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*Chapter 4*

# USE OF ALPHA-FETOPROTEIN AND OTHER BIOMARKERS FOR ASSESSING HEPATOCELLULAR CARCINOMA RISK IN VIRAL HEPATITIS

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## ABSTRACT

Hepatocellular carcinoma (HCC) diagnosis after symptom onset carries a grim prognosis with five year survival of < 20%. Thus, in a population with risk factors for HCC, our goal should be to diagnose it in an early stage and manage the patient with curative intent. The currently available screening and surveillance modalities, including ultrasound (US), are underutilized in the clinical setting. Current AASLD guidelines only recommend US every 6 months for surveillance of high-risk patients. The biomarkers that have been studied to date are only risk biomarkers and of those discussed here, only alpha-fetoprotein (AFP), AFP-L3%, and des-gamma carboxyprothrombin (DCP) are FDA-qualified as biomarkers for HCC risk. Due to low sensitivity and specificity as screening tests, they should not be used as sole screening or surveillance tests and are not used to diagnose HCC. HCC is diagnosed by imaging with CT scan or MRI with contrast using Liver Imaging Reporting and Data System (LI-RADS) criteria and, rarely, by liver biopsy. One or more of the biomarkers may, however, complement US for screening and surveillance, potentially triggering a CT or MRI with contrast. Elevated or increasing biomarkers should trigger a CT or MRI. AFP has been shown to be a strong prognostic indicator and to complement US for surveillance. A higher AFP value has clearly been

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associated with poor clinical outcome while AFP reduction > 50% after treatment predicts an improved response. The pre-treatment AFP value may identify patients who will benefit more from loco-regional therapy than transplant. A higher pre-transplant AFP level has been associated with tumor recurrence and thus may help identify patients who will need close observation for early HCC recurrence. The AFP level (with a ceiling of < 500 ng/ml) has been utilized in some liver allocation models to grant MELD exception points. AFP-L3% has been found to complement AFP in HCC surveillance, especially when the AFP value is indeterminate. As with AFP, a higher AFP-L3% level may be reflective of either advanced disease or aggressive HCC. Elevated levels of both AFP-L3% and DCP have been associated with vascular invasion by the tumor. A high post-ablation AFP-L3% value may be reflective of disease recurrence. Similar to AFP, AFP-L3% may help determine treatment modality. DCP can help differentiate HCC from non-malignant chronic liver disease. Its re-emergence after treatment can be reflective of HCC recurrence. DCP can complement AFP in HCC risk assessment. Data for other biomarkers are limited, and no firm recommendation can currently be made for their use. Several risk assessment models have been proposed to help with some of the limitations of these biomarkers. Multiple large studies are underway on the clinical utility of HCC biomarkers and on the proposed models that will ultimately help answer remaining questions on their usefulness. In the meantime, guidelines from the Japan Society of Hepatology recommend AFP, AFP-L3% and DCP for HCC surveillance. Experts in the field agree that until better screening and surveillance tools are available for HCC, we should utilize the available biomarkers to complement US and help identify patients who would benefit from further radiological workup.

## INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common primary cancer of the liver, the fifth most common cancer in men and seventh in women worldwide, and the second leading cause of cancer-related mortality worldwide with more than 650,000 new cases every year [1]. The majority of HCC cases are diagnosed in developing countries where endemic hepatitis B virus (HBV) infection is the most prevalent risk factor [2]. Developing countries account for 85% of the worldwide HCC burden, with more than 80% of cases found in sub-Saharan Africa and Eastern Asia where the incidence rate is more than 20 per 100,000 people, and in Mongolia where the rate is over 60 per 100,000 [3]. The incidence of HCC is on the rise in most parts of the world [4]. Infection with hepatitis C virus (HCV) is the leading cause of HCC in Japan although there has been a recent decrease in HCC incidence attributed to HCV treatment responders [5]. In a recent retrospective study of U.S. veterans, the incidence of HCC and HCC-related mortality has risen since 2001 [6]. There is an alarming 2.5 fold increase in HCC incidence, most of which is attributed to HCV infection. The American Cancer Society predicted 35,660 new liver and intrahepatic bile duct cancers in 2015 in the United States, approximately three-fourths of which would be HCC [7].

Because of its prevalence and high mortality, it is imperative to identify the predisposing factors for the development of HCC. This risk stratification will help identify patients who would benefit from HCC screening and surveillance. The American Association for the Study of Liver Diseases (AASLD) defines screening as “application of diagnostic tests in patients at risk for HCC, but in whom there is no a priori reason to suspect that HCC is present” while surveillance is ‘‘the repeated application of screening tests’’ in patients at risk for a disease

[4]. Cirrhosis is the major risk factor for the development of HCC with the five-year cumulative risk in the cirrhotic patient varying from 5 to 30%, depending upon the etiology of the liver disease [8]. In the U.S. the incidence of HCC is higher in patients with advanced cirrhosis and high Child-Pugh class, highest in patients with chronic HCV infection, and second-highest in those with chronic HBV (CHB) [8]. The 5-year cumulative incidence for common risk factors is summarized in Table 1. In subjects with compensated liver cirrhosis, HCC is the most common cause of death, accounting for 54-70% of mortality [8]. At autopsy, 80-90% of patients with HCC are found to have liver cirrhosis. It is important for surveillance guidelines to recognize that in approximately 10-20% of patients HCC develops in the absence of cirrhosis [9]. In patients who develop HCC in the absence of cirrhosis there is almost always chronic inflammation; thus, the necroinflammatory process appears to be the most important predisposing factor [8]. The common causes for viral and non-viral chronic hepatitis and HCC are detailed in Table 2.

**Table 1. Common risk factors and incidence of HCC [8]**

| **Risk factors** | **5-year cumulative incidence*** |
|---|---|
| HCV | 17% in Europe and United states |
| | 30% in japan |
| Hemochromatosis | 21% |
| Chronic HBV infection | 10% in Europe and United States |
| | 15% in sub-Saharan Africa and Eastern Asia |
| Alcohol related cirrhosis | 8% ** |
| Biliary Cirrhosis | 4% |

* Cumulative incidence refers to the proportion of population at risk that will develop an outcome in a given period of time.

