Curr Hepatology Rep (2017) 16:137–145
DOI 10.1007/s11901-017-0349-7

HEPATIC CANCER (A SINGAL AND A MUFTI, SECTION EDITORS)

Should AFP (or Any Biomarkers) Be Used for HCC Surveillance?

Hager F. Ahmed Mohammed[1,2] · **Lewis R. Roberts**[1]

Published online: 28 April 2017

Abstract
Purpose of Review The aim of this review is to address the controversy around the use of biomarkers for hepatocellular carcinoma (HCC) surveillance in individuals with cirrhosis or chronic hepatitis B who are at risk for development of liver cancer.
Recent Findings Recent studies suggest that surveillance for hepatocellular carcinoma is beneficial, even after adjustment for lead time and other biases. Alpha fetoprotein (AFP) is complementary to ultrasound (US) in surveillance, particularly in obese patients and patients with infiltrative tumors. US and AFP are both associated with harms to patients from false-positive overdiagnosis, with US appearing to cause greater harms. Including patient demographic characteristics and additional biomarkers into diagnostic models is beneficial. Recent studies emphasize the advantage of time trends in biomarkers over single cross-sectional measurements.
Summary AFP and other biomarkers are complementary to US in surveillance for HCC, especially when applied in models including patient variables and incorporating time trends in biomarker levels. With advances in genetic and molecular analysis of tumors, we may be poised at the cusp of a revolution in HCC surveillance.

This article is part of the Topical Collection on *Hepatic Cancer*

✉ Lewis R. Roberts
roberts.lewis@mayo.edu

[1] Division of Gastroenterology and Hepatology, Mayo Clinic College of Medicine and Science, 200 First Street SW, Rochester, MN 55905, USA

[2] Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA

Keywords Des-gamma carboxy prothrombin · AFP-L3 · GALAD · Liver cancer · Hepatocellular carcinoma · Screening · Cirrhosis

Abbreviations

AFP	Alpha fetoprotein
DCP	Des-gamma carboxy prothrombin
AFP-L3	*Lens culinaris* agglutinin-bound fraction of AFP
AASLD	American Association for the Study of Liver Diseases
EASL	European Association for the Study of the Liver
HCV	Hepatitis C virus
US	Ultrasonography
BCLC	Barcelona Clinic Liver Cancer

Introduction

While it has been generally accepted by the hepatology community that surveillance for hepatocellular carcinoma (HCC) in patients who are at risk is justified, the quality of evidence supporting this recommendation is generally perceived to be low. The available randomized controlled trial and comparative cohort studies have been criticized as being limited by methodological flaws [1–3]. While liver ultrasonography (US) is well accepted as an effective modality for screening for HCC, the use of AFP has been less well accepted, and there has been strong judgment expressed by some experts against the use of serum AFP test as a screening biomarker for HCC [4]. On the other, hand, surveys of practicing hepatologists suggest that the majority of hepatologists in Asia, Europe, and North America routinely use AFP as a screening test, typically in combination with US [5–8]. Further, Japan and Taiwan, countries that have documented

the best long-term outcomes for HCC through nationwide surveillance programs apply a combination of US, AFP, as well as other biomarkers (*Lens culinaris* binding AFP (AFP-L3%) and des gamma carboxy prothrombin (DCP)) [9, 10].

Thus, while there appears to be a tacit acceptance of a presumed utility of AFP in HCC surveillance, the issue is controversial. Consequently, there are discrepancies between different guideline recommendations regarding the use of AFP and other biomarkers. The lack of uniformity may contribute to the observed low rates of surveillance by primary care practitioners in many countries, so that in most countries, the majority of HCC patients have intermediate to advanced stage disease at the time of diagnosis [9, 11, 12]. These concerns have fueled the development of studies that have more rigorously examined the performance and cost-effectiveness of both US and blood biomarkers. Recent studies have also begun to incorporate other risk factors such as age and gender into combined models with blood biomarkers.

Recent studies suggest the performance of AFP can be optimized by stratifying the population under surveillance by etiology, receipt of therapy for chronic viral hepatitis—which may influence hepatic inflammation and liver regeneration, and liver synthetic function as measured by serum albumin [13–15]. Further, recent studies also suggest that performance of liver US, which has been considered the more sensitive and specific screening modality, may not be as high in real-life practice as previously thought [16]. US appears to have lower performance in imaging of individuals with central obesity, in whom it is difficult to accurately image the deeper parts of the liver, and for more aggressive, infiltrative and metastatic HCCs with high AFP that do not form distinct nodules in the liver [17, 18]. For many clinicians, these factors appear to justify use of US and AFP in combination for surveillance for HCC. After a number of years in which the primary debates about HCC surveillance were anchored around the perceived flaws in the supporting literature, such as lack of adjustment for lead time and length time bias, we are beginning to see results of a number of studies from Asia, North America, and Europe that are addressing these concerns and providing more robust confirmation of surveillance benefits [8, 19–26]. In addition, recent advances in next-generation sequencing and molecular analysis of tumors, blood, stool, and other analytes are producing what potentially may prove to be very exciting breakthroughs in biomarker technology, particularly assays of differentially methylated DNA regions and microRNAs. In parallel, we are seeing advances in imaging technology, such as studies of limited MRI exams performed using hepatobiliary contrast agents, and the development of novel ultrasound-based technologies [27–30]. These newer modalities may provide substantial advances over current US screening at similar or reduced cost. Thus, we may be poised at the cusp of a revolution in surveillance for HCC.

Additional considerations that have been expressed in recent studies are the potential harms of ineffective screening tests, articulation of the concept of efficacy vs. effectiveness which emphasizes the importance of ensuring full or nearly full population coverage of every step along the prevention → screening → diagnosis → treatment continuum for achieving cost-effective surveillance on a population basis, an appreciation of the importance of repeated screening for achieving optimal performance of surveillance, the importance of trends in biomarker levels over time, reflecting the fact that individuals are their own best control, and the effect of the improvements in treatment of viral hepatitis on the utility of blood-based biomarkers, which can be falsely elevated in patients with active hepatitis and liver regeneration.

