Serum Biomarkers For Hepatocellular Carcinoma (Short Review)

INDRA BACHTIAR, ANDI UTAMA, SUSAN TAI
Mochtar Riady Institute for Nanotechnology

ABSTRACT

Hepatocellular carcinoma (HCC) is one of the most prevalent cancers and the major cause of cancer death in Asian countries. HCC typically develops in patients with chronic liver disease and cirrhosis. HCC surveillance with α-fetoprotein (AFP) and ultrasonography has been recommended for persons with cirrhosis. However, AFP level is insensitive for the early detection of HCC, and ultrasonography is expensive and operator dependent. Clearly, there is a need for novel strategies for the early detection of HCC. The ideal biomarker assay for HCC would be sensitive, specific, noninvasive, reproducible, inexpensive, and acceptable to patients. We performed a review of the literature (2001–2009) of traditional and novel serum markers for hepatocellular cancer. Several biomarkers, such as AFP-L3, des-gamma carboxyprothrombin (DCP), golgi protein 73 (GP73), glypican-3 (GPC3), squamous cell carcinoma antigen (SCCA), transforming growth factor-$\beta b1$ (TGF b1), insulin-like growth factor-II (IGF-II), insulin-like growth factor-binding protein-2 (IGFBP-2), human cervical cancer oncogene (HCCR), hepatocyte growth factor (HGF), KL-6 and α-acid glycoprotein (AAG) are promising, but none of these markers has been validated for clinical use. Limitations of the current literature include inadequate sample size, heterogeneity in biomarker assay methods and result reporting, limited analysis of demographics and cause of liver disease as covariates in the expression of these markers, and a scarcity of longitudinal studies evaluating the ability of biomarkers to detect preclinical disease. A new generation of HCC serum markers awaits validation in properly controlled clinical studies.

Key words: Hepatocellular carcinoma (HCC), biomarkers, assay methods

ABSTRAK

Hepatocellular carcinoma (HCC) atau kanker hati adalah salah satu dari kanker yang paling umum dan menjadi penyebab utama kematian di negara-negara Asia. HCC biasanya berkembang pada pasien dengan penyakit hati kronis dan sirosis. Pengamatan HCC pada pasien sirosis biasanya dilakukan menggunakan α-fetoprotein (AFP) dan ultrasonografi. Akan tetapi, sensitivitas AFP untuk deteksi HCC sangat rendah, sedangkan penggunaan ultrasonografi cukup mahal dan sangat tergantung pada keahlian operator. Hal inilah yang menjadi ide dasar tentang perlunya mencari suatu strategi baru (biomarker baru) dalam mendeteksi HCC secara dini. Biomarker yang ideal harus lebih sensitif, spesifik, noninvasif, murah, dan dapat diterima oleh pasien. Berdasarkan tinjauan literatur yang kami lakukan (2001-2009), dilaporkan beberapa penemuan biomarker baru yang cukup menjanjikan, seperti AFP-L3, des-gamma carboxyprothrombin (DCP), golgi protein 73 (GP73), glypican-3 (GPC3), squamous cell carcinoma antigen (SCCA), transforming growth factor-β1 (TGFβb1), insulin-like growth factor-II (IGF-II), insulin-like growth factor-binding protein-2 (IGFBP-2), human cervical cancer oncogene (HCCR), hepatocyte growth factor (HGF), KL-6 dan α-acid glycoprotein (AAG). Akan tetapi, penggunaannya secara klinis atau uji validasi belum pernah dilaporkan. Beberapa kendala dan keterbatasan yang dilaporkan dalam penemuan biomarker ini antara lain jumlah sampel yang digunakan kurang memadai, metoda analisis yang beragam (heterogenity), terbatas pada wilayah tertentu, predictive analysis biomarker, dan masih sedikitnya studi longitudinal untuk mengevaluasi kemampuan biomarkers dalam mendeteksi penyakit pada taraf preklinik. Uji klinik dan metoda validasi yang tepat saat ini menunggu lahirnya suatu generasi biomarker baru untuk pasien HCC.