** In the absence of HBV and HCV infection.

**Table 2. Risk factors for cirrhosis and HCC [2, 8, 10, 66, 180-182]**

| |
|---|
| HBV |
| HCV |
| Coinfection or tri-infection with HCV/HBV/HIV |
| Aflatoxin in patients with HBV and HCV infection |
| Non-viral hepatitis-related risk factors |
| Alcohol-related liver disease |
| Nonalcoholic fatty liver disease |
| Hemochromatosis |
| Primary biliary cirrhosis |
| Alpha-1 antitrypsin deficiency |
| Primary sclerosing cholangitis |
| Autoimmune hepatitis |
| Wilson disease |
| Cryptogenic cirrhosis |
| Hereditary tyrosinemia |
| Porphyria |
| Aflatoxin |

HCC diagnosed after symptom onset carries a very grim prognosis, with five-year survival less than 10% [4]. Thus, the approach to the population at risk should be two-pronged:

A. Identification and treatment of the predisposing liver conditions.
B. Initiation of surveillance programs in those patients at risk for HCC.

The main emphasis on preventing HCC should be on identifying modifiable risk factors and treating them promptly. Obesity, diabetes, smoking, and infection with HBV, HCV and human immunodeficiency virus (HIV) infection have been recognized as major risk factors for HCC [10]. Universal vaccination against HBV infection has been shown to decrease the incidence of HCC [11-14]. The differential risks for HCC with CHB are summarized in Table 3. There is conflicting evidence for chemoprevention of HCC with nucleos(t)ide analogues [15]. The Greece cohort study reported that antiviral therapy in cirrhotic patients with HBeAg-negative CHB does not decrease the risk of HCC [16]. Although the nucleoside analogue entecavir is a potent antiviral that can fully suppress HBV, studies assessing its effectiveness as a prophylactic against the development of HCC have yielded conflicting results. Studies from Japan [17] and Hong Kong [18] reported that the use of entecavir in chronic HBV was associated with reduced risk of HCC. Trinchet et al. reported similar findings in France where treatment of viral hepatitis was associated with low HCC incidence [19]. This positive response was not observed in a study by Lampertico et al. [20]. Although controversy has existed about whether antiviral therapy should be recommended for CHB patients with compensated cirrhosis who have detectable, but low, serum HBV DNA levels, a recent analysis found that the 5-year cumulative HCC incidence rate was 2.2%, 8.0%, and 14.0% for patients with undetectable HBV DNA (<12 IU/mL), low HBV DNA levels (<2000 IU/mL) plus normal alanine aminotransferase (ALT) levels, and low HBV DNA levels plus elevated ALT levels at baseline, lending support for initiating antiviral therapy in this patient population [21].

Achieving a sustained viral response (SVR) with HCV treatment has been shown to decrease HCC incidence [22] and all-cause mortality [23] in patients with chronic HCV infection. The substantially higher SVR rates now being achieved with the new direct acting antiviral agents [24] may result in significant reduction of HCC incidence and mortality in this population in future. However, it has been suggested that even with DAA-associated SVRs, HCC incidence could continue to increase until 2030 [25] and that the risk of HCC may persist for 8-10 years after SVR achievement [26].

**Table 3. Incidence of HCC in the HBV-infected population [183]**

| **Hepatitis B infection status-Untreated** | **Incidence in Western population at risk** | **Incidence in Asian population at risk** |
|---|---|---|
| Inactive carriers | 0.2% | 0.2% |
| Chronic hepatitis without cirrhosis | 0.3% | 0.6% |
| Compensated cirrhosis | 2.2% | 3.7% |

Prorok has proposed that there are two components for an effective surveillance program [27]: (1) the surveillance test should detect cancer early; and (2) therapy initiated as a result of early detection by screening must be more effective than therapy undertaken in later stages of disease. Using these criteria, screening and surveillance are justified for HCC [28]. The first randomized controlled trial of HCC screening, a study in which liver ultrasound (US) and AFP testing were offered to the surveillance group every six months, found that, after five years of follow up, biannual surveillance reduced HCC mortality by 37% [29].

Multiple studies have shown that HCC surveillance is underutilized in populations at risk, with 20% or fewer of patients receiving surveillance that follows guidelines [30, 31]. Current AASLD guidelines do not recommend any biomarkers for HCC surveillance although the omission of AFP from surveillance has been questioned [32]. Some authors have concluded that US or AFP are suboptimal surveillance tools if used individually; for optimal sensitivity and specificity imaging and biomarkers should be used in combination [33, 34]. The main goal of surveillance is to find HCC in an earlier stage with small tumor burden to enhance the chance of cure or prolonging life. Increased tumor size alone worsens prognosis. Five-year survival has been shown to be significantly lower in small HCC with tumor size 3.5-5cm compared to smaller tumor mass (< 3.5 cm) [35]. The current recommendations for HCC screening and surveillance from several societies are summarized in Table 4.

**Table 4. Surveillance recommendations from liver societies [10, 24, 66, 184-186]**

| Liver societies | Current surveillance recommendations |
|---|---|
| AASLD*(2010) | US every 6 months. |
| | Biomarkers are currently not recommended. |
| EASL ** (2012) | US every 6 months. |
| | Biomarkers are currently not recommended. |
| APASL*** | US every 6 months |
| | AFP every 6 months |
| WGO**** | Ultrasound every 4-12 months based on severity of liver disease. Shorter interval is recommended for advanced cirrhotic patients. |
| JSH % | US every 3-4 months in extremely high risk groups or every 6 months in high risk groups. |
| | AFP/AFP-L3%/DCP every 3-4 months in extremely high risk groups and every 6 months in high risk groups. |

* American Association for the Study of Liver Diseases. **European Association for the Study of the Liver. *** Asian Pacific Association for the Study of the Liver. **** World Gastroenterology Organization. % Japanese Society of Hepatology. DCP, AFP and AFP-L3 are defined elsewhere in the text.

## HCC Biomarkers

Cancer biomarkers either reflect the presence of tumor in the body or predict the risk of developing cancer [36]. The Early Detection Research Network (EDRN) established by the

National Cancer Institute proposed that a biomarker needs to pass through five phases to produce a useful population-screening tool [37]:

Phase 1: Promising directions identified.

Phase 2: Clinical assay detects established disease.

Phase 3: Biomarker detects disease early before it becomes clinical and a "screen positive" rule is defined.

Phase 4: Extent and characteristics of disease detected by the test and the false referral rate are identified.

Phase 5: Impact of screening on reducing the burden of disease on the population is quantified.

