Serum Markers for Hepatocellular Carcinoma

Paul Clark, M.B.B.S. (Hons), M.P.H., Ph.D., F.R.A.C.P.

Hepatocellular carcinoma (HCC) accounts for a significant and increasing burden of disease globally.¹ Challenges exist to improve outcomes for patients with HCC using serum markers to achieve earlier diagnosis by screening at-risk patients, earlier identification of recurrence after treatment, and better phenotyping of tumor characteristics to target therapy.²

A working group of the NIH on biomarkers and surrogate endpoints defined a biomarker as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention.³ In this article, the clinical questions related to biomarker application in HCC are reviewed.

CAN SERUM MARKERS BE USED IN SURVEILLANCE OF AT-RISK POPULATIONS FOR EARLY DETECTION OF LIVER CANCER?

Disease screening relies on well-established principles.⁴ Screening should be undertaken for an important medical condition, using an acceptable, sensitive test, in a programmatic rather than *ad hoc* fashion. Preferably, diagnosis should occur in an early or latent phase, so that benefits accrue from earlier diagnosis, through treatment using established algorithms.⁴

HCC is certainly a recognized problem, but is a heterogeneous tumor occurring in a heterogeneous “at-risk” population. Although serum tests are minimally invasive and acceptable, the sensitivity and specificity of markers such as alpha-fetoprotein (AFP) are marginal,⁵ and comparisons of efficacy and utility between populations is

Abbreviations: AASLD, American Association for the Study of Liver Disease; AFP, alpha-fetoprotein; AFP-L3%, lens culinaris agglutinin-reactive fraction of alpha-fetoprotein; APASL, Asian Pacific Association for the Study of the Liver; BCLC, Barcelona Clinic Liver Cancer; CI, confidence interval; CLIP, Cancer of the Liver Italian Program; DCP, des-gamma-carboxyprothrombin; EASL, European Association for the Study of the Liver; GPC3, glypican-3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; USS, ultrasound scan.

From the School of Medicine, University of Queensland; Cancer Control Unit, QIMR-Berghofer Medical Research Institute; and Department of Gastroenterology, Princess Alexandra Hospital, Queensland, Australia.

Potential conflict of interest: Nothing to report.

Received 29 February 2016; accepted 6 May 2016

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TABLE 1. RECOMMENDATIONS FOR HCC SCREENING FROM GUIDELINES

Guideline	Screened Population	Role for Serum Markers
EASL	Cirrhotic CP A and B Cirrhotic CP C awaiting liver transplant Noncirrhotic HBV carriers with active hepatitis or family history of HCC Noncirrhotic HCV with advanced liver fibrosis	Not recommended
AASLD ^{6,7}	Hepatitis B carriers Asian males ≥40 years old Asian females ≥50 years old All cirrhotic hepatitis B carriers Family history of HCC Africans older than 20 years Personalized approach for other patients with noncirrhotic hepatitis B depending on the severity of the underlying liver disease, and current and past hepatic inflammatory activity, HBV DNA concentrations, and hepatic inflammatory activity Nonhepatitis B cirrhosis Hepatitis C Alcoholic cirrhosis Genetic hemochromatosis Primary biliary cirrhosis (cholangitis)	No role for HCC surveillance
APASL	Cirrhotic hepatitis B-- and hepatitis C--infected patients	Use in conjunction with ultrasound every 6 months;diagnosis cutoff 200 ng/mL; sensitivity increased with adjunctive serum marker DCP

hazardous (eg, when comparing the utility of ultrasound screening in a noncirrhotic, low body mass index population of hepatitis B virus (HBV)-infected Chinese with an overweight Western population with macronodular cirrhosis from hepatitis C virus [HCV]). HCC screening obeys the principle that most HCC is asymptomatic and preclinical, and that earlier diagnosis increases likelihood of curative treatment and thus confers a more favorable prognosis. All guidelines advocate the use of ultrasound every six months, but differ in their advice regarding the use of serum markers (Table 1).