There are two major challenges to development of the perfect biomarker: (1) genetic and molecular heterogeneity of individuals, which means that levels of any single biomarker in a population of unaffected individuals will be variable, and (2) genetic and molecular heterogeneity of HCC, which means there will be variation in the levels of almost all biomarkers produced by different cancers. Thus, while the ideal biomarker would be able to perfectly discriminate between cases and controls (Fig. 1a), in practice, most real-life biomarkers show overlap between cases and controls (Fig. 1b). This is exemplified by the experience in HCC, in which there is incomplete overlap between patients with elevated AFP, AFP-L3%, and DCP [31]. These variations in baseline normal biomarker levels and in tumor biomarker secretion lead to the problems of imperfect specificity, the difficulty in determining which biomarker values are falsely positive, and imperfect sensitivity, the difficulty in determining which biomarker values are falsely negative. Solutions for the first problem may be the use of serial measurements beginning before onset of cancer development, so that the individual serves as their own control [32], and for the second problem, the use of multiple biomarkers, so that all possible biomarkers that may be elevated can be interrogated at the same time [31, 33]. There are active efforts underway to achieve these goals for blood-based biomarkers, but we are still far from achieving them. Until we do, the practical alternative is the use of the appropriate clinical and demographic variables, in addition to the available biomarkers and imaging studies, in a multidimensional construct that optimizes the available information (Fig. 1c) [34], and the use of these constructs or models in a longitudinal, serial manner, so that the results of earlier studies inform the interpretation of subsequent studies (Fig. 1d) [35••]. Many experienced, thoughtful clinicians intuitively use this approach [36, 37].

The evidential basis for the practice of HCC surveillance is gradually accumulating in the USA and other Western countries. Interestingly, this is occurring at the same time as, contrary to recent trends for most cancer types, we are seeing an increase in the incidence of liver cancer and deaths from liver

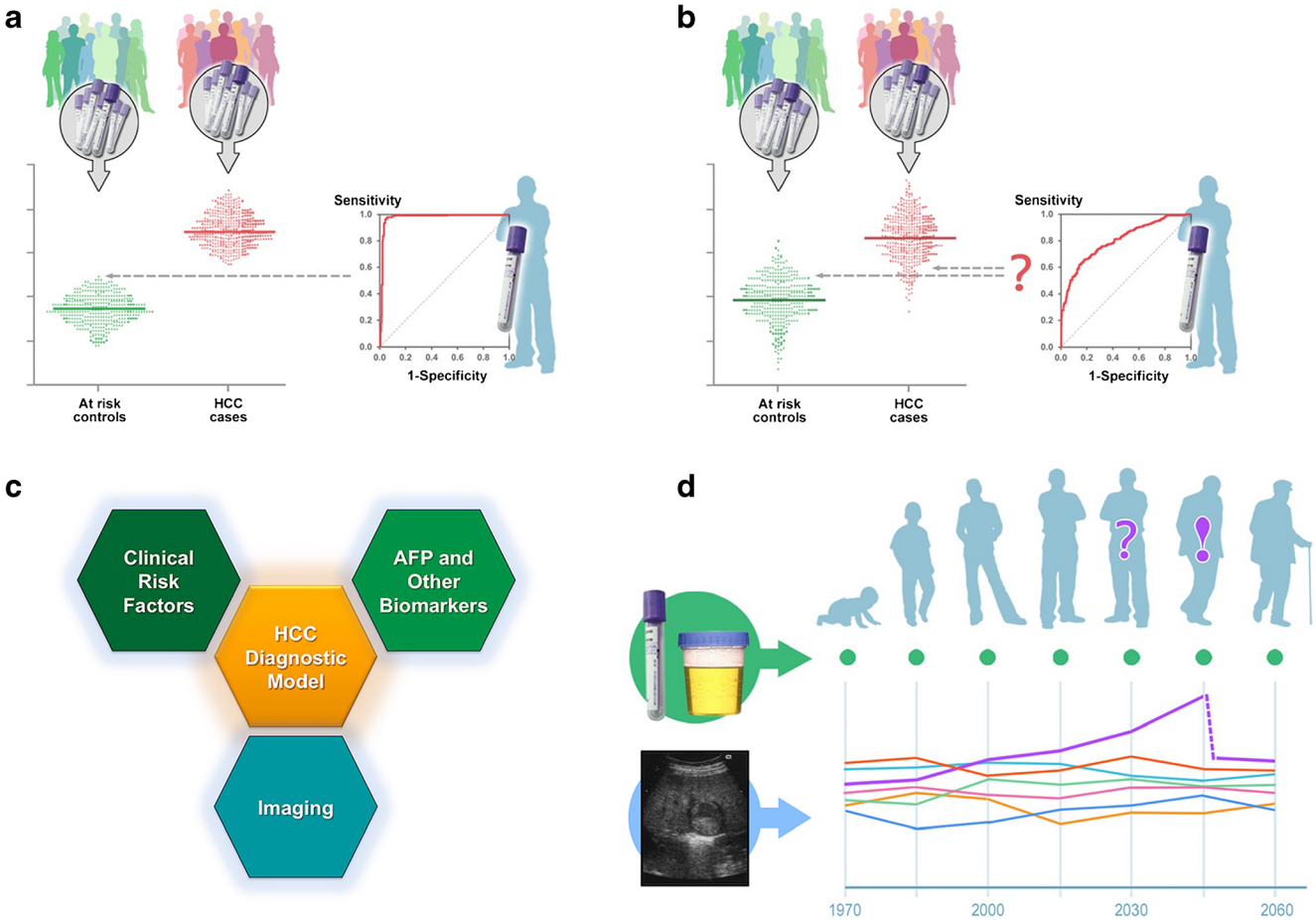

Fig. 1 **a** The ideal HCC biomarker would distinguish perfectly between HCC cases and unaffected controls because there is no overlap between the levels of the biomarker in cases and the levels in controls, resulting in an almost perfect area under the receiver operating characteristic (AUROC) curve, with an area of nearly 1. **b** In real life, most biomarkers have levels in cancer cases that partially overlap with the levels in controls, resulting in variably imperfect AUROC curves with areas typically between 0.6–0.85. **c** Newer diagnostic models such as the GALAD model incorporate patient clinical characteristics to improve the performance of biomarkers and imaging studies. **d** Methods that take longitudinal trends in biomarker levels into account can use each individual as their own control, further enhancing the combined performance of biomarkers, imaging, and diagnostic models

cancer, with liver cancer now recognized as the fastest rising cause of death from cancer in the USA. Of particular concern is the rise in the incidence of HCC associated with nonalcoholic fatty liver disease, and the increasing appreciation that a substantial number of patients with the metabolic syndrome or nonalcoholic steatohepatitis (NASH) develop HCC in the absence of cirrhosis, further complicating efforts to identify the high-risk population for surveillance [38–40].