The first disease biomarker discovered was the Bence-Jones protein. It was discovered in 1840 [38] and reported as a cancer biomarker in 1848 [39]. With advancements in technology, a growing number of biomarkers have been identified for HCC. Most of these biomarkers are in an early phase of development. Currently identified HCC biomarkers along with the EDRN phase for each are shown in Table 5.

**Table 5. EDRN phase and current status of HCC biomarkers [36, 37]**

| **Phase** | **Category** | **HCC biomarkers** | |
|---|---|---|---|
| | | **Serum/plasma/blood** | **Tissue** |
| 1 | Preclinical exploratory studies | Circulating miR-16<br>EpCAM positive circulating tumor cells<br>IL-6 and IL-10 [187] | Five-gene score, FGF3/FGF4 amplification<br>High Met expression, miR-185 and miR-201, SOUX, AKR1B10, CD34 expression,<br>Human carbonyl reductase2, |
| 2 | Clinical assay development and validation, case control studies | DCP (PIVKAII), Glypican 3, GP73, SCCA, Osteopontin, AFP-L3, Canavaninosuccinate | |
| 3 | Retrospective longitudinal repository studies | AFP | |
| 4 | Prospective screening studies | AFP | |
| 5 | Cancer control randomized studies | AFP | |

Genome-wide association studies have identified single nucleotide polymorphisms (SNPs), which are associated with HCC susceptibility [36]. Although potentially informative, studies on these SNPs are in preliminary stages. To date, the SNPs known to be associated with increased HCC susceptibility are rs7574865, rs9272015, rs2596542, rs9275319, rs101206, and rs2551677; among those found to be associated with decreased susceptibility are rs2880301 and rs455804 [36]. Three loci, rs17401966, rs7574865 and rs9275319, have

been shown to be associated with HBV-related HCC; two loci, rs2596542 and rs9275572, have been shown to be associated with HCV-related HCC [40-44]. At present, SNPs do not provide good prediction at either the individual or population level [45]. These sequencing tests are not yet available clinically.

## ROLE OF CURRENTLY AVAILABLE BIOMARKERS AND THEIR UTILIZATION IN HCC MANAGEMENT

The clinical utility and evidence based utilization of currently available HCC biomarkers are summarized in Table 9. Here we discuss the available biomarkers with a special emphasis on those which are currently recommended in society guidelines. Several models of the probability of HCC development have been developed. The GALAD model uses gender, age, AFP-L3% (*AFP-lens culinaris agglutinin),* AFP and DCP (Des-gamma-carboxythrombin). The initial case control and validation study showed promising results [46], and recent validation of this model in a large multinational cohort showed its high sensitivity (82.2-91.6%) and specificity (87.6-89.7) [47].

It is important to note that the biomarkers discussed here are only risk biomarkers. Due to low sensitivity and specificity as screening tests, they should not be used as sole screening or surveillance tests and are not used to diagnose HCC. HCC is diagnosed by imaging with CT scan or MRI with contrast using Liver Imaging Reporting and Data System (LI-RADS) criteria and, rarely, by liver biopsy. One or more of the biomarkers may, however, complement ultrasound (US) for screening and surveillance, potentially triggering a CT or MRI with contrast when either US imaging indicates the need or HCC biomarkers are elevated. Elevated or increasing biomarkers should trigger a CT or MRI. Of the biomarkers discussed here, only AFP, AFP-L3%, and DCP are FDA-cleared as biomarkers for HCC risk.

## ALPHA-FETOPROTEIN

The modern era of cancer biomarkers dawned with the discovery of alpha-fetoprotein (AFP), an oncofetal protein with a molecular weight of 68,000 [48] that is a member of the albuminoid gene superfamily [49]. Abelev et al. reported a protein in hepatoma bearing mice that migrated to the alpha 1 region in protein electrophoresis [50]. This protein was later found in the serum of a patient with primary liver cancer [51]. In 1970, the first international multicenter study on AFP reported that it was detected in the serum of approximately 75% of patients with primary liver cancer [52]. AFP is known to be present in small quantities in normal adults [49], some fetal organs, proliferating hepatocytes, and several cancers, including HCC, yolk sac tumors, and germ cell tumors [48]. One of the main arguments against the use of AFP as a surveillance biomarker is its presence in multiple conditions, summarized in Table 6.

**Table 6. Causes of Elevated AFP in non-pregnant adults [188]**

| Liver related conditions | Non-liver related conditions |
|---|---|
| Hepatocellular carcinoma | Gastric cancer |
| Acute hepatitis | Esophageal cancer [189] |
| Chronic hepatitis | Pancreatic cancer [190] |
| Fatty liver disease [191] | Colon and rectal cancer [189, 192] |
| Acute liver failure [193] | Germ cell tumors |
| Cirrhosis [194, 195] | Colitis |
| Intrahepatic cholangiocarcinoma [196] | Ataxia telangiectasia |

AFP is the only HCC biomarker that has been studied in all 5 EDRN phases. AFP is not FDA-cleared as a biomarker for HCC risk when used alone; it is only cleared if used in combination with AFPL3%. Its usefulness may range from identifying the population at risk to predicting response to treatment [36]. AFP's association with the long term risk of HCC was evaluated in a retrospective longitudinal cohort study in which 617 patients with CHB were followed for 22 years (median 6.2 years) [53]. The cumulative incidence of HCC after 18 years of follow up was 40.8% in the higher AFP group vs 17.9% in the lower AFP group. The authors concluded that elevated serum AFP was associated with increased risk of HCC and that this increased risk can persist for many years after the index AFP measurement. The risk was independent of other demographic factors.

Because of sensitivity and specificity issues, trials assessing AFP's association with HCC have used a variety of cutoffs for the AFP level. In a large phase II trial of HCC biomarkers in which chronic viral hepatitis was the main cause of liver disease, an AFP cutoff of 10.9 ng/ml had 66% sensitivity and 81% specificity [54]. A systematic review of AFP studies showed that a cutoff value of 20 mcg/L resulted in sensitivity of 41-65% and specificity of 80-94% while with a cutoff value of 200 mcg/L the sensitivity decreased to 20-45% and the specificity increased to 99-100% [55]. Researchers have reported that combining AFP with US for HCC surveillance improves sensitivity and specificity [33, 34].

