Role of Biomarkers and Biopsy in Hepatocellular Carcinoma

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KEYWORDS

• AFP • AFP-L3 • DCP • PIVKA-II • Liquid biopsy • Sequencing

KEY POINTS

- Hepatocellular surveillance with alpha-fetoprotein and ultrasound has substantially greater sensitivity for early stage hepatocellular carcinoma (HCC) than with ultrasound alone.
- Combining alpha-fetoprotein with other biomarkers (eg, GALAD score) may further improve early detection.
- Biopsy of hepatocellular carcinoma lesions is associated with risk, and there is little evidence that tumor sequencing can be used to determine systemic therapy decisions in HCC.
- More frequent use of biopsy in a research setting may improve our understanding of HCC biology and assist with development of targeted therapy in the future.

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third leading cause of cancer death worldwide.[1] HCC prevalence and attributable mortality in the United States are increasing rapidly,[2,3] driven largely by increasing prevalence in alcoholic liver disease and nonalcoholic fatty liver disease, as well as peaking hepatitis C virus prevalence.[4,5]

HCC usually arises in the setting of cirrhosis, and international liver societies recommend screening for HCC in at-risk patients using biannual ultrasound (US) with or without alpha-fetoprotein (AFP) measurement.[6–8] The motivation behind these guidelines is to increase the probability of detection of early stage HCC that is amenable to curative therapy.[9,10] However, commonly used methods of HCC screening have inadequate sensitivity, especially for early stage cancer: the combination of US and AFP results in only a 63% sensitivity for detection of early stage HCC.[11] Given this

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limitation, there has been interest in developing and validating improved biomarkers for early detection of HCC, prognostication, and management.

Unlike other common malignancies, HCC can be diagnosed based on imaging characteristics without a biopsy, which reduces risks of biopsy-related complications such as tumor seeding and bleeding.[12] However, this practice may result in some limitations. First, greater than 5% of MRI-diagnosed HCC may be false positive or non-HCC lesions.[13] Second, the lack of routine liver biopsies has resulted in limited understanding of HCC at a molecular level that has hampered drug development.[14]

The purpose of this article is to review the utility of serum biomarkers in HCC detection and prognostication and potential use of liver biopsy to guide therapy decisions in HCC.

HEPATOCELLULAR CARCINOMA BIOMARKERS

HCC biomarkers include AFP, Lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and des-gamma-carboxyprothrombin (DCP).

Diagnosis and Detection

The role of AFP in HCC diagnosis has evolved over time. In early American and European HCC guidelines, high AFP levels were used as an adjunct to imaging for HCC diagnosis.[12,15] With advances in imaging methods, AFP is no longer used for HCC diagnosis but may have a role in HCC detection in conjunction with US.[6–8] There has been some controversy on the role of AFP in HCC screening. Recent American, European, and Asia-Pacific HCC guidelines are agnostic as to whether AFP should be included in HCC surveillance programs.[6–8] AFP concentration elevations greater than 20 ng/mL have been reported in up to 10% to 20% of patients with viral hepatitis or cirrhosis and may be more common with active hepatitis.[16,17] Conversely, the sensitivity of AFP alone is relatively low in early stage HCC.[17] The cost-effectiveness of AFP in addition to US-only surveillance has also been questioned,[18] although, of note, the study used estimates of sensitivity/specificity that were not restricted to early stage HCC.

More recent studies have supported the use of AFP in HCC surveillance. A recent meta-analysis found that AFP greatly increased sensitivity for early HCC over US alone from 45% to 63%, and this increase in sensitivity was robust across several subgroups including prospective studies, post-2000 studies, and studies only including patients with cirrhosis.[11] Although screening strategies with US plus AFP had lower specificity for early HCC than did US alone (84% vs 92%),[11] this relatively small decrease in specificity would likely be offset by increased probability of early detection. Trends in AFP over time may also be informative in HCC detection. One recent study of 1050 patients with hepatitis C found that an empirical Bayes model incorporating not only US and absolute AFP level but also the average of prior AFP values in that same patient had superior performance characteristics for identifying patients who went on to develop HCC than did US and AFP alone.[19] AFP is frequently incorporated into HCC surveillance in real-world cohorts,[20–24] suggesting that despite the controversy over the utility of AFP in HCC surveillance, many providers consider it useful in clinical practice.