Differences in Current Guideline Recommendations for HCC Surveillance

The ultimate cancer surveillance modality should have high sensitivity and specificity, should be applicable for individuals at high risk for cancer in a cost-effective manner, and should be proven to reduce mortality when applied in the at-risk population. The effect of surveillance in reducing the mortality associated with HCC has been demonstrated in only a few studies, each of which has flaws and none of which are completely generalizable to individuals with all chronic liver diseases or cirrhosis of all etiologies [1, 2]. The most widely accepted surveillance modality is US as recommended by the American Association for Study of Liver Disease (AASLD), the European Association for the Study of the Liver (EASL), and the Asian Pacific Association for the Study of Liver Disease (APASL) [41••, 42, 43]. The rational for discouraging use of AFP in the AASLD 2010 guidelines was the lack of evidence supporting its high sensitivity and specificity as an effective surveillance and diagnostic tool for HCC [44], while the EASL-EORTC concluded that the surveillance value of AFP with or without US is unsatisfactory [42]. Despite the more limited evidence for the effectiveness of biomarkers in HCC surveillance, national surveillance programs in countries like Japan and Taiwan use biomarkers as routine tools for HCC surveillance, including AFP, AFP-L3%, and DCP [45].

The recently published 2017 AASLD guidelines for HCC reviewed the evidence for surveillance and recommended the use of ultrasound with or without AFP for surveillance [41••]. In the evidence review, no studies were found that directly compared the performance of US to AFP in surveillance. However, some studies compared US to the combination of US and AFP. Overall, there was no difference between the two approaches in the likelihood of diagnosis at an early stage to facilitate curative treatment. The combination of AFP and US was associated with a higher survival rate, but this did not reach statistical significance. The studies reviewed had many limitations: they did not take into account the limitations of US and were underpowered. In addition, early detection of HCC was often not compared between the two different modalities. Thus further studies are needed to determine whether the use of AFP or US alone would lead more frequently to a diagnostic workup. Similar considerations led to the recommendation for combined use of US with AFP in HCC surveillance by the WHO Guidelines for the Prevention Care and Treatment of Persons with Chronic Hepatitis B Infection [46].

Pros and Cons of Current Methods of HCC Surveillance

Ultrasonography

Ultrasonography is the current mainstay for HCC surveillance. The sensitivity of US for HCC detection is approximately 60%, with a specificity of 85–90% [47]. Despite its capability for high sensitivity and specificity, US is operator dependent. Moreover, US is affected by the body habitus of the patient, with suboptimal performance in patients with central obesity, as well as by liver nodularity in patients with cirrhosis [48]. Compared to US, serum biomarkers provide more standardized results regardless of operator expertise or patient body habitus. US also has very limited capability for distinguishing between different types of liver tumors. A surveillance study from Japan showed that 4.4% of hepatic tumors detected by US were cholangiocarcinomas [10]. On the other hand, a higher percentage of patients underwent unneeded diagnostic imaging based on indeterminate lesions detected by US compared to false positive AFP results (26% compared to 2.7%) [49]. Atiq et al. found that the stage of HCC tumor detected by US did not differ significantly from that detected by AFP [50]. It further revealed that false positive surveillance by US is more likely to be associated with harm than that with AFP (22.8% compared to 11.4%, $P < 0.001$). Harm was defined as performance of CT, MRI, biopsy, or other procedures in patients without HCC. The sensitivity of US decreases as tumors become smaller in size, with a resultant drop in sensitivity from 94% for all HCC stages down to only 63% for early tumors, defined as a single nodule <5 cm or 3 nodules each <3 cm without vascular invasion. Double-contrast US was found to have higher sensitivity than B-mode US in Japan. A retrospective study conducted for tumors less than 2 cm in transplant patients revealed sensitivities of 21, 40, and 47% for US, CT, and MRI, respectively. On the other hand, the specificities were 82, 74, and 77%, respectively [51].

Computed Tomography and Magnetic Resonance Imaging

Cross-sectional multiphasic contrast-enhanced CT and MRI are generally not recommended as screening studies despite their high diagnostic value due to the high cost of these tests, as well as the additional potential harm due to repeated exposure to irradiation or intravenous contrast. In an effort to maximize the potential value of cross-sectional MRI while minimizing contrast exposure, scanning time, and cost, abbreviated MRI examination protocols are being developed and tested in a number of centers [27, 28, 30, 52]. Ongoing studies may clarify the most appropriate niche for cost effective use of these modalities, perhaps particularly in those settings where US performs the least reliably, such as in individuals with truncal obesity.

Alpha Fetoprotein (AFP)

AFP is the most commonly studied biomarker for HCC, with the available studies including a phase 5 biomarker trial assessing the impact of AFP on survival of patients with HCC [1]. In general, AFP level is positively associated with tumor size, restricting its utility in detecting smaller HCC tumors. The utility of AFP is limited by its low sensitivity, and specificity for early stage disease when its performance is considered in a cross-sectional manner at a single point in time. At a cutoff value of 20 ng/mL, AFP was found to have a sensitivity between 49 and 71% and a specificity between 49 and 86% in detecting HCC tumors <5 cm in size [53]. AFP can be falsely elevated in conditions other than HCC, e.g., hepatitis C infection, or cholangiocarcinoma [54–56]. The accuracy of AFP in HCC surveillance has been shown to improve with measuring an increasing trend of AFP rather than a one-time assessment [32]. Additionally, accounting for other patient factors such as ALT level and etiology of liver disease in assessing AFP result can substantially aid interpretation [13, 14, 35••]. Newer statistical approaches that use prior AFP levels in a longitudinal manner, such as the parametric empirical Bayesian (PEB) screening algorithm, also show promise in enhancing the performance of AFP [57••, 58].

The combination of AFP and US has higher sensitivity than AFP or US alone [49]. AFP increased the US sensitivity of HCC detection and early HCC detection by 7 and 6%, respectively [47]. In a prospective study, AFP increased the sensitivity of early detection of HCC from 32 to 63% [16]. Another prospective study of the HALT-C cohort showed that while US detected 58% of early HCC cases before AFP elevation, AFP was elevated in 21% of early HCC cases before a nodule

was detected on US [59]. A cost-effectiveness analysis was conducted by Gounder et al. comparing surveillance by US alone versus AFP with transition to US if AFP level was found to be more than 10 ng/ml [60]. The AFP-US method was associated with lower cost per early stage tumor detected and years of life gained (YLG) compared to US alone. The cost of AFP-US surveillance was found to be $375,000 ($36,000/early stage tumor detected and $13,000/YLG) vs. $814,000 ($59,000/early stage tumor detected and $21,000/YLG) by US alone. Thus, the AFP-US method was associated with an additional 27.8 YLG compared to 38.9 YLG by US alone. The relatively low cost of the AFP-US strategy could help broaden surveillance for HCC [60].

Other Biomarkers in Clinical Use

Besides AFP, other serum biomarkers have not been rigorously studied beyond phase III biomarker studies.