To overcome the low sensitivity issue, El-Serag et al. proposed an AFP-based algorithm to predict HCC risk [56]. They found that a predictive model that included data on levels of AFP, ALT, and platelets, along with age at time of AFP test, and interaction terms between AFP and ALT and between AFP and platelets best discriminated between HCV-infected cirrhotic patients who would and would not develop HCC within six months. The authors envisioned that application of this modified AFP model would decrease both false positives and false negatives. In a study of AFP in cirrhotic patients Oka et al. found that the cumulative incidence of HCC was significantly higher in patients who had AFP levels of 20 ng/ml or more but below 200 ng/ml at baseline compared to those with levels below 20 ng/ml, as well as in patients who had repeated transient increases in AFP to above 100 ng/ml compared to those who had levels consistently below 20 ng/ml [57]. The researchers concluded that such patients should be treated as being in a super-high-risk group for HCC and should be provided frequent US examination.

AFP has been shown to increase hepatoma cell proliferation in vitro [58] and cytoplasmic AFP has been shown to function on retinoic acid receptor signaling and thus promote the human hepatoma [59]. The suppression of AFP mRNA expression may inhibit the tumor cell growth [48]. In a preliminary study, epitope-optimized AFP activated CD8 T cells and

generated strong antitumor effects in the carcinogen-induced autochthonous HCC mouse model [60], leading researchers to hypothesize that the same type of epitope optimization might enable the development of effective human vaccines to prevent HCC recurrence after liver resection [61]. In a phase I human AFP vaccine study in which 6 patients with advanced HCC were given vaccine twice weekly for three weeks all patients generated T cell responses [62]. However, the T cell responses did not result in any anti-tumor activity in a later study [63].

## AFP for Surveillance of CHB Patients

The annual HCC risk for CHB patients with cirrhosis is estimated to be 3-5% [64]. The annual HCC risk in patients without cirrhosis has been estimated to be 0.4–0.6% in Asian males > 40 years of age and 0.3–0.6% in Asian females > 50 years of age [64]. In the United States, the annual HCC incidence in CHB patients has been reported to be 387 per 100,000 for men and 63 per 100,000 for women in an Alaska native cohort [65] and 470 per 100,000 overall and 657 per 100,000 in men only in a heterogeneous urban North American population [65]. The AASLD IDSA HCV Guidance Panel has concluded that surveillance with dual modality AFP and US becomes cost effective when the incidence of HCC exceeds 0.2% [66]. Carr et al. [67] found that compared to HCV-associated HCC, HBV-associated HCC has larger tumor size and higher AFP level [67]. HCC surveillance using AFP has been assessed in several CHB populations. The first surveillance study in a CHB population was conducted in China using combined AFP and US every six months [29]. In the surveillance arm, there was a 37% reduction in HCC-associated mortality. In a prospective 16-year study of 1,487 Alaska natives with CHB, surveillance was done with AFP every 6 months; men and non-pregnant women with an elevated AFP level were evaluated with US. In a comparison of the long-term survival rate for patients whose HCC was detected by the surveillance program to a historical control group of Alaska native HCC patients, screening was shown to be effective in detecting most HCC tumors at a resectable stage and to significantly prolong survival rates [68]. In a recent review of 132 patients diagnosed with HCC during regular surveillance, the primary mode of tumor detection was US only in 51.5%, US and AFP in 22.0%, AFP only in 19.7%, and incidental in 6.8% of patients. It was found that AFP increased sensitivity by 19.7% for all patients and 28.0% for HBV-related early stage HCC patients, suggesting that AFP may play a significant role in increasing sensitivity in HCC surveillance, especially for detecting early stage HBV-related HCC [69]. Using a computerized decision-analytic model to compare various surveillance strategies Thompson et al. concluded that in a mixed-etiology cohort, the most effective surveillance strategy is to survey with AFP and US every 6 months, but noted that cost effectiveness varied considerably depending on the cirrhosis etiology, with surveillance much more likely to be cost-effective in those with HBV-related cirrhosis [70]. El Serag et al. recommend HCC surveillance every six months with the combination of AFP and US in all patients with cirrhosis or advanced hepatic fibrosis irrespective of etiology, and in adult CHB patients irrespective of cirrhosis [64].

## AFP for Surveillance of Patients with Chronic Hepatitis C

HCV infection is associated with a 15-20 fold higher risk of HCC compared to non-HCV patients, with patients with advanced fibrosis or cirrhosis at the highest risk [3]. The rate of HCC among patients with chronic hepatitis C (CHC) ranges from 1% to 3% over 30 years [3]. Any level of HCV viremia is a strong risk factor for HCC while treatment that results in a sustained viral response (SVR) substantially decreases the risk; studies have reported a 57% to 75% reduction in risk in patients who achieved an SVR with interferon-based therapy [3]. However, because some level of risk persists for years after treatment, patients should have continued surveillance after achieving an SVR. Post-interferon treatment, a high AFP level has been associated with a higher incidence of HCC [71].

In a comparison of biomarker levels in patients in the HALT-C trial in whom HCC did or did not develop, it was found that mild to moderate elevations in total AFP occur frequently in patients with CHC and advanced fibrosis, appear to relate to factors other than HCC, and are poor predictors of HCC [72]. In patients without HCC, 24.5% had at least one AFP between 20 and 199, and 2.3% had at least one AFP value ≥ 200 ng/ml. The authors reported that if the higher cutoff of 200 ng/ml is used then the specificity of AFP increases to 99% but the sensitivity falls to less than 20%. In a retrospective case-control study that was designed to assess whether AFP levels might accurately detect HCC in subgroups of patients, it was found that AFP most accurately detects HCC in patients without HCV infection [73]. AFP levels of 59 ng/mL or greater most accurately detected HCC in patients with HCV-associated cirrhosis; levels of AFP of 11 ng/mL or greater accurately identified HCC in HCV-negative patients. Chang et al. reported that the addition of AFP (cutoff value of 20 ng/ml) to US for surveillance increased sensitivity from 92 (US alone) to 99.2 (US combined with AFP) without significantly affecting the specificity of surveillance (68.3% vs 71.5%) [74]. Other studies have also shown that AFP is complementary to US in surveillance [75, 76]. In a retrospective study of patients with biopsy-proven cirrhosis who were evaluated for high hepatocyte proliferation (S phase fraction), 74% of subjects had hepatitis C; 39% of patients who had high AFP (> 20ng/ml) and high S phase fraction (≥ 1.8) developed HCC compared to 1% of those with normal AFP and low S phase fraction [77], emphasizing the usefulness of AFP as a risk marker for future HCC.