Two other commonly used biomarkers are AFP-L3 and DCP, also known as protein induced by vitamin K absence or antagonist II. AFP comprises 3 glycoforms with distinct binding affinity to *Lens culinaris agglutinin*. The glycoform with the greatest affinity, AFP-L3, is upregulated in HCC compared with nonmalignant liver disease.[25] DCP is an abnormal form of prothrombin that does not undergo posttranslational

modification with gamma-carboxylation. DCP levels are higher levels in patients with HCC than in those with nonmalignant liver disease. The most commonly used cut-offs are greater than 40 mAU/mL for DCP and greater than 5% to 10% for AFP-L3.[26] One meta-analysis found that for distinguishing early stage HCC from controls, AFP and DCP had similar area under the receiver operating characteristic curve (AUC) (0.75 vs 0.70), whereas that of AFP-L3 was marginally lower (0.67).[26] Notably, though, AFP-L3 and DCP are complementary to AFP, as they may be abnormal even when AFP is not.[27] In one study, AFP-L3 and DCP demonstrated similar predictive power for distinguishing HCC from chronic liver disease in patients with HCC and AFP less than 20 ng/mL (AUC 0.63 and 0.74, respectively) compared with that in all patients with HCC, and the combination of AFP, AFP-L3, and DCP had greater predictive power than did any individual marker.[26] Note that similar to prothrombin time, DCP is affected by vitamin K antagonists and cannot be used as an HCC biomarker in patients taking warfarin.

The fact that AFP-L3 and DCP provide information beyond AFP alone suggests that scores combining multiple patient characteristics and biomarkers may have greater sensitivity than individual markers in early detection of HCC. One notable recent score that has combined multiple markers with demographics is the GALAD score (*g*ender, *a*ge, AFP-*L*3%, *A*FP, and *D*CP). Overall, GALAD has been shown to have excellent performance characteristics with AUC greater than 0.90 at distinguishing HCC from nonmalignant liver disease (**Table 1**).[28–32] Importantly, most of the studies on GALAD for HCC detection specifically evaluated the most clinically relevant problems of detection at an early stage and/or whether GALAD offers incremental benefit to the most commonly used HCC biomarker, AFP. In addition, GALAD scores seem to increase months or even years before clinically apparent HCC is present,[30,31] so trends in GALAD scores rather than merely absolute scores may be useful in HCC detection, similar to what has been demonstrated for AFP.[19] GALAD has shown excellent promise in large phase 2 studies (case-control) and to an extent in phase 3 (retrospective longitudinal) studies, but further validation in larger phase 3 and phase 4 (prospective screening) studies is required.[33] The Roche Elecsys GALAD score recently received Breakthrough Device Designation by the US Food and Drug Administration, allowing for streamlined market clearance/approval.

Prognosis

AFP has value both as a prognostic marker (ie, impacts survival regardless of treatment type) and predictive marker (ie, predicts response to therapy). This finding may be related to differences in cancer biology: AFP-secreting HCC tumors are more often associated with *TP53* mutations and poor differentiation.[34–36] Elevated AFP is associated with poorer outcomes in patients receiving resection,[37] liver transplantation,[38] tumor ablation,[39] transarterial chemoembolization,[40] and sorafenib.[41] In addition, AFP trends have been used to monitor response to treatments including systemic chemotherapy and ablation, and improvement in AFP has been associated with improved outcomes.[42,43]

Perhaps the best-established use of AFP as a predictive marker is with ramucirumab, a VEGFR2 inhibitor. In a randomized controlled trial, ramucirumab did not improve survival over placebo as second-line therapy in the overall cohort but on subgroup analysis and in a subsequent study led to a survival advantage (and is approved for use solely) in patients with AFP greater than 400 ng/mL.[44,45] Likewise, survival benefit to cabozantinib was greater in patients with HCC with serum AFP greater than or equal to 200 ng/mL.[46]