Kata kunci: Hepatocellular carcinoma (HCC), biomarker, metoda pengujian

Correspondence:
Indra Bachtiar Ph.D,
Mochtar Riady Institute
for Nanotechnology
Jl. Boulevars Jend.
Sudirman 1688
Lippo Karawaci,
Indonesia, 15811
Phone: +62-21-5421-
0123 ext. 8108
Fax. : +62-21-5421-0110
Email:
ibachtiar@mrinstitute.org

INTRODUCTION

Primary Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world. Because of the global pandemic of hepatitis B and C viral infections, the incidence of HCC is rapidly increasing in Asian and Western countries, and this trend is expected to continue for the next 50 years because of the long latency between infection and the development of HCC. The prognosis of advanced HCC remains poor and novel treatment and diagnosis strategies are urgently needed.[1] Currently, the only available tools for HCC surveillance are testing for serum α-fetoprotein (AFP) level and hepatic ultra-sonography. However, AFP level has poor sensitivity and specificity for HCC and ultrasonography is operator dependent and limited in its ability to differentiate HCC from such non-neoplastic lesions as regenerative nodules. Newer methods for the early detection of HCC are needed in the form of either biomarkers or radiological tests. Biomarkers are defined as indicators of cellular, biochemical, molecular, or genetic alterations by which normal or abnormal biological processes can be recognized.[2]

The ideal marker for HCC would be specific for HCC and not detected in premalignant liver disease (ie, cirrhosis regardless of the cause). It should be sensitive, enabling detection of HCC at an early stage, when curative treatment is possible. The marker should be easily measurable, and the test should be reproducible, minimally invasive, and acceptable to patients and physicians. There have been many reports of novel biomarkers for HCC.[3] When searching PubMed with a combination of the key words "hepatocellular carcinoma" and "biomarkers," 2794 reports were identified in 2000—2009. Recent developments of gene-expression microarrays, proteomics, and tumor immunology permit thousands of genes and proteins to be screened simultaneously. With the growing application of these techniques, it is anticipated that there will be an explosion of new biomarkers for cancer screening, including HCC, in the next decade. This review summarizes recent studies of the specific molecular markers in diagnosis of HCC.

SINGLE MARKERS

α-Fetoprotein

Alpha-fetoprotein (AFP) is an oncofetal glycoprotein of unknown function that is present at increased levels in sera of patients with cirrhosis and HCC. So far AFP, the only serological marker commonly used in diagnosis, has failed to be a reliable marker mainly because it shows poor sensitivity, ranging from 39% to 65% and a

specificity ranging from 76% to 97%.[4] This high variability is because different cutoffs are used in the different studies and also because these data were mainly obtained from retrospective studies. AFP seems to be reliable at values over 400 IU/ml, but the percentage of patients with such high levels is very small; this represents one of the most important limits of this marker.

AFP-L3

The fucosylated variant of the AFP glycoprotein, having a high affinity of the sugar chain to Lens culinaris (AFP-L3), has been proposed to be more specific than AFP for HCC diagnosis. The percentage of AFP-L3 was calculated as the ratio between AFP-L3 and total AFP using lectin-affinity electrophoresis, and the intensity of the band analyzed by a densitometer. Initially, AFP-L3 was proposed as a better marker of HCC, having 55.3% sensitivity and 93.9% specificity, at a 15% cut-off value.[5] In another study, a better sensitivity and specificity (75% and 83%) led to a reliable diagnostic accuracy, but using a 35% AFP-L3 cut-off value.[6] More recently, using a 15% cut-off value, a sensitivity of 96% and specificity of 92% have been reported. However, these very promising results were not reproduced in another study, in which the sensitivity and specificity were 36.1% and 93.4%, respectively, using a 10% AFP-L3 cut-off, versus 30.9% and 99.5% using a 15% cut-off. In any case, the diagnostic accuracy was still poor: 53.2%.[7] The inconsistency of the data is very likely explained by the reading assay used to determine the AFP-L3 percentage, since it is very difficult to standardize the conversion of a qualitative technique like immunoblotting into a quantitative technique by densitometry.

