilan JC, Ojeda G, Arnedo R, et al. Predictive factors of risk of hepatocellular carcinoma in chronic hepatitis C. Eur J Intern Med. 2013
8. Lee MH, Yang HI, Liu J, et al. Prediction models of long-term cirrhosis and hepatocellular carcinoma risk in chronic hepatitis B patients: risk scores integrating host and virus profiles. Hepatology. 2013; 58:546–54. [PubMed: 23504622]
9. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004; 130:417–22. [PubMed: 15042359]
10. McMahon BJ, Bulkow L, Harpster A, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology. 2000; 32:842–6. [PubMed: 11003632]
11. Singal AG, Conjeevaram HS, Volk ML, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. Cancer Epidemiol Biomarkers Prev. 2012; 21:793–9. [PubMed: 22374994]
12. Zhang B, Yang B. Combined alpha fetoprotein testing and ultrasonography as a screening test for primary liver cancer. J Med Screen. 1999; 6:108–10. [PubMed: 10444731]
13. Marrero JA, Feng Z, Wang Y, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009; 137:110–8. [PubMed: 19362088]
14. Marrero JA, Su GL, Wei W, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. Hepatology. 2003; 37:1114–21. [PubMed: 12717392]
15. Nakamura S, Nouso K, Sakaguchi K, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. Am J Gastroenterol. 2006; 101:2038–43. [PubMed: 16848811]
16. Baek YH, Lee JH, Jang JS, et al. Diagnostic role and correlation with staging systems of PIVKA-II compared with AFP. Hepato-gastroenterology. 2009; 56:763–7. [PubMed: 19621698]
17. Xu C, Yan Z, Zhou L, et al. A comparison of glypican-3 with alpha-fetoprotein as a serum marker for hepatocellular carcinoma: a meta-analysis. J Cancer Res Clin Oncol. 2013
18. Chong CC, Lee KF, Ip PC, et al. Pre-operative predictors of post-hepatectomy recurrence of hepatocellular carcinoma: can we predict earlier? Surgeon. 2012; 10:260–6. [PubMed: 22959159]
19. Giannini EG, Marenco S, Borgonovo G, et al. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. Hepatology. 2012; 56:1371–9. [PubMed: 22535689]
20. Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. Liver Transpl. 2013; 19:634–45. [PubMed: 23536495]
21. Memon K, Kulik L, Lewandowski RJ, et al. Alpha-fetoprotein response correlates with EASL response and survival in solitary hepatocellular carcinoma treated with transarterial therapies: a subgroup analysis. J Hepatol. 2012; 56:1112–20. [PubMed: 22245905]
22. Chan SL, Mo FK, Johnson PJ, et al. New utility of an old marker: serial alpha-fetoprotein measurement in predicting radiologic response and survival of patients with hepatocellular carcinoma undergoing systemic chemotherapy. J Clin Oncol. 2009; 27:446–52. [PubMed: 19064965]