Lectin-Binding Alpha Fetoprotein (AFP-L3%)

AFP L3 is a subfraction of the total AFP that binds to *Lens culinaris* agglutinin and has been shown to be a highly specific biomarker for HCC (99.4% specificity) [61]. Although more sensitive versions of the test have been developed, it has lower sensitivity than AFP (sensitivity is 18.8%) [61, 62]. AFP-L3 is more likely to be elevated in infiltrative tumors and advanced HCC stages, and therefore predicts tumor recurrence and overall patient survival. It can be falsely elevated in liver failure [61]. Even for small HCCs, elevated AFP-L3% is associated with more proliferative tumors with moderately differentiated or poorly differentiated histology, rich arterial neovascularization, and decreased portal supply, all consistent with a poorer prognosis [63–65]. Because of its high specificity for HCC, AFP-L3% can be of particular value in circumstances in which the total AFP is nonspecifically elevated by inflammation or enhanced liver regeneration, such as in patients with chronic HCV infection [66].

Des-Gamma-Carboxy Prothrombin (DCP) Also Called Protein Induced by Vitamin K Absence/Antagonist-II (PIVKA-II)

DCP/PIVKA-II is an abnormal form of the coagulant protein prothrombin that is missing the normal gamma carboxyl moiety with which prothrombin is modified posttranslationally in the liver by the vitamin K-dependent enzyme gamma-glutamyl carboxylase, prior to secretion of prothrombin into the plasma [67]. DCP has been shown to detect HCCs with similar sensitivity to AFP but higher specificity [68, 69]. DCP can be elevated in patients with normal AFP levels, thus increasing the sensitivity of biomarker detection of HCC when used in combination with AFP [68–70]. Vitamin K is required for the normal action of gamma-glutamyl carboxylase; thus, individuals with vitamin K deficiency or those using vitamin K antagonists such as warfarin have artificially high DCP levels [68]. Using a cutoff level of 40 mAU/ml for tumors <5 cm, the sensitivity, specificity, and likelihood ratio (LR) of DCP for HCC diagnosis were found to be 0.14–0.54, 0.95–0.99, and 6.86–29.7, respectively [71]. After initial discovery and clinical evaluation in the USA and Taiwan, the clinical utility of DCP has been most appreciated in Japan, where highly sensitive assays were developed and it has become part of the standard HCC surveillance regimen [45, 72–74]. While DCP has been in routine use in Japan for many years, it has only more recently been rigorously evaluated in Western countries [33, 75–77]. These studies have generally confirmed the utility of DCP as a biomarker, although there are only limited studies testing its performance in prospective studies [59]. At least one study suggests that DCP may be a better marker in HCCs developing in patients with NASH [77]. DCP is currently approved by the US FDA as a marker of risk for HCC and its use in diagnostic and prognostic models such as the GALAD and BALAD scores is increasing interest in its biomarker performance [78••, 79, 80].

Emerging Biomarkers Not Yet in Clinical Use

Other emerging biomarkers include osteopontin (OPN) [81–83], latent TGF-beta binding protein I (LTBP1) [84], latent TGF-beta binding protein II (LTBP2) [83], DKK1 [85], midkine (MDK) [86, 87], GP73 [88, 89], glypican 3 (GPC3) [90], and a variety of miRNAs [91, 92]. These markers are under active investigation and none of them have been approved for clinical use.

Combination of Biomarkers with Clinical Features, the GALAD Serologic Model

GALAD is a statistical model to assess the likelihood of HCC in patients with chronic liver disease. The GALAD model was developed initially in the UK with a subsequent large multicenter cohort validation including Hong Kong, Japan, and Germany [78••, 80]. The model combines patient demographics (gender and age) with the serum biomarkers (AFP-L3%, AFP, and DCP); a Web-based calculator is available at: http://www.mayoclinic.org/medical-professionals/model-end-stage-liver-disease/galad [93]. The validation study revealed that the GALAD model achieves a larger area under the receiver operating characteristic curve (AUROC 0.93–0.97) compared to the individual biomarkers in all three countries. This suggests that it achieves more accurate early detection of HCC in the setting of chronic liver disease. Unlike the performance of US in previous studies, GALAD has the ability to distinguish HCC from other hepatobiliary cancers (AUROC 0.95). The etiology of the underlying liver

disease did not have a significant impact on the GALAD result. Small size single tumors were associated with lower AUROC (0.85–0.95), with the overall AUROC for tumors <2 cm between 0.89 and 0.93 [94, 80].

Conclusion

Despite the imperfect sensitivity and specificity of individual blood biomarkers, they still appear to have an important role in HCC surveillance. Biomarkers provide standardized surveillance performance when US performance is suboptimal due to an inexperienced operator or in obese patients. While the utility of biomarkers and ultrasound or other imaging studies are often cast in a competitive framework between biomarkers or between biomarkers and US, optimal models will need to be developed that integrate these modalities in the most effective way. In real-life practice, US and AFP have been shown to be complementary in surveillance for HCC [16]. In addition, the combination of patient demographic characteristics with biomarkers in models such as the GALAD model show early promise for further enhancing the value of biomarker measurements in HCC surveillance. Combining these models with integration of the longitudinal changes in biomarker levels and the results of ultrasound or enhanced ultrasound-based imaging may present the next step forward for improvements in HCC surveillance. In the near term, studies prospectively validating the GALAD and similar scores and other new biomarkers are urgently needed.

Compliance with Ethical Standards

Conflict of Interest Hager F. Ahmed Mohammed declares no conflict of interest. Lewis R. Roberts has received grant funding from BTG, Gilead Sciences, and Wako Diagnostics and has served on an advisory board for Bayer.

Human and Animal Rights and Informed Consent All reported studies/experiments with human or animal subjects performed by the authors have been previously published and complied with all applicable ethical standards (including the Helsinki declaration and its amendments, institutional/national research committee standards, and international/national/institutional guidelines).

Sources of Funding Mayo Clinic Cancer Center (CA15083), Mayo Clinic Center for Cell Signaling in Gastroenterology (NIDDK P30DK084567), Mayo Clinic Center for Clinical and Translational Science (NCATS UL1 TR000135), and the Mayo Foundation.