Des-gamma Carboxy Prothrombin (DCP)

DCP is an abnormal prothrombin lacking carboxy-lation of the 10 glutamic-acid residues in the N-terminus, and is the result of an acquired post-translational defect of the prothrombin precursor in HCC cells, and therefore used as HCC marker. DCP, also known to be induced by Vitamin K absence or antagonist-II (PIVKA-II), does not have any biological coagulative activity. In 1984, Liebman first reported higher DCP in patients with HCC and also higher levels in patients with recurrence of the disease after surgical resection, suggesting the use of DCP as an HCC marker. After that, DCP was investigated in several studies but very different results were obtained depending on the cut-off value used. Currently, 40mAU/ml is the cut-off most widely adopted and based on this value, sensitivity ranges between 48% and 62% and specificity between 81% and 98%.[8] In the same studies DCP did not appear markedly better than AFP, which had a sensitivity of 40–54% and specificity of 88–97%. The use of DCP in

combination with AFP is also suggested by the fact that the two markers are not correlated. In other studies, it has been reported that DCP seems to be particularly related to the intrahepatic invasiveness of the tumour in particular toward the portal vein.[9] In conclusion, DCP is more reliable than AFP as a prognostic tool for predicting the clinical outcome of patients with HCC, rather than as a diagnostic tool for early detection of the cancer. For this reason DCP is not an adequate diagnostic marker.

Golgi Protein 73 (GP73)

GP73 is a resident Golgi protein, shown to be up-regulated in HCC patients. Marrero reported a sensitivity of 69% and a specificity of 75% in HCC versus cirrhotic patients, using 10 relative units as cut-off, calculated by densitometric scanning of immunoblotting.[10] Although this is a promising study, more investigations are required to confirm these data and clarify the role of this marker in detecting early cancer, considering that these data seem better than those for AFP but not dramatically so. However, it is very unlikely that this marker could be used in clinical practice because the technique used to quantify it does not make the molecule suitable as a routine biomarker, since it does not fit the ideal technical criteria defining a reliable clinical marker for large-scale use in diagnosis.

Glypican-3 (GPC3)

GPC3 is an oncofoetal protein, being a member of the glypican family of heparin sulphate proteoglycans. GPC3 has been reported to be down-regulated in breast cancer, ovarian cancer and lung adenocarcinoma but upregulated in HCC. GPC3 has been mainly investigated at the tissue levels, although some studies have reported the presence of GPC3 in the sera of about 50% of HCC patients but absence in healthy subjects.[11] In HCC, GPC3 has been shown to stimulate growth forming complexes with Wingless-type MMTV (Wnt) interaction site family. No systematic data are available concerning its sensitivity and specificity, furthermore the use of this marker mainly in histological procedures hampers its use in surveillance programs and in the clinical setting.

Squamous Cell Carcinoma Antigen (SCCA)

SCCA is a member of the high molecular weight family of serin protease inhibitors (serpins). Two different isoforms encoded by two highly homologous genes, SCCA1 and SCCA2, have been identified. Both proteins are physiologically expressed in the supra basal layer of multi-stratified squamous epithelium. SCCA has been detected in a number of different multi-stratified epithelium-derived malignancies, including the cervix, lung, head and neck. Pontisso et al. were the first to report the expression of SCCA variants in HCC tissues at the protein and translational levels.[12]