Table 1
Selected studies validating the GALAD score

Study, Ref	Location	HCC Patients	Controls	Results
Johnson et al,[28] 2014	UK: Birmingham and Newcastle	N = 394 27% alcohol, 11% HCV, 8% HBV	N = 439 17% HCV, 16% alcohol, 13% HBV	Birmingham: AUC 0.97 overall and 0.96 for early stage Newcastle: AUC 0.95
Berhane et al,[29] 2016	UK, Germany, Japan, Hong Kong	N = 2430 49% HCV, 21% HBV	N = 4404 40% HCV, 26% HBV, 34% other liver disease, 5% non-HCC cancer, 2% healthy	All patients: AUC 0.97/0.93/0.94 in UK/Japan/Germany Early HCC: AUC 0.93/0.91 in UK/Japan
Berhane et al,[30] 2017	Japan	N = 119	N = 2128	2247 patients under surveillance. Increase in GALAD score preceded HCC development
Best et al,[31] 2019	Germany, Japan	N = 126 (Germany) N = 26 (Japan) 100% NAFLD	N = 231 (Germany) N = 363 (Japan) 100% NAFLD	Germany: AUC 0.92 (early stage HCC), 0.93 (cirrhosis only), 0.85 (early stage HCC and cirrhosis) Japan: increase in GALAD score preceded HCC development
Yang et al,[32] 2019	US	N = 111 43% HCV, 27% NAFLD, 13% alcohol, 10% HBV	N = 180 27% NAFLD, 21% alcohol, 18% HCV, 15% HBV	AUC 0.95 overall, 0.92 for BCLC stage 0/A, 0.90 for AFP <20 ng/mL

More recently, the BALAD and BALAD-2 scores, which incorporate *b*ilirubin, *a*lbumin, AFP-*L*3, *A*FP, and *D*CP, have also been used for prognostication in HCC (**Table 2**).[29,47–51] BALAD and BALAD-2 include the same variables, but BALAD considers them as either normal or abnormal, whereas BALAD-2 uses them as continuous variables. Most studies on BALAD and BALAD-2 have been conducted in patients with chronic hepatitis C in Japan, although it has been studied internationally as well. BALAD and BALAD-2 may add information beyond treatment type and conventional staging alone,[29,49–51] although this benefit is likely to be small.[48]

Other novel protein and nonprotein biomarkers

Identifying non-AFP biomarkers to predict response to systemic therapy has been challenging. The SHARP study did not identify any serum biomarkers predictive of treatment response to sorafenib.[52] Similarly, neither AFP nor c-Met were associated with response to regorafenib treatment.[53] A recent study identified a set of microRNAs and plasma proteins associated with response to regorafenib, although this requires further validation.[54]

Table 2
Selected studies validating the BALAD and BALAD 2 scores

Study, Ref	Location	HCC Patients	Results
Toyoda et al,[47] 2006	Japan, 5 institutions	N = 2600 75% HCV, 14% HBV, 2% HBV + HCV, 9% nonviral	Patients with high BALAD score were less likely to receive resection or ablation and more likely to receive systemic or no therapy. Similar prognostic significance as TNM staging
Kitai et al,[48] 2008	Japan, 5 institutions	N = 1173 75% HCV, 13% HBV, 2% HBV + HCV	BALAD was inferior to conventional and biomarker-combined Japan Integrated Staging scores
Chan et al,[49] 2015	Hong Kong, 1 institution	N = 198 100% HBV	BALAD added additional prognostic information to BCLC stage
Berhane et al,[29] 2016	UK, Germany, Japan, Hong Kong	N = 2430 49% HCV, 21% HBV	Higher BALAD-2 score associated with poorer prognosis in all countries and treatment types
Toyoda et al,[50] 2017	Japan, >750 institutions	N = 24,029 70% HCV, 16% HBV	Both BALAD and BALAD-2 associated with prognosis in multivariable analysis. BALAD-2 had predictive power across treatment types and disease causes
Wongjarupong et al,[51] 2018	US, 1 institution	N = 113 58% HCV, 12% alcohol, 12% NAFLD/cryptogenic, 10% HBV 100% liver transplant recipients	Both BALAD and BALAD-2 associated with recurrence and survival posttransplant. Tumor size plus BALAD/2 showed the best test characteristic.

Glycoprotein biomarkers, other than AFP, AFP-L3 and DCP, are also under investigation as HCC biomarkers. Glycosylation of proteins is altered in malignant transformation, including in HCC, and there has been interest in evaluating them as biomarkers for HCC detection. In a study of 42 patients with HCC and 53 patients with viral hepatitis, multifucosylated alpha-1-acid glycoprotein has been shown to have very high predictive power for HCC (AUC 0.93 for HCC vs hepatitis B).[55] Altered N-glycosylation and fucosylation of haptoglobin has been noted in HCC compared with chronic liver disease controls.[56,57] Multimarker studies are also possible in glycoproteomics. One group collected serum from 8 patients with HCC and 14 healthy controls, performed affinity chromatography followed by liquid chromatography/mass spectrometry, and identified 21 liver-expressed candidate biomarkers that were mostly found in higher levels in HCC.[58] Existing studies on glycoprotein markers in HCC have been limited in scope and require external validation before they can be recommended for clinical use.