## AFP as Prognostic Indicator for Outcomes after the Diagnosis of HCC

Pre-operative serum AFP level can be predictive of the malignant features and prognosis of HCC. In a retrospective study, patients who had liver resection for HCC were classified based on AFP level into 3 categories: AFP level of ≤ 20, 20-400 and > 400 ng/ml [78]. It was found that pre-operative AFP and tumor size were closely related to HCC post-operative survival. Furthermore, hepatectomy was less effective in patients with AFP value of > 400. The authors concluded that HCC patients with serum AFP higher than 20 ng/mL need comprehensive therapy besides surgical resection and require close follow up. Similar results for HBV-associated HCC have been reported by other investigators [79-82].

A higher AFP level has been reported to be predictive of survival. In a retrospective analysis of HCC patients with baseline AFP > 200 ng/ml that compared patients with AFP value of > 10,000 and < 10,000, it was found that AFP > 10,000 was associated with

significantly worse one-month and six-month overall survival and a higher chance of distant metastatic disease [83]. In a study of Egyptian patients with HCV-associated HCC who underwent hepatic resection, elevated AFP was one of the significant variables predicting tumor recurrence [84]. Serum AFP level at the time of diagnosis with HCV-related HCC has also been shown to be an independent predictor of mortality [85]. In a systematic review of 72 studies, Tandon et al. reported that in HCC patients with cirrhosis the predictors of mortality were AFP, portal vein thrombosis, tumor size, and Child-Pugh class [86].

In patients with HCV-related HCC, it has been reported that the two factors associated with post-resection survival are vascular invasion and AFP level [87]. An AFP value of ≥ 1000 ng/ml and the presence of vascular invasion were independent unfavorable prognostic factors affecting overall survival; AFP of ≥ 1000 ng/ml was an independently significant predictor of poor disease-free survival. It has also been reported that pre-operative AFP of > 100 ng/ml, as well as multifocal lesions and history of tumor rupture, were independent risk factors for recurrence of HCC after hepatectomy [88]. Liu et al. reported that AFP > 400 ng/ml was associated with low overall survival post-hepatectomy in a cohort of Chinese HCC patients, most of whom had CHB (89.7% CHB, 2.4% CHC, and 4.2% both CHB and CHC) [89]. In their review, Singhal et al. noted that AFP > 400 ng/ml has been associated with high tumor burden, bilobar disease, portal vein thrombosis and lower median survival [90]. The aggressive nature of AFP producing tumor may be partially attributed to ephrin-A1, an angiogenic factor [91]. Ephrin A-1 induces the expression of AFP by HCC cells and also over expresses matrix metalloproteinase-2 (MMP-2). MMP-2 overexpression by ephrin-A1 may be involved in promoting HCC invasion and metastasis in AFP-producing tumors.

Studies have also evaluated pre-transplant AFP level as a predictor of post-transplant survival. In one study, an AFP value of > 15 was independently associated with low post-transplant survival: the higher the pre-transplant AFP value, the lower the post-transplant survival independent of Milan criteria [92]. In addition, changes in serum AFP level while on the waiting list corresponded closely to changes in post-transplant mortality. In an assessment of 6478 adult transplant recipients registered in the Scientific Registry of Transplant Recipients (SRTR) it was found that only total tumor volume (TTV) and AFP > 400 predicted post-transplant patient survival, leading the authors to recommend that eligibility criteria be extended beyond the Milan criteria, with inclusion of HCC with TTV of 115 cm3 if AFP is < 400 ng/ml [93]. An analysis of 2253 post-transplant patients from the United Network for Organ Sharing (UNOS) registry divided them into low (53.7%), medium (35.7%), and high (10.6%) pre-transplant AFP groups. The low AFP group demonstrated the best 4-year survival (76%) compared with the medium (65%) and high (57%) AFP groups [94].

The utility of AFP for assessing response to treatment has also been assessed. In a study of 51 single tumor HCC patients (61% related to chronic viral hepatitis) with baseline AFP > 200 ng/ml who had received transarterial locoregional therapy the response to treatment was defined as reduction in AFP of > 50% [95]. AFP responders to locoregional therapy were found to have a high likelihood of showing EASL response (> 50 reduction in viable tumor size). Survival correlated better with AFP response than EASL response. In a prospective study, Wang et al. reported that AFP status one month after radiofrequency ablation was a strong predictor of short term HCC recurrence [96].

The AFP response to sorafenib treatment and its correlation with survival is controversial. Personeni et al. suggested that AFP response (> 20% reduction in AFP value during 8 weeks treatment) can help identify a subgroup of advanced HCC patients who can

achieve longer survival while on sorafenib therapy [97]. Llovet et al. reported that although baseline AFP predicted survival in the placebo cohort of the Sorafenib HCC Assessment Randomized Protocol (SHARP) trial, change in AFP value neither predicted survival nor time to progression in the sorafenib treatment cohort [98].

AFP has been incorporated in multiple HCC staging systems, summarized in Table 7. A comparison study of the current staging systems for advanced HCC that used survival analysis and relative operating characteristic (ROC) to access the prognostic value of each scoring system found that the Advanced Liver Cancer Prognostic System (ALCPS) performed best, with the largest area under the ROC curve in predicting 3-month overall survival (sensitivity 76.32%, specificity 78.72%) [99]. Cancer of the Liver Italian Program (CLIP) and Chinese University Prognostic Index (CUPI) were similar to ALCPS in prognostic discrimination but had relatively lower power. In 2006, Japanese investigators proposed the BALAD (bilirubin, albumin, Lens culinaris agglutinin-reactive alpha-fetoprotein/AFP-L3%, AFP and DCP) score [100]. In a recent study of patients with HBV-related HCC, it was found that the BALAD score could stratify the cohort into different patient groups with distinct median overall survival, and further stratify outcomes in each Barcelona Clinic Liver Cancer (BCLC) subgroup [101].