In a subsequent study, a strong difference was reported between SCCA expression in HCC and peritumoural tissues in the same patients, suggesting the usefulness of this marker either in immuno histochemical diagnosis of HCC, or in the search for micrometastases. The detection of SCCA in HCC tissues is quite surprising since no squamous epithelial cells are present in the liver, although they are in other epithelial malignancies with which the liver shares a common embryogenic origin. A fascinating hypothesis, that still needs to be demonstrated, is that SCCA could represent a biological fingerprint of the dedifferentiation that commonly occurs in HCC, like AFP production. Furthermore, SCCA has been detected at higher levels in the sera of HCC than cirrhotic patients, although no clear correlation was found between tissue and serological levels, likely because SCCA is present at the cytosol level, not being associated with membrane-bound organelles. Thus, circulating SCCA is a result of cell lysis rather than of a secretory process. Nevertheless, SCCA has been used in HCC diagnosis and yielded a sensitivity of 84.2% and a specificity of 48.9%; for this reason SCCA seems to be the perfect partner to be used together with AFP, that has low sensitivity but high specificity.

Transforming Growth Factor-β1 (*TGFβ1*)

TGFβ1 is a multifunctional cytokine with increased expression levels in HCC cells. Elevated levels of circulating TGFβ1 have been noted in HCC patients. A recent report by Song compared TGFβ1 and AFP levels in patients with small HCCs and cirrhotic controls. TGFb1 was measured using a commercial ELISA for active TGFβ1. The area under the ROC curve was significantly higher for TGFβ1 than AFP, suggesting increased sensitivity for TGFβ1. However, the authors caution that TGFβ1 levels might increase in cirrhotic patients, due to its decreased hepatic clearance. This feature might limit its usefulness in patients with advanced liver disease. Another concern is the lack of disease-specificity since TGFβ1 expression is up-regulated in wound healing, angiogenesis, fibrosis, and extra-hepatic tumors.[13]

Insulin-like Growth Factor-II (IGF-II)

Members of the family of insulin-like growth factors are thought to be involved in the pathogenesis of HCC. Increased hepatic IGF-II levels have been found in humans and experimental animals with liver cancer. Based on earlier reports demonstrating IGF-II overexpression in HCC, Tsai performed two comparative studies of AFP and IGF-II serum levels in HCC patients and cirrhotic or normal control subjects.[14] IGF-II was quantitated by

immunoradiometry, and its levels were expressed in relation to serum prealbumin to account for the effect of nutritional status on IGF-II serum levels. IGF-II/prealbumin ratios were increased in HCC patients as compared to cirrhotic and normal controls. However, the sensitivity, specificity, and overall diagnostic accuracy of IGF-II (42, 96 and 69%) were inferior to that of AFP (73, 98 and 86%). Due to its unimpressive performance in these studies and the confounding metabolic influence on circulating IGF-II levels, the future of this candidate marker is doubtful.

Insulin-like Growth Factor-Binding Protein-2 (IGFBP-2)

IGFBP-2 is a secretory protein that is involved in insulin like growth factor signaling. IGFBP-2 is produced by a wide range of tumor cells, and elevated serum levels have previously been demonstrated in patients with prostate cancer. Based on a previous report of IGFBP-2 mRNA over expression in a mouse model of HCC, Ranke et al. measured IGFBP-2 protein levels in the sera of 50 HCC patients.[15] IGFBP-2 levels were increased above the age adjusted normal values in 37 of the 50 patients. This increase was not attributable to tumor-induced weight loss, since it did not correlate with the patients' body mass index. In contrast, the serum levels of IGFBP-3 and IGF-1 – two related IGF signaling proteins that are predominantly produced by hepatocytes – were low. The sensitivity and specificity of IGFBP-2 for HCC were not reported, and remain to be determined in future studies.