References

Papers of particular interest, published recently, have been highlighted as:
•• Of major importance

1. Zhang BH, Yang BH, Tang ZY. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417–22. doi:10.1007/s00432-004-0552-0.
2. McMahon BJ, Bulkow L, Harpster A, Snowball M, Lanier A, Sacco F, et al. Screening for hepatocellular carcinoma in Alaska natives infected with chronic hepatitis B: a 16-year population-based study. Hepatology. 2000;32(4 Pt 1):842–6. doi:10.1053/jhep.2000.17914.
3. Kansagara D, Papak J, Pasha AS, O'Neil M, Freeman M, Relevo R, et al. Screening for hepatocellular carcinoma in chronic liver disease: a systematic review. Ann Intern Med. 2014;161(4):261–9. doi:10.7326/M14-0558.
4. Sherman M. Alphafetoprotein: an obituary. J Hepatol. 2001;34(4):603–5.
5. Chalasani N, Said A, Ness R, Hoen H, Lumeng L. Screening for hepatocellular carcinoma in patients with cirrhosis in the United States: results of a national survey. Am J Gastroenterol. 1999;94(8):2224–9. doi:10.1111/j.1572-0241.1999.01297.x.
6. Van Kleek EJ, Schwartz JM, Rayhill SC, Rosen HR, Cotler SJ. Liver transplantation for hepatocellular carcinoma: a survey of practices. J Clin Gastroenterol. 2006;40(7):643–7.
7. El-Serag HB, Alsarraj A, Richardson P, Davila JA, Kramer JR, Durfee J, et al. Hepatocellular carcinoma screening practices in the Department of Veterans Affairs: findings from a national facility survey. Dig Dis Sci. 2013;58(11):3117–26. doi:10.1007/s10620-013-2794-7.
8. van Meer S, de Man RA, Coenraad MJ, Sprengers D, van Nieuwkerk KM, Klumpen HJ, et al. Surveillance for hepatocellular carcinoma is associated with increased survival: results from a large cohort in the Netherlands. J Hepatol. 2015;63(5):1156–63. doi:10.1016/j.jhep.2015.06.012.
9. Park JW, Chen M, Colombo M, Roberts LR, Schwartz M, Chen PJ, et al. Global patterns of hepatocellular carcinoma management from diagnosis to death: the BRIDGE Study. Liver Int. 2015;35(9):2155–66. doi:10.1111/liv.12818.
10. Kudo M, Izumi N, Ichida T, Ku Y, Kokudo N, Sakamoto M, et al. Report of the 19th follow-up survey of primary liver cancer in Japan. Hepatol Res. 2016;46(5):372–90. doi:10.1111/hepr.12697.
11. Singal AG, Yopp A, Skinner CS, Packer M, Lee WM, Tiro JA. Utilization of hepatocellular carcinoma surveillance among American patients: a systematic review. J Gen Intern Med. 2012;27(7):861–7. doi:10.1007/s11606-011-1952-x.
12. Ahmed Mohammed HA, Yang JD, Giama NH, Choi J, Ali HM, Mara KC, et al. Factors influencing surveillance for hepatocellular carcinoma in patients with liver cirrhosis. Liver Cancer. 2017;6(2):126–36. doi:10.1159/000450833.
13. Gopal P, Yopp AC, Waljee AK, Chiang J, Nehra M, Kandunoori P, et al. Factors that affect accuracy of alpha-fetoprotein test in detection of hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014;12(5):870–7. doi:10.1016/j.cgh.2013.09.053.
14. Yang JD, Dai J, Singal AG, Gopal P, Addissie B, Nguyen MH, et al. Improved performance of serum alpha-fetoprotein for hepatocellular carcinoma diagnosis in HCV cirrhosis with normal alanine transaminase. Cancer Epidemiol Biomark Prev. 2017; doi:10.1158/1055-9965.EPI-16-0747.
15. Chung JW, Kim BH, Lee CS, Kim GH, Sohn HR, Min BY, et al. Optimizing surveillance performance of alpha-fetoprotein by