**Table 7. Different staging systems utilizing AFP**

| **Cancer of the Liver Italian Program (CLIP) variables** [197] | **Chinese University Prognostic Index (CUPI) variables** [198] | **French score variables** [198] |
|---|---|---|
| Portal vein thrombosis | TNM ***stage | Portal vein thrombosis |
| Child-Turcotte Pugh | Asymptomatic disease on presentation | |
| | Ascites | |
| Tumor morphology | Total bilirubin | Total bilirubin |
| | Alkaline phosphatase | Alkaline phosphatase |
| AFP (ng/ml) | AFP | AFP |

## AFP for Allocation of Liver Organ for Liver Transplantation

When the Model for End-Stage Liver Disease (MELD) scoring system was first introduced in 2002 to prioritize candidates for liver transplantation, MELD exception points were given to patients with HCC by Organ Procurement and Transplantation Network (OPTN) imaging criteria and patients with high AFP (> 500) without any radiological evidence of HCC. A study in 2006 using the UNOS registry revealed that the majority of patients who received extra MELD points based on an elevated AFP did not have HCC, leading the researchers to conclude that AFP correlates poorly with the presence of HCC in patients awaiting liver transplantation [102]. Since then AFP has not been used for determining organ allocation.

UNOS recently approved a proposal to delay granting MELD exception points to HCC patients for 6 months irrespective of their MELD score or AFP level, based on multiple studies showing the advantage gained by HCC patients when compared to non-HCC patients;

specifically, candidates will now be registered at their calculated MELD/PELD scores for the first three months (initial application) as well as for the first three-month extension, as long as the candidate continues to meet the policy criteria; at six months (the second extension), candidates will receive a score of 28 [103]. Currently, in the OPTN allocation system, patients with AFP over 500 ng/mL are not to receive organs until the AFP is under 500. HCC exception points allocation models used in selected organ allocation systems are summarized in Table 8**.**

**Table 8. HCC exception points models used in selected organ allocation systems [104]**

| **Organization** | **MELD exception points at listing** | **Points allocation based on AFP level** |
|---|---|---|
| OPTN*/UNOS | 22 | No |
| Eurotransplant | 22 | No |
| Human Organ Procurement and Exchange Program | 22 | Yes |
| Organització Catalana de Trasplantaments | 19 | Yes |

***** Organ Procurement and Transplantation Network.

AFP values or the log of AFP values have been used in calculating scores in newly proposed allocation models such as HCC-MELD, deMELD, MELD-HCC, and new deMELD [104]. A recently published study used the log of AFP values along with the MELD score and the number and size of tumors to develop the MELD equivalent score (MELDEQ) and applied it to UNOS data on adult patients who were added to the wait list between January 22, 2005 and September 30, 2009 [105]. The authors concluded that HCC patients with a combination of a low biochemical MELD score and a low AFP level (MELDEQ $\leq$ 15) would receive a substantial advantage in contrast to patients with chemical MELD scores in a similar range and that a delay of 6 months for listing might be appropriate; however, the survival of patients with MELDEQ scores > 15 would probably be adversely affected by a universal 6-month delay in listing.

**Table 9. Clinical utility and evidence based utilization of currently available HCC biomarkers**

| **Risk prediction** | **Screening and diagnosis** | **Prognostic indicator** | **Risk of PVT** | **HCC recurrence** | **Response to treatment** |
|---|---|---|---|---|---|
| AFP | AFP | AFP | AFP-L3 | AFP | AFP |
| AFP-L3 | AFP-L3 | AFP-L3 | DCP | AFP-L3 | AFP-L3 |
| DCP | DCP | DCP | High Met expression | DCP | FGF |
| SNP | OPN | OPN | | Five gene score | Five gene score |
| | GPC3 | GPC3 | | OPN | High Met expression |
| | FGF | FGF | | | |
| | GP73 | High Met expression | | | |
| | CSA | | | | |

## AFP-L3%

AFP shows differential affinity for the lectin *Lens culinaris* agglutinin (LCA) [106]. Based on this affinity, AFP electrophoresis results in three distinct glycoforms: AFP-L1, AFP-L2, and AFP-L3. AFP-L1 does not have affinity for LCA and is present in chronic hepatitis and liver cirrhosis; AFP-L2 has moderate affinity for LCA and is predominantly produced by yolk sac tumors; AFP-L3 is more prevalent in HCC patients. Results for AFP-L3 are shown as the ratio of LCA-reactive AFP to total AFP (AFP-L3%). In a cohort of chronic hepatitis patients, AFP-L3% was found to be more sensitive than AFP for HCC surveillance [107]. In the subgroup with AFP < 20, AFP-L3% levels were significantly higher in HCC patients than non-HCC patients; the sensitivity for HCC detection was more than 70%. The sensitivity increased further when the combination of AFP-L3% and des-gamma-carboxy prothrombin (DCP) was used. A systemic review of 12 studies suggested that AFP-L3% can be complementary to AFP in diagnosing HCC [108]. In particular, AFP-L3% may add to the clinical utility of AFP if the AFP level is indeterminate. In a retrospective study that assessed blood samples from subjects with benign and malignant liver disease, using a 35% cutoff for AFP-L3% in a subgroup of patients (predominantly CHC patients) whose AFP level was considered indeterminate (10-200 ng/ml) increased the diagnostic utility and helped diagnose an additional 10% of patients not diagnosed using AFP [109]. AFP-L3% increases early in HCC and has been reported to diagnose HCC earlier than imaging based diagnosis [110]. Sato et al. [111] and Kumada et al. [112] reported similar findings in their prospective studies of cirrhotic patients (predominantly caused by HCV and HBV infection).

AFP-L3% is FDA-cleared as a biomarker for HCC risk when used in combination with AFP. AFP-L3% elevation can be reflective of either advanced HCC or more aggressive tumor. In a multicenter study of newly diagnosed HCC patients, 90% of whom had chronic viral hepatitis, AFP-L3% of 15% or more was found to be indicative of advanced HCC even with small tumor size or low AFP concentration [113]. In this study, elevated AFP-L3% was associated with malignant portal vein thrombosis and overall poor prognosis. Elevated AFP-L3% can be reflective of poor HCC differentiation along with other prognostic indicators of poor prognosis, including malignant portal vein thrombosis [114-116].

With solid tumors there is always a concern that there may be tumor recurrence at the primary target organ or a distant metastatic lesion. AFP-L3% may identify patients with a higher risk of early recurrence. In a single center study, 416 newly diagnosed HCC patients were identified, more than 90% of whom had underlying HCV or HBV infection [117]. All the patients received ablative therapy with curative intent. A positive AFP-L3% value post-ablation was the strongest predictor of recurrence, with all patients with AFP-L3% $\geq$ 15% experiencing recurrence within 18 months.