Human Cervical Cancer Oncogene (HCCR)

Two splice isoforms of HCCR have been identified. HCCR-1, a 360 amino acid protein, has the characteristic features of a membrane protein. The shorter isoform, HCCR-2, lacks a transmembrane domain and appears to be located within the cytoplasm. The functions of the two HCCR isoforms are unknown, although one study suggested that HCCR-2 may function as an oncoprotein. Increased levels of HCCR have been detected in cancerous tissues from breast, kidney, ovary, stomach, and colon. Yoon and colleagues documented robust HCCR expression in hepatocellular carcinoma cells without expression in surrounding cirrhotic tissue.[16]

HCCR was absent in normal hepatocytes, a surprising finding in light of the fact that the authors had previously demonstrated substantial hepatic HCCR mRNA levels in normal livers. The authors developed an ELISA assay for the detection of the shared C-terminal domains of HCCR1 and -2 in serum, and reported modest (20–40%) increases in HCC patients as compared to cirrhotic controls. No differences were found between normal subjects and non-cirrhotic patients with chronic hepatitis. In comparison with AFP, HCCR was slightly more sensitive (78% versus 46%) but similar in specificity and overall accuracy. A

trend towards improved sensitivity for the detection of small (< 2 cm) HCCs (69% versus 46%) did not achieve statistical significance. Further studies will be needed to identify the circulating serum form of HCCR, and to evaluate its clinical utility.

Hepatocyte Growth Factor (HGF)

Hepatocyte growth factor is a multi-functional cytokine that affects mitogenesis, cell motility, matrix invasion, and epithelial carcinogenesis. Based on earlier reports of increased serum HGF levels in patients with chronic HCV infection, Yamagamim et al. prospectively collected blood samples from patients with chronic hepatitis, liver cirrhosis, and newly diagnosed hepatocellular cancer.[17] Since prolonged storage of venous blood and repeat freezing and thawing results in HGF activation by serine proteases and artificially high measurements, samples were frozen in the presence of a serine protease inhibitor, and analyzed within 48 h. Serum HGF levels were significantly higher in HCC patients (0.53- 0.17 ng/ml) than in patients with liver cirrhosis (0.38-0.08; p < 0.05) or chronic hepatitis (0.38 - 0.05). Unfortunately, data on sensitivity and specificity of the HGF assay were not provided.

The prospective component of the study suggested that high HGF concentrations were associated with a significantly increased risk of HCC development. However, increased HGF serum levels have also been reported in patients with extrahepatic malignancies including squamous cell carcinoma of the esophagus and lymphomas, as well as coronary syndrome, aortic dissection, pulmonary thromboembolism, cerebral infarction, and sepsis.[18] Additional studies will be needed to determine whether inflammatory changes rather than hepatic carcinogenesis are responsible for increased serum HGF levels in patients with chronic hepatitis and HCC.

KL-6

Kl-6 (also known as mucin 1 or MUC-1) is a membrane protein expressed in many epithelial cells. It binds extracellular pathogens, and is involved in cell signaling and carcinogenesis. Alterations in KL-6 expression levels, cellular localization, and glycosylation have been reported in breast cancer and in patients with inflammatory lung diseases. Recently, Moriyama et al. demonstrated KL-6 expression in hepatocellular cancer cells and elevated KL-6 serum levels in patients with HCV-related HCC.[19] Arase et al. studied serum KL-6 levels in 502 consecutive HCV-positive patients, and found elevated levels in 20% of HCC cases.[20] The performance of KL-6 (34% sensitivity with a specificity of 80%) was inferior to that of DCP and AFP, respectively. Despite the

excellent reported specificity (99%) and sensitivity (87%) of combined KL-6, DCP, and AFP, the future of KL-6 as an individual marker of HCC appears doubtful.