Finally, the authors briefly discuss nonprotein biomarkers.[59] Neutrophil-lymphocyte ratio reflects systemic inflammation, which is thought to play an important role in carcinogenesis by inhibiting apoptosis and promoting angiogenesis.[60] Elevated neutrophil-lymphocyte ratio has been associated with poorer overall survival and disease-free survival in patients treated with surgical therapy and is also associated with poorer overall survival in patients receiving palliative therapy or ablation.[61] Circulating tumor cells are malignant cells that have been detached from the primary tumors and released into the circulation and can be found in most solid tumors.[62] Cell-free DNA is released into the circulation by cells and can be quantified or sequenced.[59] Extracellular vesicles are formed when cell membranes, apoptotic bodies, or lysosomes bud off and can be isolated from serum/plasma.[63] Circulating tumor cells have only modest sensitivity (70%) for HCC detection. The literature on extracellular vesicles for early HCC detection is limited by heterogeneity on which property of extracellular vesicles is evaluated: most studies investigated microRNAs, but there has been little consistency in which microRNAs were studied.[64,65] In comparison, cell-free DNA methylation profiles have demonstrated greater potential: 2 large studies from China and the United States found sensitivity and specificity of greater than 90% for distinguishing HCC from chronic liver disease and sensitivity greater than 75% in identifying early stage HCC.[66,67] Whether these methylation scores can be validated across disease causes and ethnicities remains to be seen.

ROLE OF BIOPSY IN HEPATOCELLULAR CARCINOMA

Diagnosis

Biopsy is not needed to establish the diagnosis of HCC because of high specificity of dynamic imaging (computed tomography and MRI) in identifying HCC greater than or equal to 2 cm.[6–8] In addition, patients with cirrhosis and coagulopathy are at increased risk of developing biopsy-related complications such as bleeding,[14] and tumor seeding after biopsy has been reported to be as high as 2.7%.[68] A recent meta-analysis reported a pooled specificity of both computed tomography and MRI of 91% to 92%, implying a greater than 5% false-positive rate of HCC diagnosis with imaging alone.[69]

The *Li*ver *R*eporting *a*nd *D*ata *S*ystem (LIRADS) represents an attempt to standardize liver imaging and may result in a lower rate of false positives.[70,71] However, entities such as combined HCC-intrahepatic cholangiocarcinoma, metastatic lesions, and dysplastic nodules may be difficult to distinguish from HCC radiographically.[72,73] Routinely conducting liver biopsy to reduce false-positive rates has not been well

studied; however, the authors acknowledge that a greater than 5% false-positive HCC diagnosis rate is significant. The standard of care in many tertiary care centers is to review most patients with a new HCC diagnosis in a multidisciplinary tumor board setting and only obtain biopsies of lesions that are not definitely HCC based on LIRADS criteria.

Biopsy and Precision Medicine

Precision oncology has attracted major interest recently and is a bedrock of several other cancer types. Her2/neu inhibitors improve survival in patients with Her2/neu-amplified breast cancer, alectinib and erlotinib are used for lung cancer with *EGFR* mutations and *EML4-ALK* fusions, and pembrolizumab is approved for any microsatellite instability-high or mismatch repair–deficient solid tumors.[74–77] Unfortunately, precision therapy for HCC is comparatively lacking, in part because of nonavailability of tissue to study tumor mutations and biology, as it is not standard of care to biopsy HCC, as detailed earlier.[12,14,78] Only recently has deep sequencing of human HCC tissue identified molecular subtypes with distinct prognoses.[34,79,80] Commercially available sequencing platforms such as Foundation One are frequently used in oncology to determine tissue of origin and even guide therapy or clinical trial eligibility.[81,82] Although there are comparatively few data on this topic in HCC, small sequencing studies have suggested that next-generation sequencing may yield additional insights into cancer biology.[83]

Molecular characterization of HCC has yielded substantial insight into driver mutations in HCC.[80,84–88] **Fig. 1** shows the frequency of common gene mutations in HCC. Genes consistently shown to be mutated in HCC include those involved in telomere maintenance (*TERT* promoter), WNT/beta-catenin pathways (*CTNNB1*, *AXIN1*), tumor suppressor genes (*TP53*, *TSC2*), cell cycle (*CDKN2A*), chromatin remodeling (*ARID1A*, *ARID2*), oxidative stress (*NFE2L2*), MAP kinase (*RPS6KA3*), and normal liver function (*ALB*, *APOB*).[80,84–88] Mutational profiles may differ based on disease cause. *TERT* promoter mutations may be more frequently found in NAFLD or alcohol-related HCC.[80,89] HBV-related HCC is associated with *TP53*-inactivating mutations[80,85] and