AFP

AFP levels may increase with hepatocyte regeneration, HCC, and embryonic carcinomas.⁶ It remains the most commonly used screening biomarker for HCC, although its role is contentious. HCC guidelines for professional societies in North America (American Association for the Study of Liver Disease [AASLD]),^{7,8} Europe (European

Association for the Study of the Liver [EASL]),⁹ and Asia (Asian Pacific Association for the Study of the Liver [APASL])¹⁰ all differ slightly. There is insufficient evidence to support the use of AFP in HCC screening,¹¹ a conclusion supported by a Cochrane meta-analytic review.¹²

AFP suffers from poor sensitivity even at relatively low cutoffs (eg, 20 ng/mL, sensitivity 64.3%), and is even more limited in smaller tumors (25% sensitivity at tumor size <3 cm).¹³ Because AFP is secreted by regenerating hepatocytes as well as hepatic tumors, specificity is limited (91.4%).¹³ In Western countries, based on retrospective case-control study data, there has been some renewed interest in the inclusion of AFP in HCC screening risk prediction models; however, these have yet to establish a clinical niche.¹⁴ Higher AFP may be associated with poorer outcome and more advanced tumor stage at diagnosis, although this feature has limited clinical utility in screening programs directed at early detection of treatable disease.

Recommendations to use AFP are limited mainly to those areas endemic with HBV,¹⁰ based on two randomized clinical trials in China.^{15,16} Each study has its own methodological flaws, and generalization outside of non-Chinese, non-HBV-infected population is limited. In brief, Zhang et al.¹⁶ found 69% sensitivity of AFP in a chronic HBV-infected population (not restricted to cirrhosis) screened with ultrasound and AFP every six months, finding significant survival advantage, despite only 58% adherence to screening protocols. In a cirrhotic population with HBV, Chen and colleagues¹⁵ found no survival benefit from AFP alone measured every six months, although HCC was diagnosed at an earlier HCC stage in the AFP screened group. One important caveat is that this study was in rural China with potentially limited access to curative treatment, thus possibly adversely impacting survival advantage from earlier stage diagnosis.¹⁶

CAN AFP BE USED AS AN AID TO DIAGNOSIS FOR A SUSPICIOUS LIVER LESION?

Indeterminate lesions more than 1 cm identified on ultrasound scan (USS) require computed tomography or magnetic resonance imaging with dynamic contrast imaging in portal, arterial, and delayed phase.⁷⁻⁹ AFP cannot be used reliably to distinguish malignant versus nonmalignant lesions, although specificity improves at higher levels. The presence of an elevated AFP, particularly an AFP that has increased from baseline, may alert the clinician to the *possibility* of HCC diagnosis; however, it does not confirm it. More importantly, a normal or low AFP should not dissuade a clinician from the possibility of HCC for a new lesion in an at-risk patient.

ARE OTHER TUMOR MARKERS USEFUL?

Des-gamma-Carboxyprothrombin

Des-gamma-carboxyprothrombin (DCP; also known as prothrombin induced by vitamin K absence II) is produced by malignant hepatocytes.⁶ The sensitivity of DCP may be better than that of AFP but not reliably so,¹⁷ with sensitivity particularly problematic for smaller tumors.¹⁸

Lens Culinaris Agglutinin-Reactive Fraction of AFP (AFP-L3%)

Meta-analysis of lens culinaris agglutinin-reactive fraction of AFP (AFP-L3%) demonstrated a pooled sensitivity

of 48.3% (95% confidence interval [CI]: 45.9-50.7) and a pooled specificity of 92.9% (95% CI: 91.6-94.0); area under the curve was 0.76.¹⁹ Thus, poor performance characteristics limit its application in screening strategies.

Glypican-3

Glypican-3 (GPC3) has been explored as a screening biomarker in retrospective data, and in meta-analysis, pooled sensitivity and specificity were 69% and 83%, respectively.^{20,21} It remains a biomarker of interest because of its more biologically plausible link to HCC, although poor sensitivity limits its application in screening.

Genomic Biomarkers

In a nested study evaluating potential serum microRNA biomarkers as screening markers for HCC, an miRNA panel outperformed AFP at detecting early preclinical HCC, but sensitivity remained too limited to ensure reliability as a screening tool.²² also offer evolving potential screening biomarker for HCC.²³ Biomarker development using long, noncoding RNA, metabolomics, and proteomics is in evolution; however, at this stage, these efforts remain exploratory.^{24,25}

CAN SERUM MARKERS BE USED TO PREDICT HCC PROGNOSIS AND DETERMINE TREATMENT?