α1-acid glycoprotein (AAG)

α1-acid glycoprotein (AAG) is a member of the lipocalins, a family that shares at least 2 structurally conserved sequence motifs. AAG is synthesized predominantly in the liver as a single polypeptide of 41–43 kDa, made up of 183 amino acids, with a hydrophobic prosthetic group, and a high content of sialic acid. The biological functions of AAG are poorly understood; it is an acute phase protein, and its plasma concentration increase as a response to inflammation is triggered by cytokines. As a consequence, AAG concentrations vary in many physiological states (age and pregnancy) and pathological conditions such as liver cirrhosis, renal disease, and cancer. Significant increases of AAG have been found in patients with active lung and gastrointestinal cancers compared with patients with inactive disease. Moreover, in patients with colorectal cancer treated with 5-fluorouracil, AAG correlates with a response to therapy, with lower AAG concentrations seen in responding patients and higher AAG concentrations found in patients with progressive disease. AAG serum concentration has been suggested as a potential marker for cirrhosis and hepatocellular carcinoma (HCC). Recently, Indra et al reported that the combination of AAG and AFP shows high sensitivity and improves the accuracy of HCC diagnosis.[21]

PHASES OF BIOMARKER VALIDATION IN CANCER SURVEILLANCE

To establish a formal framework to guide the process of biomarker evaluation and development, a 5-phase program is used by the Early Detection Research Network (EDRN) of the National Cancer Institute. These 5 phases help define criteria to determine the current status of biomarkers in the published literature, assess how close these biomarkers are to clinical application, and serve as a guide for future biomarker development.

Phase 1: Preclinical Exploratory Studies

The aim of phase 1 studies is to identify biological characteristics unique to HCC that may lead to assays for future clinical use. For each biomarker studied, the key question is how well it can distinguish between cases and controls. Studies in this phase are exploratory in nature and may use tissue, serum, plasma, or urine. Assays should be reliable and reproducible. If the biomarker is measured in a binary scale (present or absent), the true positive rate and false-positive rate should be determined to summarize its ability to discriminate between disease and non disease. If the assay involves continuous variables, a receiver operating characteristic (ROC) curve should be used, and an optimal cutoff value that will differentiate cases from controls should be determined.[22] Criteria for selection of a biomarker for further development have not been established, although it has been suggested that the area under the ROC curve be used for ranking biomarkers. This review focuses on biomarkers that can be detected in serum because the clinical utility of any biomarker assay is dependent on the use of readily available biological samples.

Phase 2: Clinical Assay and Validation

The aim of phase 2 studies is to identify biomarkers that can distinguish subjects with cancer from those without cancer. An important aspect of phase 2 studies is to identify biomarkers that can detect early-stage cancer; therefore, the specimen used for biomarker assay must be obtained non invasively. Aims of this phase are to estimate true-positive and false-positive rates for the clinical assay and assess the ability of the biomarker assay to differentiate patients with HCC, in particular, those with early HCC, from at-risk subjects, ie, patients with cirrhosis. The biomarker assay should be simple; and its intra-assay and interassay variability should be reported. It is important at this phase to correlate results of the biomarker assay with such covariates as demographics, cause of liver disease, and other known risk factors for HCC. The design of these studies should be case-controlled studies. Inclusion of an adequate number of cases in the early stage is important to determine the diagnostic capability of the biomarker for early-stage HCC. Controls should be patients for whom surveillance will ultimately be applied, ie, patients with compensated cirrhosis without known HCC. Combinations of markers also can be evaluated at this stage. Validation of novel biomarkers should be compared with standard methods for HCC detection: AFP level and ultrasound. The sample size at this phase should have sufficient power to allow for random variation of the biomarker assay and confidence in the results.

Phase 3: Retrospective Longitudinal Studies

Studies in this phase aim to evaluate the capacity of the biomarker(s) to detect preclinical disease. These studies involve the collection of specimens from a cohort of patients with cirrhosis who are prospectively followed up to determine whether the biomarker can detect HCC cases before clinical diagnosis of the tumor. Samples from patients who subsequently developed HCC are tested retrospectively to determine whether levels of the biomarker were elevated before HCC was diagnosed by using available clinical tools. Studies in this phase also

should identify such variables (covariates) as demographics, exposure to alcohol and tobacco, family history, cause of liver disease, and prior treatment of underlying liver disease that may affect the ability of the biomarker to detect early HCC. Other aims include the comparison of various biomarkers singly and in combination. Data analysis includes determining ROC curves at different intervals (every 6–12 month before clinical diagnosis of HCC) to evaluate the performance of a biomarker to identify at-risk subjects destined to develop cancer.[23] No biomarker has been identified that meets the goals of phase 3 studies.