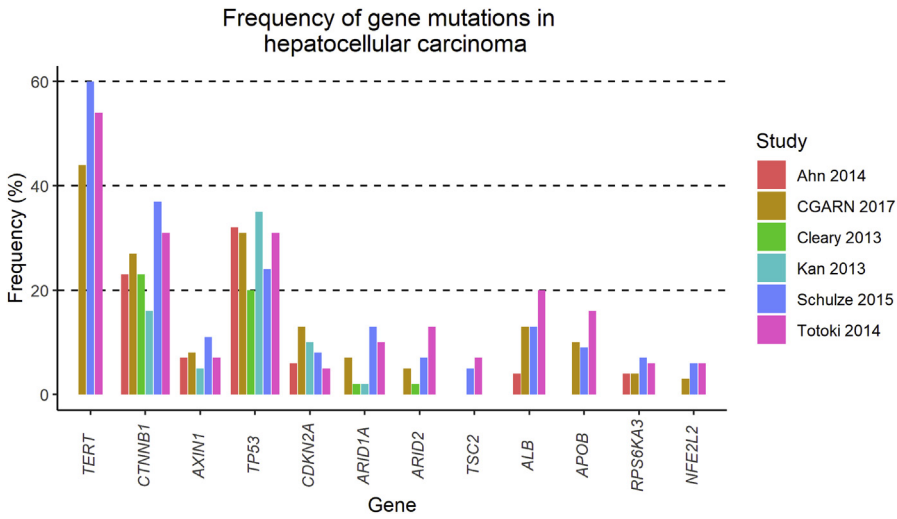

Fig. 1. Frequency of common gene mutations in HCC.

more frequently has *RB1* and less frequently has *CTNNB1* mutations compared with HCC of other causes.[87]

Recently, investigators have divided HCC into subgroups based on a combination of driver mutations and histology.[34] One subgroup is characterized by *TP53* mutations, poorly differentiated histology, and elevated AFP and carries a poorer prognosis, whereas another more often involves *CTNNB1* mutations and well-differentiated histology, with more favorable prognosis. One large recent study evaluated mutations in 801 tumors from 720 patients and found that presence of a high-risk gene expression profile was associated with poorer survival in patients undergoing resection, ablation, or noncurative therapy.[90]

Whether mutational profiles are useful in guiding therapy is an emerging topic of investigation. In one study, patients receiving sorafenib whose tumors carried *PI3K-mTOR* pathway mutations had shorter progression-free and overall survival, whereas WNT/beta-catenin mutations were also associated with poorer prognosis among patients receiving checkpoint inhibitors.[91] *FGF19* copy number amplification may also be associated with improved response to sorafenib in HCC.[92] These findings require external validation but (if patients have undergone molecular characterization of tumors) may help guide therapy, as the list of systemic therapeutics against HCC becomes increasingly diverse. Although it has been reported that greater than 20% of early stage HCC have mutations potentially targetable by Food and Drugs Administration–approved medications,[80] phase 2 studies for several of these medications in overall HCC populations have already been unsuccessful.[93–95] Whether these medications are effective among individuals carrying specific mutations remains to be determined, and ongoing studies are evaluating this possibility.[96]

SUMMARY

AFP is the oldest biomarker still in use for HCC detection and prognostication, and we support its use in HCC surveillance given the improvement in early detection sensitivity with AFP plus US over US alone. The GALAD score that combines AFP, AFP-L3, DCP, age, and sex may be an even more promising surveillance tool, although validation in larger phase 3 and 4 studies is still required. Recent research on sequencing HCC tumors has yielded substantial insights into HCC tumor biology and has raised the possibility of precision oncology in which therapy decisions are guided by cancer genetics. At this point, though, no mutational profile has been convincingly shown to predict response to HCC therapy. Given this, it has been believed that routine biopsy with sequencing of HCC is unlikely to change patient management in the short term. However, biopsy in a research setting to expand the understanding of HCC tumor genetics may assist in development of future, more effective systemic therapy for HCC.

DISCLOSURE

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