selection of proper target population in chronic hepatitis B. PLoS One. 2016;11(12):e0168189. doi:10.1371/journal.pone.0168189.
16. Singal AG, Conjeevaram HS, Volk ML, Fu S, Fontana RJ, Askari F, et al. Effectiveness of hepatocellular carcinoma surveillance in patients with cirrhosis. Cancer Epidemiol Biomark Prev. 2012;21(5):793–9. doi:10.1158/1055-9965.EPI-11-1005.
17. Del Poggio P, Olmi S, Ciccarese F, Di Marco M, Rapaccini GL, Benvegnu L, et al. Factors that affect efficacy of ultrasound surveillance for early stage hepatocellular carcinoma in patients with cirrhosis. Clin Gastroenterol Hepatol. 2014;12(11):1927–1933 **e2**. doi:10.1016/j.cgh.2014.02.025.
18. Simmons O, Fetzer DT, Yokoo T, Marrero JA, Yopp A, Kono Y, et al. Predictors of adequate ultrasound quality for hepatocellular carcinoma surveillance in patients with cirrhosis. Aliment Pharmacol Ther. 2017;45(1):169–77. doi:10.1111/apt.13841.
19. Singal AG, Pillai A, Tiro J. Early detection, curative treatment, and survival rates for hepatocellular carcinoma surveillance in patients with cirrhosis: a meta-analysis. PLoS Med. 2014;11(4):e1001624. doi:10.1371/journal.pmed.1001624.
20. Cucchetti A, Trevisani F, Pecorelli A, Erroi V, Farinati F, Ciccarese F, et al. Estimation of lead-time bias and its impact on the outcome of surveillance for the early diagnosis of hepatocellular carcinoma. J Hepatol. 2014;61(2):333–41. doi:10.1016/j.jhep.2014.03.037.
21. Thein HH, Campitelli MA, Yeung LT, Zaheen A, Yoshida EM, Earle CC. Improved survival in patients with viral hepatitis-induced hepatocellular carcinoma undergoing recommended abdominal ultrasound surveillance in Ontario: a population-based retrospective cohort study. PLoS One. 2015;10(9):e0138907. doi:10.1371/journal.pone.0138907.
22. Wu CY, Hsu YC, Ho HJ, Chen YJ, Lee TY, Lin JT. Association between ultrasonography screening and mortality in patients with hepatocellular carcinoma: a nationwide cohort study. Gut. 2016;65(4):693–701. doi:10.1136/gutjnl-2014-308786.
23. Cucchetti A, Garuti F, Pinna AD, Trevisani F. Italian liver Cancer g. Length time bias in surveillance for hepatocellular carcinoma and how to avoid it. Hepatol Res. 2016;46(12):1275–80. doi:10.1111/hepr.12672.
24. Oeda S, Iwane S, Takasaki M, Furukawa NE, Otsuka T, Eguchi Y, et al. Optimal follow-up of patients with viral hepatitis improves the detection of early-stage hepatocellular carcinoma and the prognosis of survival. Intern Med. 2016;55(19):2749–58. doi:10.2169/internalmedicine.55.6730.
25. Johnson P, Berhane S, Kagebayashi C, Satomura S, Teng M, Fox R, et al. Impact of disease stage and aetiology on survival in hepatocellular carcinoma: implications for surveillance. Br J Cancer. 2017;116(4):441–7. doi:10.1038/bjc.2016.422.
26. Singal AG, Mittal S, Yerokun OA, Ahn C, Marrero J, Yopp A, et al. Hepatocellular carcinoma screening associated with early tumor detection and Improved survival among patients with cirrhosis in the United States. Am J Med. 2017; doi:10.1016/j.amjmed.2017.01.021.
27. Marks RM, Ryan A, Heba ER, Tang A, Wolfson TJ, Gamst AC, et al. Diagnostic per-patient accuracy of an abbreviated hepatobiliary phase gadoxetic acid-enhanced MRI for hepatocellular carcinoma surveillance. AJR Am J Roentgenol. 2015;204(3):527–35. doi:10.2214/AJR.14.12986.
28. Besa C, Lewis S, Pandharipande PV, Chhatwal J, Kamath A, Cooper N, et al. Hepatocellular carcinoma detection: diagnostic performance of a simulated abbreviated MRI protocol combining diffusion-weighted and T1-weighted imaging at the delayed phase post gadoxetic acid. Abdom Radiol (NY). 2017;42(1):179–90. doi:10.1007/s00261-016-0841-5.
29. Jo PC, Jang HJ, Burns PN, Burak KW, Kim TK, Wilson SR. Integration of contrast-enhanced US into a multimodality approach to imaging of nodules in a cirrhotic liver: how I do it. Radiology. 2017;282(2):317–31. doi:10.1148/radiol.2016151732.
30. Sutherland T, Watts J, Ryan M, Galvin A, Temple F, Vuong J, et al. Diffusion-weighted MRI for hepatocellular carcinoma screening in chronic liver disease: direct comparison with ultrasound screening. J Med Imaging Radiat Oncol. 2017;61(1):34–9. doi:10.1111/1754-9485.12513.
31. Toyoda H, Kumada T, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, et al. Prognostic significance of simultaneous measurement of three tumor markers in patients with hepatocellular carcinoma. Clin Gastroenterol Hepatol. 2006;4(1):111–7.
32. Lee E, Edward S, Singal AG, Lavieri MS, Volk M. Improving screening for hepatocellular carcinoma by incorporating data on levels of alpha-fetoprotein, over time. Clin Gastroenterol Hepatol. 2013;11(4):437–40. doi:10.1016/j.cgh.2012.11.029.
33. Marrero JA, Feng Z, Wang Y, Nguyen MH, Befeler AS, Roberts LR, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. Gastroenterology. 2009;137(1):110–8. doi:10.1053/j.gastro.2009.04.005.
34. El-Serag HB, Kanwal F, Davila JA, Kramer J, Richardson P. A new laboratory-based algorithm to predict development of hepatocellular carcinoma in patients with hepatitis C and cirrhosis. Gastroenterology. 2014;146(5):1249–1255 **e1**. doi:10.1053/j.gastro.2014.01.045.
35.•• White DL, Richardson P, Tayoub N, Davila JA, Kanwal F, El-Serag HB. The updated model: an adjusted serum alpha-fetoprotein-based algorithm for hepatocellular carcinoma detection with hepatitis C virus-related cirrhosis. Gastroenterology. 2015;149(7):1986–7. doi:10.1053/j.gastro.2015.10.004. **An adjusted AFP screening algorithm incorporating longitudinal biomarker information showed excellent performance characteristics.**
36. Yapali S, Talaat N, Lok AS. Management of hepatitis B: our practice and how it relates to the guidelines. Clin Gastroenterol Hepatol. 2014;12(1):16–26. doi:10.1016/j.cgh.2013.04.036.
37. El-Serag HB, Kanwal F. alpha-Fetoprotein in hepatocellular carcinoma surveillance: mend it but do not end it. Clin Gastroenterol Hepatol. 2013;11(4):441–3. doi:10.1016/j.cgh.2012.12.046.
38. Paradis V, Zalinski S, Chelbi E, Guedj N, Degos F, Vilgrain V, et al. Hepatocellular carcinomas in patients with metabolic syndrome often develop without significant liver fibrosis: a pathological analysis. Hepatology. 2009;49(3):851–9. doi:10.1002/hep.22734.
39. Mittal S, Sada YH, El-Serag HB, Kanwal F, Duan Z, Temple S, et al. Temporal trends of nonalcoholic fatty liver disease-related hepatocellular carcinoma in the veteran affairs population. Clin Gastroenterol Hepatol. 2015;13(3):594–601 **e1**. doi:10.1016/j.cgh.2014.08.013.
40. Piscaglia F, Svegliati-Baroni G, Barchetti A, Pecorelli A, Marinelli S, Tiribelli C, et al. Clinical patterns of hepatocellular carcinoma in nonalcoholic fatty liver disease: a multicenter prospective study. Hepatology. 2016;63(3):827–38. doi:10.1002/hep.28368.
41.•• Heimbach J, Kulik LM, Finn R, Sirlin CB, Abecassis M, Roberts LR, et al. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2017; doi:10.1002/hep.29086. **New AASLD guidelines for screening, diagnosis and treatment of hepatocellular carcinoma.**
42. European Association For The Study Of The L, European Organisation For R, Treatment Of C. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2012;56(4):908–43. doi:10.1016/j.jhep.2011.12.001.
43. Omata M, Lesmana LA, Tateishi R, Chen PJ, Lin SM, Yoshida H, et al. Asian Pacific Association for the Study of the Liver consensus recommendations on hepatocellular carcinoma. Hepatol Int. 2010;4(2):439–74. doi:10.1007/s12072-010-9165-7.
44. Bruix J, Sherman M. American Association for the Study of Liver D. Management of hepatocellular carcinoma: an update. Hepatology. 2011;53(3):1020–2. doi:10.1002/hep.24199.