Prognosis and treatment in liver cancer is determined by tumor size, number, and distribution, which must be considered in the clinical context of the patient (compensation of liver disease, portal hypertension, and performance status). The central role of these factors is represented in such algorithms as the Barcelona Clinic Liver Cancer (BCLC) staging system and the Mazzaferro (Milan) criteria for liver transplantation.⁷⁻⁹ Serum markers therefore do not play a primary role in prognostication in HCC.

Serum markers may be adjunctive to guide HCC prognosis.^{6,26} High AFP levels (eg, >1000 ng/mL) are predictive of poorer post-liver transplant survival for patients with HCC, allowing stratification independently of traditional characteristics such as tumor size.^{27,28} Most data on prognostic staging systems suffer from retrospective, case-control design. Of prognostic models, the Cancer of the Liver Italian Program (CLIP) score is the most widely used and best validated in both Western and Asian cohorts, integrating AFP (>400 ng/mL) with Child-Pugh

class, tumor morphology, and portal vein thrombus.^{26,29} The CLIP score may have utility in differentiating prognosis in advanced tumors to better identify treatment futility.²⁶ Generally, identifying poorer prognosis tumors has less clinical utility than identifying patients with good prognosis, particularly in the screening setting.

CAN SERUM MARKERS BE USED TO ASSESS TREATMENT RESPONSE OR HCC RECURRENCE?

Diagnosis of response or recurrence should be based primarily on radiological criteria (eg, modified RECIST).⁵ For patients with HCC and high baseline levels of serum markers, marker surveillance may be an adjunct to identify recurrence, and should be a stimulus for earlier diagnostic imaging. Use of scores such as CLIP may help to integrate serum markers into the monitoring approach for recurrence and aid prognostication in the setting of recurrence.²⁶

CAN SERUM MARKERS HELP TO PERSONALIZE HCC THERAPY?

Perhaps the most exciting potential application of biomarkers is their use to “personalize” HCC therapy. Current personalization strategies such as the BCLC algorithm use a range of characteristics to direct treatment intent (curative versus noncurative) and treatment modality (transplant, surgery, locoregional, sorafenib, or supportive care).

Personalized medicine with targeted biological/chemotherapy, based on biomarkers that reflect tumor biology, is not yet possible. Personalized therapy for HCC remains limited by: (1) poor biomarker characterization of a biologically heterogeneous cancer, and (2) limited therapeutic options to reply to any potential biomarker signature.³⁰ Molecular profiling with biomarkers acting as a “liquid biopsy”³¹ for HCC and linking this to HCC therapy remains an elusive but important goal.

CONCLUSION

The clinical application of biomarkers for HCC remains limited. The poor sensitivity and specificity of currently available biomarkers such as AFP, DCP, AFP-L3%, and GPC3 make them unreliable for HCC screening in most populations, particularly in the west. AFP may have a role in prognostication, although it must be considered

in relation to other important tumor and patient characteristics, as reflected in indices such as the CLIP score. Presently, no biomarker panel characterizes tumor biology sufficiently to allow personalization of HCC chemotherapy, and biologic and chemotherapeutic treatment options remain limited. The evolution of genomic profiles and protein signatures combined with efforts to expand therapies for HCC will help inform diagnostic and treatment approaches in the future. As biological understanding and clinical need converge, the role of biomarkers in HCC is yet to be fully defined.

CORRESPONDENCE

Paul Clark, M.B.B.S. (Hons), M.P.H., Ph.D., F.R.A.C.P., GI/Hepatology, Duke Clinical Research Institute, P.O. Box 17969, Durham, NC. Email: paul.j.clark@uq.edu.au

REFERENCES

- 1) Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *Int J Cancer* 2015;136:E359-E386.
- 2) McMahon B, Block J, Block T, Cohen C, Evans AA, Hosangadi A, et al. Hepatitis-associated liver cancer: gaps and opportunities to improve care. *J Natl Cancer Inst* 2016;108:djv359.
- 3) Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. *Clin Pharmacol Ther*. 2001;69:89–95.
- 4) Wilson JMG, Jungner G. Principles and Practices of Screening for Disease. Geneva: World Health Organization Public Health Papers; 1968.
- 5) Bruix J, Reig M, Sherman M. Evidence-based diagnosis, staging, and treatment of patients with hepatocellular carcinoma. *Gastroenterology* 2016;150:835-853.
- 6) Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellular carcinoma serum markers. *Semin Oncol* 2012;39:410-433.
- 7) Bruix J, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011;53:1020-1022.
- 8) Bruix J, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005;42:1208-1236.
- 9) European Association for the Study of the Liver-European Organisation for the Research and Treatment of Cancer. EASL-EORTC Clinical Practice Guidelines: management of hepatocellular cancer. *J Hepatol* 2011;56:908-943.
- 10) 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;4:439-474.
- 11) Aghoram R, Cai P, Dickinson JA. Alpha-fetoprotein and/or liver ultrasonography for screening of hepatocellular carcinoma in