Changes in AFP-L3% may reflect treatment response. In a study of HCC patients, AFP-L3% level was more reflective of clinical response than AFP [118]. Tamura et al. showed that AFP-L3% status 1 month after treatment with curative intent was an independent predictor of HCC recurrence [119]. The status of HCC biomarkers may help decide the preferred treatment option. In a retrospective study of patients with single nodular < 5cc HCC without any vascular invasion the positive status of HCC biomarkers significantly predicted HCC recurrence in the radiofrequency ablation group [120]. The authors suggested that positive

status of three markers may be reflective of micro-invasiveness and, hence, surgical resection might be a preferred treatment modality as compared to radiofrequency ablation.

## DCP

Des-gamma carboxyprothrombin (DCP), also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II) [121], is a prothrombin precursor produced in HCC [122]. The exact mechanism of DCP production in HCC has not been fully clarified [123].

First reported as a tumor biomarker in 1984, DCP was detected in the sera of 69 out of 76 patients with biopsy-proven HCC [124]. In three patients there was a decrease in DCP level with treatment and re-elevation in the level was associated with disease recurrence. Marrero et al. [125] and Durazo et al. [126] have reported that DCP was better for differentiating HCC from nonmalignant chronic liver disease than AFP. However, whether DCP is superior to AFP for screening and surveillance of HCC is controversial [64].

Because the mechanisms of DCP and AFP production by HCC are mutually independent [122] the combination of these biomarkers can be complementary [127] and will help increase the sensitivity of screening for early phase HCC. In a study that evaluated the sensitivity and specificity of DCP, alone or in combination with AFP, for the detection of HCC in CHB patients, the combination had optimal sensitivity and specificity for detecting early stage HCC [128]. Similarly, DCP can increase HCC detection in combination with AFP-L3% [129]. In an HCC surveillance study of patients, 70% of whom had chronic viral hepatitis, the combination of DCP and AFP-L3% had optimal sensitivity and specificity for detecting early HCC independent of AFP level [130]. High DCP levels are predictive of tumor aggressiveness [131] and microvascular [129] or portal vein invasion [132]. A retrospective cohort study of HCC patients found that pre-transplant AFP, AFP-L3%, and DCP all predicted HCC recurrence after transplantation and showed that combining biomarkers with Milan criteria may be better than using Milan criteria alone for optimizing liver transplantation eligibility [133]. DCP is FDA-cleared as a biomarker for HCC risk.

## OSTEOPONTIN

Osteopontin (OPN) is an acidic glycoprotein which has varied physiological functions. Its link to carcinogenesis was described by Senger et al. [134]. Increase in the serum OPN level has been reported in multiple myeloma and colon, pancreatic, breast and ovarian cancer [135-137]. OPN over-production has been reported in HCC [138] and has been associated with poor prognosis [139]. In a study that included HCC patients, patients with chronic liver diseases, and healthy controls Kim et al. reported higher OPN levels in HCC patients compared to patients in the other two groups [140]. In the HCC patients, the OPN level increased significantly with advancing degree of tumor stage and Child-Pugh class. Diagnostic sensitivity and specificity of OPN for HCC were 87% and 82%, respectively. OPN had a greater area under curve value (0.898) than either AFP (0.745) or DPC (0.578), which the researchers concluded suggested the superior diagnostic accuracy of OPN. In a study that measured OPN and AFP levels in patients with HCV-related liver cirrhosis (with

and without HCC) and in healthy controls, OPN levels were significantly higher in cirrhotic patients with HCC [141]. In these patients, OPN levels increased significantly with late tumor stage, advanced Child-Pugh class, larger tumor size (≥ 5 cm) and high tumor grade. The sensitivity and specificity of OPN for HCC were 88.3% and 85.6%, respectively, and its diagnostic accuracy was superior to AFP. In a study that assessed postoperative changes in OPN level it was found that OPN levels significantly decreased after curative resection of HCC and that postoperative OPN might serve as a surrogate biomarker for monitoring treatment response and tumor recurrence after resection [142]. OPN has been shown to play an important role in HCC metastasis and tumor growth of HCC [143] and in disease progression [144] and may be a potential therapeutic target for combating these. Osteopontin is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## GLYPICAN

Glypican-3 (GPC3) is a member of the glypican family of heparin sulfate proteoglycans that are anchored to the cell surface by glycosyl-phosphatidylinositol [145]. GPC3 is expressed in up to 72% of HCC but is not detectable in sera of healthy donors and patients with hepatitis [146, 147]. High GPC3 expression has been reported in HCV-related HCC [148, 149] and in a study of HBV- and HCV-related HCC patients, GPC 3 was found to be a sensitive and specific marker for diagnosis of early stage HCC [150]. In a similar study of HCC (predominantly due to CHB and CHC), the GPC3 level was significantly elevated in both early and advanced HCC [151]. GPC3 appears to be complementary to AFP for diagnosis of HCC [151, 152]. Positive GPC3 status in HCC is associated with an overall poor prognosis [149, 153] and with early HCC recurrence after resection [153, 154]. Glypican is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## GOLGI PROTEIN 73

Golgi protein 73 (GP73) is a Golgi protein of unknown function which has been found to be upregulated in viral hepatitis [155]. In normal livers, GP73 is constitutively expressed at low levels by biliary epithelial cells but not by hepatocytes. In advanced liver disease due to both viral causes (HBV, HCV) and non-viral causes (alcohol-induced liver disease, autoimmune hepatitis) hepatocyte expression of GP73 is dramatically up-regulated [156]. In advanced liver disease, the GP73 level is significantly higher in HCC patients compared to non-HCC patients and has significantly higher sensitivity than AFP for diagnosing early HCC [157-159]. It also has better sensitivity and specificity than AFP for diagnosing HBV-related HCC [160, 161]. The combination of AFP and GP73 has been reported to be more sensitive than AFP alone for HCC diagnosis [152]. The conclusion of a systematic review of studies assessing the accuracy of GP73 and AFP in the diagnosis of HCC was that GP73 has accuracy comparable to AFP for HCC diagnosis, but that determining the value of the combination of GP73 and AFP for HCC detection will require further investigation [162]. To date, data is limited and does not satisfy the criteria for a biomarker put forward by EDRN. GP73 is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## FIBROBLAST GROWTH FACTOR (FGF) AMPLIFICATION