Phase 4: Prospective Screening Studies

Studies in phase 4 aim to determine operating characteristics of the biomarker-based surveillance in the relevant population by determining the cancer detection rate and false-referral rate. Other aims would be to evaluate the stage of the tumors detected and proportion of patients with tumors amenable to curative treatment, assess the feasibility and costs of implementing the surveillance program, and monitor for tumors that may be missed. Ideally, phase 4 studies involve randomization of an at-risk population to surveillance with the biomarker under study versus the standard of care. Because the incidence rate of HCC among patients with cirrhosis is relatively low (1%– 5%/years), a large sample size is required. No phase 4 studies have been conducted. As new biomarkers with promising results become available, a phase 4 study that involves randomization of patients with cirrhosis to surveillance with AFP level and ultrasound versus testing for newer biomarkers would be warranted.

Phase 5: Cancer Control Studies

Studies in phase 5 aim to determine whether surveillance of an at-risk population by using the new biomarker reduces the cancer burden compared with no surveillance. Such logistics as the large sample size and long duration required, costs of the study, acceptability by patients and physicians, ethical considerations, and adherence to protocol, including no surveillance in the control group, make phase 5 studies extremely challenging. No phase 5 study has been conducted for HCC to date.

CONCLUSIONS

In summary, although many studies of newer markers for HCC have been published, the existing literature has many limitations. Most studies were underpowered. The performance of the biomarker assays, such as reproducibility, was seldom described. Analyses of covariates, such as demographics and cause of liver disease, were rarely performed. As a consequence, most new biomarkers for HCC have remained stagnant in phase 1 or 2 studies, although some were first reported as potential HCC markers 15 years ago. For progress to be made in biomarker validation, federally funded collaborative research networks should be established so that promising biomarkers identified in phase 1 and 2 studies can be evaluated further.

Several newer markers, including AFP-L3, des-gamma carboxyprothrombin (DCP), golgi protein 73 (GP73), glypican-3 (GPC3), squamous cell carcinoma antigen (SCCA), transforming growth factor-$\beta 1$ (*TGF$\beta 1$*), insulin-like growth factor-II (IGF-II), insulin-like growth factor-binding protein-2 (IGFBP-2), human cervical cancer oncogene (HCCR), hepatocyte growth factor (HGF), KL-6 and α-*acid* glycoprotein (AAG) appear to be promising and should be evaluated further in properly designed phase 2 studies to determine their ability to detect early-stage HCC, followed by phase 3 studies that will retrospectively determine whether they can detect preclinical disease. If results hold up, phase 4 studies to assess prospectively their ability to detect early HCC, then phase 5 studies to confirm that surveillance using these markers can reduce morbidity and mortality from HCC, should be conducted. This structured approach is important because many new biomarkers are likely to be discovered based on gene microarray, proteomics, and tumor immunology during the next decade. ❖

REFFERENCES

1. K. Okuda, Hepatocellular carcinoma, J. Hepatol. 32., 2000., 225– 237.
2. Srinivas PR, Kramer BS, Srivastava S. Trends in biomarker research for cancer detection. *Lancet Oncol* 2001;2:698–704.
3. Pepe MS, Etzioni R, Feng Z, Potter JD, Thompson ML, Thornquist M, Winget M, Yasui Y. Phases of biomarker development for early detection of cancer. *J Natl Cancer Inst* 2001;93:1054–61.
4. Collier J, Sherman M. Screening for hepatocellular carcinoma. Hepatology 1998;27:273–8.
5. Taketa K, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, et al. A collaborative study for the evaluation of lectin-reactive alphafetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993;53:5419-23.
6. Wang SS, Lu RH, Lee FY, Chao Y, Huang YS, Chen CC, et al. Utility of lentil lectin affinity of alpha-fetoprotein in the diagnosis of hepato-cellular carcinoma. *J Hepatol* 1996;25:166–71.
7. Oka H, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, et al. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutin-inreactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; 16:1378–83
8. Tsai SL, Huang GT, Yang PM, Sheu JC, Sung JL, Chen DS. Plasma des-gamma-carboxyprothrombin in the early stage of hepatocellular car-cinoma. Hepatology 1990;11:481–8.