45. Kokudo N, Hasegawa K, Akahane M, Igaki H, Izumi N, Ichida T, et al. Evidence-based clinical practice guidelines for hepatocellular carcinoma: the Japan Society of Hepatology 2013 update (3rd JSH-HCC guidelines). Hepatol Res. 2015;45(2) doi:10.1111/hepr.12464.
46. World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. WHO, Geneva 2015.
47. Singal A, Volk ML, Waljee A, Salgia R, Higgins P, Rogers MA, et al. Meta-analysis: surveillance with ultrasound for early-stage hepatocellular carcinoma in patients with cirrhosis. Aliment Pharmacol Ther. 2009;30(1):37–47. doi:10.1111/j.1365-2036.2009.04014.x.
48. Tong MJ, Blatt LM, Kao VW. Surveillance for hepatocellular carcinoma in patients with chronic viral hepatitis in the United States of America. J Gastroenterol Hepatol. 2001;16(5):553–9.
49. Chang TS, Wu YC, Tung SY, Wei KL, Hsieh YY, Huang HC, et al. Alpha-fetoprotein measurement benefits hepatocellular carcinoma surveillance in patients with cirrhosis. Am J Gastroenterol. 2015;110(6):836–844; **quiz 45**. doi:10.1038/ajg.2015.100.
50. Atiq O, Tiro J, Yopp AC, Muffler A, Marrero JA, Parikh ND, et al. An assessment of benefits and harms of hepatocellular carcinoma surveillance in patients with cirrhosis. Hepatology. 2016; doi:10.1002/hep.28895.
51. Kim DY, Han KH. Epidemiology and surveillance of hepatocellular carcinoma. Liver Cancer. 2012;1(1):2–14. doi:10.1159/000339016.
52. Kim SY, An J, Lim YS, Han S, Lee JY, Byun JH, et al. MRI with liver-specific contrast for surveillance of patients with cirrhosis at high risk of hepatocellular carcinoma. JAMA Oncol. 2016; doi:10.1001/jamaoncol.2016.3147.
53. Song PP, Xia JF, Inagaki Y, Hasegawa K, Sakamoto Y, Kokudo N, et al. Controversies regarding and perspectives on clinical utility of biomarkers in hepatocellular carcinoma. World J Gastroenterol. 2016;22(1):262–74. doi:10.3748/wjg.v22.i1.262.
54. Di Bisceglie AM, Hoofnagle JH. Elevations in serum alpha-fetoprotein levels in patients with chronic hepatitis B. Cancer. 1989;64(10):2117–20.
55. Di Bisceglie AM, Sterling RK, Chung RT, Everhart JE, Dienstag JL, Bonkovsky HL, et al. Serum alpha-fetoprotein levels in patients with advanced hepatitis C: results from the HALT-C Trial. J Hepatol. 2005;43(3):434–41.
56. Zhou XD, Tang ZY, Fan J, Zhou J, Wu ZQ, Qin LX, et al. Intrahepatic cholangiocarcinoma: report of 272 patients compared with 5,829 patients with hepatocellular carcinoma. J Cancer Res Clin Oncol. 2009;135(8):1073–80. doi:10.1007/s00432-009-0547-y.
57.•• Tayob N, Lok AS, Do KA, Feng Z. Improved detection of hepatocellular carcinoma by using a longitudinal alpha-fetoprotein screening algorithm. Clin Gastroenterol Hepatol. 2016;14(3):469–75. doi:10.1016/j.cgh.2015.07.049. **e2. New parametric empirical Bayesian (PEB) screening algorithm incorporating longitudinal biomarker information into surveillance.**
58. McIntosh MW, Urban N. A parametric empirical Bayes method for cancer screening using longitudinal observations of a biomarker. Biostatistics. 2003;4(1):27–40. doi:10.1093/biostatistics/4.1.27.
59. Lok AS, Sterling RK, Everhart JE, Wright EC, Hoefs JC, Di Bisceglie AM, et al. Des-gamma-carboxy prothrombin and alpha-fetoprotein as biomarkers for the early detection of hepatocellular carcinoma. Gastroenterology. 2010;138(2):493–502. doi:10.1053/j.gastro.2009.10.031.
60. Gounder PP, Bulkow LR, Meltzer MI, Bruce MG, Hennessy TW, Snowball M, et al. Cost-effectiveness analysis of hepatocellular carcinoma screening by combinations of ultrasound and alpha-fetoprotein among Alaska Native people, 1983-2012. Int J Circumpolar Health. 2016;75:31115. doi:10.3402/ijch.v75.31115.
61. Kudo M. Alpha-fetoprotein-L3: useful or useless for hepatocellular carcinoma? Liver Cancer. 2013;2(3–4):151–2. doi:10.1159/000343847.
62. Yi X, Yu S, Bao Y. Alpha-fetoprotein-L3 in hepatocellular carcinoma: a meta-analysis. Clin Chim Acta. 2013;425:212–20. doi:10.1016/j.cca.2013.08.005.
63. Chaiteerakij R, Zhang X, Addissie BD, Mohamed EA, Harmsen WS, Theobald PJ, et al. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. Liver Transpl. 2015;21(5):599–606. doi:10.1002/lt.24117.
64. Hayashi K, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, et al. Usefulness of measurement of *Lens culinaris* agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and recurrence of small hepatocellular carcinoma. Am J Gastroenterol. 1999;94(10):3028–33. doi:10.1111/j.1572-0241.1999.01378.x.
65. Kumada T, Nakano S, Takeda I, Kiriyama S, Sone Y, Hayashi K, et al. Clinical utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in small hepatocellular carcinoma: special reference to imaging diagnosis. J Hepatol. 1999;30(1):125–30.
66. Leerapun A, Suravarapu SV, Bida JP, Clark RJ, Sanders EL, Mettler TA, et al. The utility of *Lens culinaris* agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. Clin Gastroenterol Hepatol. 2007;5(3):394–402; **quiz 267**. doi:10.1016/j.cgh.2006.12.005.
67. Wallin R, Prydz H. Studies on a subcellular system for vitamin K-dependent carboxylation. Thromb Haemost. 1979;41(3):529–36.
68. Liebman HA, Furie BC, Tong MJ, Blanchard RA, Lo KJ, Lee SD, et al. Des-gamma-carboxy (abnormal) prothrombin as a serum marker of primary hepatocellular carcinoma. N Engl J Med. 1984;310(22):1427–31. doi:10.1056/NEJM198405313102204.
69. Tsai SL, Huang GT, Yang PM, Sheu JC, Sung JL, Chen DS. Plasma des-gamma-carboxyprothrombin in the early stage of hepatocellular carcinoma. Hepatology. 1990;11(3):481–8.
70. Cui R, Wang B, Ding H, Shen H, Li Y, Chen X. Usefulness of determining a protein induced by vitamin K absence in detection of hepatocellular carcinoma. Chin Med J. 2002;115(1):42–5.
71. Tateishi R, Yoshida H, Matsuyama Y, Mine N, Kondo Y, Omata M. Diagnostic accuracy of tumor markers for hepatocellular carcinoma: a systematic review. Hepatol Int. 2008;2(1):17–30. doi:10.1007/s12072-007-9038-x.
72. Mita Y, Aoyagi Y, Yanagi M, Suda T, Suzuki Y, Asakura H. The usefulness of determining des-gamma-carboxy prothrombin by sensitive enzyme immunoassay in the early diagnosis of patients with hepatocellular carcinoma. Cancer. 1998;82(9):1643–8.
73. Ikoma J, Kaito M, Ishihara T, Nakagawa N, Kamei A, Fujita N, et al. Early diagnosis of hepatocellular carcinoma using a sensitive assay for serum des-gamma-carboxy prothrombin: a prospective study. Hepato-Gastroenterology. 2002;49(43):235–8.
74. Makuuchi M, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, et al. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. Hepatol Res. 2008;38(1):37–51. doi:10.1111/j.1872-034X.2007.00216.x.
75. Marrero JA, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, et al. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. Hepatology. 2003;37(5):1114–21. doi:10.1053/jhep.2003.50195.
76. Volk ML, Hernandez JC, Su GL, Lok AS, Marrero JA. Risk factors for hepatocellular carcinoma may impair the performance of biomarkers: a comparison of AFP, DCP, and AFP-L3. Cancer Biomark. 2007;3(2):79–87.
77. Ertle JM, Heider D, Wichert M, Keller B, Kueper R, Hilgard P, et al. A combination of alpha-fetoprotein and des-gamma-carboxy