FGF3 and FGF4 are receptors for fibroblast growth factor and promote HCC [36]. FGF2 has also been reported to be highly expressed in HCC [163]. In a study of HCC patients undergoing resection a high pre-operative FGF level was found to be predictive of invasive tumor and early postoperative recurrence [164]. FGFR4 contributes significantly to HCC progression [165]. An *in vitro* study that investigated the expression of human FGF19 and its receptor, FGFR4, in HCC specimens found that FGF19 was significantly overexpressed in HCC and that FGF19 recombinant protein could increase the proliferation and invasion capabilities of HCC cell lines and inhibit their apoptosis while decreasing FGF19 and FGFR4 expression by siRNA significantly inhibited proliferation and increased apoptosis [166]. The researchers concluded that FGF19 inhibition might be a therapeutic strategy for HCC. High FGF3/4 amplification has been shown to be an indicator of poor prognosis and there is limited evidence that its expression may predict response to sorafenib in some patients; however, the use of high levels of FGF3/4 expression as a general purpose marker is problematic because the FGF3/4 mutation has only been observed in a very small percentage of HCC patients [36, 167, 168]. Blockade of FGFR4-mediated signaling might be a potential therapeutic target [169] but because FGFR4 plays an important physiological role in the regulation of hepatic bile acid synthesis any possible use of therapeutic FGF19-FGFR4 inhibition must be considered in conjunction with the potential safety implications of FGF19-FGFR4 blockade [170]. Due to limited data, it is difficult to ascertain whether there are any differential changes in FGF3/4 amplification in viral hepatitis-related HCC compared to other etiologies. FGFs are not FDA-qualified as biomarkers for HCC risk and are not currently available.

## CANAVANINOSUCCINATE

Metabolomic profiling using gas chromatography has been shown to differentiate individuals with HCC from non-HCC subjects using a panel of metabolite biomarkers which could be particularly helpful in subjects with AFP < 20 ng/ml [171]. Canavaninosuccinate (CSA) is an organic acid metabolite produced in liver [36] that has been identified as a metabolite biomarker which could differentiate HCC from cirrhotic and healthy individuals [172]. The combination of AFP and CSA has been shown to have high sensitivity and specificity (92% and 100%, respectively) for discriminating HCC from cirrhosis [36]. CSA is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## FIVE GENE SCORE

A score using 5 genes (TAF9, RAMP3, HN1, KRT19 and RAN) was developed to predict disease-free survival after curative resection [36]. These genes are involved in dysregulated pathways of HCC development. The score was developed and then validated in HCV-related HCC in a Western population and HBV-related HCC in an Asian population [173]. The score has been shown to accurately predict disease free survival and early

recurrence and with its highly predictive accuracy it might help guide therapy [174]. It could stratify the type of therapy based on the score. For example, HCC patients with good genetic prognostic parameters might benefit from resection while in patients with bad prognostic parameters resection would not improve the outcome [174]. Patients with a score of ≥ zero have worse survival compared to HCC patients with a score < zero [36]. The 5-gene score is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## HIGH MET EXPRESSION

It has been shown that high expression of c-Met, a high affinity receptor for hepatocyte growth factor, is associated with poor prognosis and HCC recurrence [175, 176] and with portal vein invasion [177]. Inhibition of c-Met may have potential as a therapy in HCC patients with strong c-Met expression [176]. In a phase 2 trial, treatment with tivantinib, a selective oral inhibitor of MET, resulted in a median time to progression for patients with MET-high tumors of 2.7 months compared to 1.4 months for patients on placebo [178]. If future trials confirm the results, it might be useful for this high c-MET subgroup of patients. It might also complement primary target therapy based on c-Met expression [179]. However, c-MET is not FDA-qualified as a biomarker for HCC risk and is not currently available.

## CONCLUSION

HCC diagnosis after symptom onset carries a grim prognosis with five-year survival of < 20%. Thus, in a population with risk factors for HCC, our goal should be to diagnose it in an early stage and manage the patient with curative intent. The currently available screening and surveillance modalities, including US, are underutilized in the clinical setting. Current AASLD guidelines only recommend US every 6 months for surveillance of high-risk patients. The biomarkers that have been studied to date are only risk biomarkers and of those discussed here, only AFP, AFP-L3%, and DCP are FDA-qualified as biomarkers for HCC risk. Due to low sensitivity and specificity as screening tests, they should not be used as sole screening or surveillance tests and are not used to diagnose HCC. HCC is diagnosed by imaging with CT scan or MRI with contrast using Liver Imaging Reporting and Data System (LI-RADS) criteria and, rarely, by liver biopsy. One or more of the biomarkers may, however, complement ultrasound (US) for screening and surveillance, potentially triggering a CT or MRI with contrast. Elevated or increasing biomarkers should trigger a CT or MRI.

AFP has been shown to be a strong prognostic indicator and to complement US for surveillance. A higher AFP value has clearly been associated with poor clinical outcome while AFP reduction > 50% after treatment predicts an improved response. The pre-treatment AFP value may identify patients who will benefit more from loco-regional therapy than transplant. A higher pre-transplant AFP level has been associated with tumor recurrence and thus may help identify patients who will need close observation for early HCC recurrence. AFP has been used in multiple HCC staging systems, including ALCPS, CLIP, CUPI and BALAD. The AFP level (with a ceiling of < 500 ng/ml) has been utilized in some liver allocation models to grant MELD exception points. AFP-L3% has been found to complement

AFP in HCC surveillance, especially when the AFP value is indeterminate. As with AFP, a higher AFP-L3% level may be reflective of either advanced disease or aggressive HCC. Elevated levels of both AFP-L3% and DCP have been associated with vascular invasion by the tumor. A high post-ablation AFP-L3% value may be reflective of disease recurrence. Similar to AFP, AFP-L3% may help determine treatment modality. DCP can help differentiate HCC from non-malignant chronic liver disease. Its re-emergence after treatment can be reflective of HCC recurrence. DCP can complement AFP in HCC risk assessment. Data for other biomarkers are limited, and no firm recommendation can currently be made for their use.

Several risk assessment models have been proposed to help with some of the limitations of these biomarkers. Multiple large studies are underway on the clinical utility of HCC biomarkers and on the proposed models that will ultimately help answer remaining questions on their usefulness. In the meantime, guidelines from the Japanese Society of Hepatology recommend AFP, AFP-L3% and DCP for HCC surveillance. Experts in the field agree that until better screening and surveillance tools are available for HCC, we should utilize the available biomarkers to complement US and help identify patients who would benefit from further radiological workup.

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