23. Yang JD, Kim WR, Park KW, et al. Model to estimate survival in ambulatory patients with hepatocellular carcinoma. Hepatology. 2012; 56:614–21. [PubMed: 22370914]
24. Llovet JM, Pena CE, Lathia CD, et al. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. Clin Cancer Res. 2012; 18:2290–300. [PubMed: 22374331]
25. Nakazawa T, Hidaka H, Takada J, et al. Early increase in alpha-fetoprotein for predicting unfavorable clinical outcomes in patients with advanced hepatocellular carcinoma treated with sorafenib. Eur J Gastroenterol Hepatol. 2013; 25:683–9. [PubMed: 23395995]
26. Tanaka T, Taniguchi T, Sannomiya K, et al. A novel des-gamma-carboxy prothrombin in serum for the diagnosis of hepatocellular carcinoma. J Gastroenterol Hepatol. 2013
27. Chen M, Li G, Yan J, et al. Reevaluation of glypican-3 as a serological marker for hepatocellular carcinoma. Clin Chim Acta. 2013; 423C:105–111. [PubMed: 23643963]
28. el-Houseini ME, Mohammed MS, Elshemey WM, et al. Enhanced detection of hepatocellular carcinoma. Cancer Control. 2005; 12:248–53. [PubMed: 16258497]
29. Clifford RJ, Zhang J, Meerzaman DM, et al. Genetic variations at loci involved in the immune response are risk factors for hepatocellular carcinoma. Hepatology. 2010; 52:2034–43. [PubMed: 21105107]
30. Zhang H, Zhai Y, Hu Z, et al. Genome-wide association study identifies 1p36.22 as a new susceptibility locus for hepatocellular carcinoma in chronic hepatitis B virus carriers. Nat Genet. 2010; 42:755–8. [PubMed: 20676096]
31. Li S, Qian J, Yang Y, et al. GWAS identifies novel susceptibility loci on 6p21.32 and 21q21.3 for hepatocellular carcinoma in chronic hepatitis B virus carriers. PLoS Genet. 2012; 8:e1002791. [PubMed: 22807686]
32. Jiang DK, Sun J, Cao G, et al. Genetic variants in STAT4 and HLA-DQ genes confer risk of hepatitis B virus-related hepatocellular carcinoma. Nat Genet. 2013; 45:72–5. [PubMed: 23242368]
33. Kumar V, Kato N, Urabe Y, et al. Genome-wide association study identifies a susceptibility locus for HCV-induced hepatocellular carcinoma. Nat Genet. 2011; 43:455–8. [PubMed: 21499248]
34. Miki D, Ochi H, Hayes CN, et al. Variation in the DEPDC5 locus is associated with progression to hepatocellular carcinoma in chronic hepatitis C virus carriers. Nat Genet. 2011; 43:797–800. [PubMed: 21725309]
35. Guyot E, Sutton A, Rufat P, et al. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. J Hepatol. 2013; 58:312–8. [PubMed: 23069476]
36. Marrero JA, Romano PR, Nikolaeva O, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol. 2005; 43:1007–12. [PubMed: 16137783]
37. Wang M, Long RE, Comunale MA, et al. Novel fucosylated biomarkers for the early detection of hepatocellular carcinoma. Cancer Epidemiol Biomarkers Prev. 2009; 18:1914–21. [PubMed: 19454616]
38. Tian L, Wang Y, Xu D, et al. Serological AFP/Golgi protein 73 could be a new diagnostic parameter of hepatic diseases. Int J Cancer. 2011; 129:1923–31. [PubMed: 21140449]
39. Qi J, Wang J, Katayama H, et al. Circulating microRNAs (cmiRNAs) as novel potential biomarkers for hepatocellular carcinoma. Neoplasma. 2013; 60:135–42. [PubMed: 23259781]
40. Tomimaru Y, Eguchi H, Nagano H, et al. Circulating microRNA-21 as a novel biomarker for hepatocellular carcinoma. J Hepatol. 2012; 56:167–75. [PubMed: 21749846]
41. Wang B, Chen D, Chen Y, et al. Metabonomic profiles discriminate hepatocellular carcinoma from liver cirrhosis by ultraperformance liquid chromatography-mass spectrometry. J Proteome Res. 2012; 11:1217–27. [PubMed: 22200553]
42. Zhang A, Sun H, Yan G, et al. Urinary metabolic profiling identifies a key role for glycocholic acid in human liver cancer by ultra-performance liquid-chromatography coupled with high-definition mass spectrometry. Clin Chim Acta. 2013; 418:86–90. [PubMed: 23313056]
43. Nault JC, De Reynies A, Villanueva A, et al. A hepatocellular carcinoma 5-gene score associated with survival of patients after liver resection. Gastroenterology. 2013; 145:176–87. [PubMed: 23567350]
44. Boyault S, Rickman DS, de Reynies A, et al. Transcriptome classification of HCC is related to gene alterations and to new therapeutic targets. Hepatology. 2007; 45:42–52. [PubMed: 17187432]