- patients with chronic hepatitis B. *Cochrane Database Syst Rev* 2012; 9:CD002799.
- 12) Wun YT, Dickinson JA. Alpha-fetoprotein and/or liver ultrasonography for liver cancer screening in patients with chronic hepatitis B. *Cochrane Database Syst Rev* 2003;(2):CD002799.
- 13) Sherman M, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995;22:432-438.
- 14) 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;146:1249-1255.e1241.
- 15) Chen JG, Parkin DM, Chen QG, Lu JH, Shen QJ, Zhang BC, Zhu YR. Screening for liver cancer: results of a randomised controlled trial in Qidong, China. *J Med Screen* 2003;10:204-209.
- 16) Zhang B-H, Yang B-H, Tang Z-Y. Randomized controlled trial of screening for hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2004;130:417-422.
- 17) 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;137:110-118.
- 18) Nakamura S, Nouse K, Sakaguchi K, Ito YM, Ohashi Y, Kobayashi Y, et al. Sensitivity and specificity of des-gamma-carboxy prothrombin for diagnosis of patients with hepatocellular carcinomas varies according to tumor size. *Am J Gastroenterol* 2006;101:2038-2043.
- 19) Yi X, Yu S, Bao Y. Alpha-fetoprotein-L3 in hepatocellular carcinoma: a meta-analysis. *Clin Chim Acta* 2013;425:212-220.
- 20) Yang SL, Fang X, Huang ZZ, Liu XJ, Xiong ZF, Liu P, et al. Can serum glypican-3 be a biomarker for effective diagnosis of hepatocellular carcinoma? A meta-analysis of the literature. *Dis Markers* 2014; 2014:127831.
- 21) 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;45:580-588.
- 22) 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;16:804-815.
- 23) He Y, Meng XM, Huang C, Wu BM, Zhang L, Lv XW, Li J. Long non-coding RNAs: novel insights into hepatocellular carcinoma. *Cancer Lett* 2014;344:20-27.
- 24) Kimhofer T, Fye H, Taylor-Robinson S, Thursz M, Holmes E. Proteomic and metabonomic biomarkers for hepatocellular carcinoma: a comprehensive review. *Br J Cancer* 2015;112:1141-1156.
- 25) Wang X, Zhang A, Sun H. Power of metabolomics in diagnosis and biomarker discovery of hepatocellular carcinoma. *Hepatology* 2013; 57:2072-2077.
- 26) Hsu CY, Hsia CY, Huang YH, Su CW, Lin HC, Lee PC, et al. Selecting an optimal staging system for hepatocellular carcinoma: comparison of 5 currently used prognostic models. *Cancer* 2010;116:3006-3014.
- 27) Hameed B, Mehta N, Sapisochin G, Roberts JP, Yao FY. Alpha-fetoprotein level > 1000 ng/mL as an exclusion criterion for liver transplantation in patients with hepatocellular carcinoma meeting the Milan criteria. *Liver Transpl* 2014;20:945-951.
- 28) Berry K, Ioannou GN. Serum alpha-fetoprotein level independently predicts posttransplant survival in patients with hepatocellular carcinoma. *Liver Transpl* 2013;19:634-645.
- 29) Liu P-H, Hsu C-Y, Hsia C-Y, Lee Y-H, Su C-W, Huang Y-H, et al. Prognosis of hepatocellular carcinoma: assessment of eleven staging systems. *J Hepatol* 2016;64:601-608.
- 30) Bruix J, Han KH, Gores G, Llovet JM, Mazzaferro V. Liver cancer: approaching a personalized care. *J Hepatol* 2015;62:S144-S156.
- 31) Crowley E, Di Nicolantonio F, Loupakis F, Bardelli A. Liquid biopsy: monitoring cancer-genetics in the blood. *Nat Rev Clin Oncol* 2013; 10:472-484.