prothrombin is superior in detection of hepatocellular carcinoma. Digestion. 2013;87(2):121–31. doi:10.1159/000346080.

78.•• Johnson PJ, Pirrie SJ, Cox TF, Berhane S, Teng M, Palmer D, et al. The detection of hepatocellular carcinoma using a prospectively developed and validated model based on serological biomarkers. Cancer Epidemiol Biomark Prev. 2014;23(1):144–53. doi:10.1158/1055-9965.EPI-13-0870. **Original description of the GALAD scoring system incorporating patient demographic information and biomarkers for surveillance.**

79. Best J, Bilgi H, Heider D, Schotten C, Manka P, Bedreli S, et al. The GALAD scoring algorithm based on AFP, AFP-L3, and DCP significantly improves detection of BCLC early stage hepatocellular carcinoma. Z Gastroenterol. 2016;54(12):1296–305. doi:10.1055/s-0042-119529.

80. Berhane S, Toyoda H, Tada T, Kumada T, Kagebayashi C, Satomura S, et al. Role of the GALAD and BALAD-2 serologic models in diagnosis of hepatocellular carcinoma and prediction of survival in patients. Clin Gastroenterol Hepatol. 2016;14(6):875–886 **e6**. doi:10.1016/j.cgh.2015.12.042.

81. Duarte-Salles T, Misra S, Stepien M, Plymoth A, Muller D, Overvad K, et al. Circulating osteopontin and prediction of hepatocellular carcinoma development in a large European population. Cancer Prev Res (Phila). 2016;9(9):758–65. doi:10.1158/1940-6207.CAPR-15-0434.

82. Shang S, Plymoth A, Ge S, Feng Z, Rosen HR, Sangrajrang S, et al. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. Hepatology. 2012;55(2):483–90. doi:10.1002/hep.24703.

83. da Costa AN, Plymoth A, Santos-Silva D, Ortiz-Cuaran S, Camey S, Guilloreau P, et al. Osteopontin and latent-TGF beta binding-protein 2 as potential diagnostic markers for HBV-related hepatocellular carcinoma. Int J Cancer. 2015;136(1):172–81. doi:10.1002/ijc.28953.

84. Cao B, Yang L, Rong W, Feng L, Han N, Zhang K, et al. Latent transforming growth factor-beta binding protein-1 in circulating plasma as a novel biomarker for early detection of hepatocellular carcinoma. Int J Clin Exp Pathol. 2015;8(12):16046–54.

85. Shen Q, Fan J, Yang XR, Tan Y, Zhao W, Xu Y, et al. Serum DKK1 as a protein biomarker for the diagnosis of hepatocellular carcinoma: a large-scale, multicentre study. Lancet Oncol. 2012;13(8):817–26. doi:10.1016/S1470-2045(12)70233-4.

86. Zhu WW, Guo JJ, Guo L, Jia HL, Zhu M, Zhang JB, et al. Evaluation of midkine as a diagnostic serum biomarker in hepatocellular carcinoma. Clin Cancer Res. 2013;19(14):3944–54. doi:10.1158/1078-0432.CCR-12-3363.

87. Vongsuvanh R, van der Poorten D, Iseli T, Strasser SI, McCaughan GW, George J. Midkine increases diagnostic yield in AFP negative and NASH-related hepatocellular carcinoma. PLoS One. 2016;11(5):e0155800. doi:10.1371/journal.pone.0155800.

88. Yang J, Li J, Dai W, Wang F, Shen M, Chen K, et al. Golgi protein 73 as a biomarker for hepatocellular carcinoma: a diagnostic meta-analysis. Exp Ther Med. 2015;9(4):1413–20. doi:10.3892/etm.2015.2231.

89. Marrero JA, Romano PR, Nikolaeva O, Steel L, Mehta A, Fimmel CJ, et al. GP73, a resident Golgi glycoprotein, is a novel serum marker for hepatocellular carcinoma. J Hepatol. 2005;43(6):1007–12. doi:10.1016/j.jhep.2005.05.028.

90. Jia X, Liu J, Gao Y, Huang Y, Du Z. Diagnosis accuracy of serum glypican-3 in patients with hepatocellular carcinoma: a systematic review with meta-analysis. Arch Med Res. 2014;45(7):580–8. doi:10.1016/j.arcmed.2014.11.002.

91. Lin XJ, Chong Y, Guo ZW, Xie C, Yang XJ, Zhang Q, et al. A serum microRNA classifier for early detection of hepatocellular carcinoma: a multicentre, retrospective, longitudinal biomarker identification study with a nested case-control study. Lancet Oncol. 2015;16(7):804–15. doi:10.1016/S1470-2045(15)00048-0.

92. Huang JT, Liu SM, Ma H, Yang Y, Zhang X, Sun H, et al. Systematic review and meta-analysis: circulating miRNAs for diagnosis of hepatocellular carcinoma. J Cell Physiol. 2016;231(2):328–35. doi:10.1002/jcp.25135.

93. GALAD Score Calculator. 2017. http://www.mayoclinic.org/medical-professionals/model-end-stage-liver-disease/galad. Accessed March 23 2017.

94. Addissie BD, Yang JD, Ward M, Algeciras-Schiminich A, Theobald JP, Roberts L. 647 validation of the GALAD score for Prediction of hepatocellular carcinoma at a US tertiary referral center and comparison of its performance to liver ultrasound. Gastroenterology. 2016;150(4):S1041.