45. Lee JS, Chu IS, Heo J, et al. Classification and prediction of survival in hepatocellular carcinoma by gene expression profiling. Hepatology. 2004; 40:667–76. [PubMed: 15349906]
46. Arao T, Ueshima K, Matsumoto K, et al. FGF3/FGF4 amplification and multiple lung metastases in responders to sorafenib in hepatocellular carcinoma. Hepatology. 2013; 57:1407–15. [PubMed: 22890726]
47. Kondo S, Ojima H, Tsuda H, et al. Clinical impact of c-Met expression and its gene amplification in hepatocellular carcinoma. International journal of clinical oncology. 2013; 18:207–13. [PubMed: 22218908]
48. Santoro A, Rimassa L, Borbath I, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. The lancet oncology. 2013; 14:55–63. [PubMed: 23182627]
49. Jiang J, Gusev Y, Aderca I, et al. Association of MicroRNA expression in hepatocellular carcinomas with hepatitis infection, cirrhosis, and patient survival. Clin Cancer Res. 2008; 14:419–27. [PubMed: 18223217]
50. Budhu A, Jia HL, Forgues M, et al. Identification of metastasis-related microRNAs in hepatocellular carcinoma. Hepatology. 2008; 47:897–907. [PubMed: 18176954]
51. Sato F, Hatano E, Kitamura K, et al. MicroRNA profile predicts recurrence after resection in patients with hepatocellular carcinoma within the Milan Criteria. PLoS One. 2011; 6:e16435. [PubMed: 21298008]
52. Schulze K, Gasch C, Staufer K, et al. Presence of EpCAM-positive circulating tumor cells as biomarker for systemic disease strongly correlates to survival in patients with hepatocellular carcinoma. Int J Cancer. 2013
53. Sun YF, Xu Y, Yang XR, et al. Circulating stem cell-like epithelial cell adhesion molecule-positive tumor cells indicate poor prognosis of hepatocellular carcinoma after curative resection. Hepatology. 2013; 57:1458–68. [PubMed: 23175471]

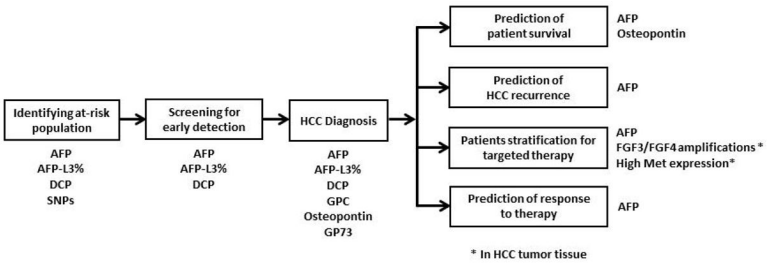

Figure 1.
Applications of established and novel HCC biomarkers in clinical care

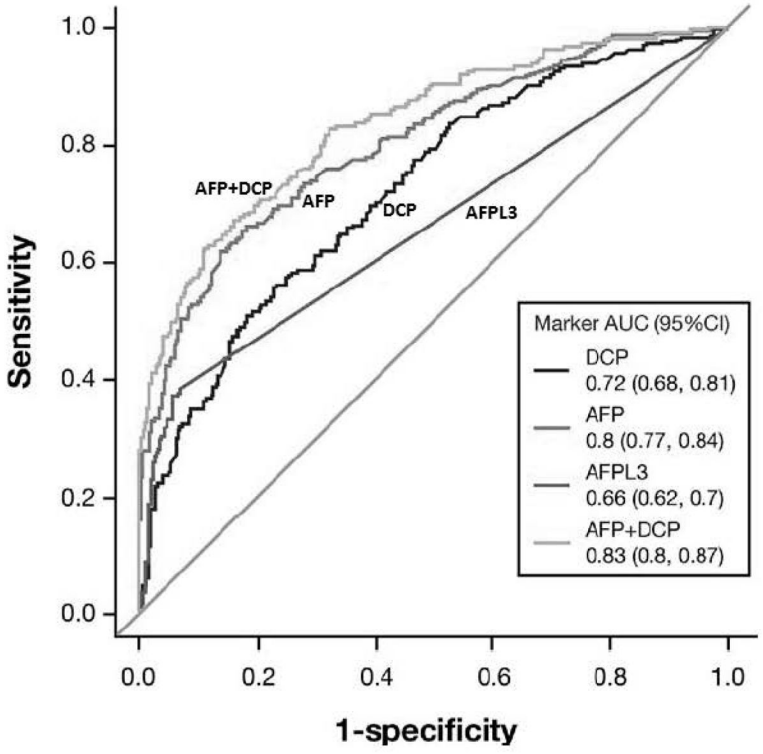

Figure 2.
Performance of AFP, AFP-L3% and DCP for early HCC diagnosis in cirrhotic patients

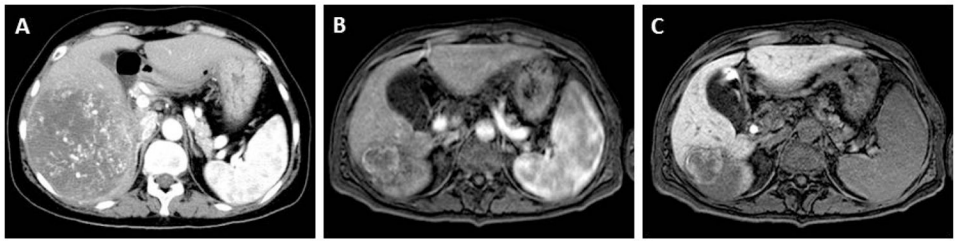

Figure 3.

Arterial phase computed tomography image of a large HCC tumor shown to have FGF3/FGF4 amplification before sorafenib treatment (A). After treatment with sorafenib for 2 months, the tumor size was substantially decreased as shown in the arterial phase (B) and hepatocyte phase (C) magnetic resonance images (Images courtesy of Dr. Masatoshi Kudo, used with permission)

Table 1

Summary of SNPs associated with HCC risk identified from GWAS

Gene	SNPs	Minor allele	Risk for HCC	Patient settings	OR (95% CI)	P value	Reference
Intron 1 of *TPTE2*	rs2880301	C	Decrease	HBV/HCV, Korea	0.27 (0.19–0.39)	1.74×10^{-12}	Clifford et al.[28]
KIF1B	rs17401966	G	Decrease	HBV, China	0.61 (0.55–0.67)	1.70×10^{-18}	Zhang et al.[29]
GRIK1	rs455804	A	Decrease	HBV, China	0.84 (0.80–0.89)	5.24×10^{-10}	Li et al.[30]
STAT4	rs7574865	G	Increase	HBV, China	1.22 (1.15–1.29)	1.66×10^{-11}	Jiang et al.[31]
HLA-DQA1/DRB1	rs9272015	A	Increase	HBV, China	1.28 (1.22–1.35)	5.24×10^{-22}	Li et al. [30]
MICA	rs2596542	A	Increase	HCV, Japan	1.39 (1.27–1.52)	4.21×10^{-13}	Kumar et al.[32]
HLA-DQ	rs9275319	A	Increase	HBV, China	1.51 (1.38–1.66)	8.65×10^{-19}	Jiang et al. [31]
DEPDC5	rs1012068	G	Increase	HCV, Japan	1.75 (1.51–2.03)	1.27×10^{-13}	Miki et al. [33]
Upstream of *DDX18*	rs2551677	A	Increase	HBV/HCV, Korea	3.38 (2.07–5.53)	1.41×10^{-10}	Clifford et al.[28]

Clin Gastroenterol Hepatol. Author manuscript; available in PMC 2016 February 01.

Table 2

Summary of known and newer HCC biomarkers

Biomarker	Source	Current Phase for Surveillance	Application of biomarker	Proven or Potential Clinical Utility
AFP	Serum	5	Risk Stratification/Surveillance/Diagnosis/Prognosis	The only HCC biomarker for which a prospective study has proven a reduction in mortality from HCC in the surveillance setting[9]
AFP-L3%	Serum	2	Risk Stratification/Diagnosis/Prognosis	Changes in AFP-L3% over time may be more sensitive than changes in AFP for predicting HCC progression after transarterial chemoembolization (personal communication from L Roberts)
DCP	Serum	2	Risk Stratification/Diagnosis/Prognosis	Combination of DCP and AFP was superior to either AFP or DCP alone for HCC diagnosis[12]
Glypican 3	Serum	2	Diagnosis	Comparable performance to AFP for HCC diagnosis[15]
Osteopontin	Plasma	2	Early detection	Slightly better than AFP in early HCC detection Performance was improved when used in combination with AFP[6]
GP73	Serum	2	Early detection	Diagnostic performance depends on the method of measurement[34–36]
Canavaninosuccinate	Serum	2	Early detection	Outperforms AFP for HCC diagnosis[39]
Five-gene score	Tissue	1	Prognosis	Predicts survival and early recurrence after curative resection[41]
FGF3/FGF4 amplification	Tissue	1	Prediction	Predicts response to sorafenib; may potentially be used to personalize therapy[44]
High *Met* expression	Tissue	1	Prediction	Associated with better response to tivantinib; may potentially be used as a biomarker for selecting patients for treatment with MET inhibitors[46]