9. Suehiro T, Matsumata T, Itasaka H, Taketomi A, Yamamoto K, Sugimachi K. Des-gamma-carboxy prothrombin and proliferative activity of hepatocellular carcinoma. Surgery 1995;117:682–91.

10. 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;43:1007–12.

11. Nakatsura T, Yoshitake Y, Senju S, Monji M, Komori H, Motomura Y, et al. Glypican-3, overexpressed specifically in human hepatocellular carcinoma, is a novel tumour marker. Biochem Biophys Res Commun 2003;306:16–25.

12. Pontisso P, Calabrese F, Benvegnu L, Lise M, Belluco C, Ruvoletto MG, et al. Overexpression of squamous cell carcinoma antigen variants in hepatocellular carcinoma. *Br J Cancer* 2004;90: 833–7.

13. Song BC, Chung YH, Kim JA, Choi WB, Suh DD, Pyo SI, et al. Transforming growth factor-beta1 as a useful serologic marker of small hepatocellular carcinoma. Cancer 2002; 94:175–80.

14. Tsai JF, Jeng JE, Chuang LY, You HL, Ho MS, Lai CS, et al. Serum insulin-like growth factor-ii and alpha-fetoprotein as tumor markers of hepatocellular carcinoma. Tumour Biol 2003; 24:291–8.

15. Ranke MB, Maier KP, Schweizer R, Stadler B, Schleicher S, Elmlinger MW, et al. Pilot study of elevated levels of insulin-like growth factor-binding protein-2 as indicators of hepatocellular carcinoma. Horm Res 2003; 60:174–80.

16. Yoon SK, Lim NK, Ha SA, Park YG, Choi JY, Chung KW, et al. The human cervical cancer oncogene protein is a biomarker for human hepatocellular carcinoma. Cancer Res 2004; 64:5434–41.

17. Yamagamim H, Moriyama M, Matsumura H, Aoki H, Shimizu T, Saito T, et al. Serum concentrations of human hepatocyte growth factor is a useful indicator for predicting the occurrence of hepatocellular carcinomas in c-viral chronic liver diseases. Cancer 2002; 95:824–34.

18. Sekine K, Fujishima S, Aikawa N. Plasma hepatocyte growth factor is increased in early-phase sepsis. *J Infect Chemother* 2004; 10:110–4.

19. Moriyama M, Matsumura H,Watanabe A, Nakamura H, Arakawa Y, Oshiro S, et al. Detection of serum and intrahepatic kl-6 in anti-hcv positive patients with hepatocellular carcinoma. Hepatol Res 2004; 30:24–33.

20. Arase Y, Ikeda K, Suzuki F, Suzuki Y, Saitoh S, Kobayashi M, et al. Usefulness of the serum kl-6 assay in patients with hepatitis c virus. Intervirology 2005; 48:400–4.

21. Indra B, Julian MS, Beni A, Rino AG, Irsan H et.al. Combination of alpha-1-acid glycoprotein and alpha-fetoprotein as an improved diagnostic tool for hepatocellular carcinoma. *Clin Chim Acta* 2009;399:97–101.

22. Baker SG. The central role of receiver operating characteristic (ROC) curves in evaluating tests for the early detection of cancer. *J Natl Cancer Inst* 2003;95:511–515.

23. Baker SG. Identifying combinations of cancer markers for further study as triggers of early intervention. Biometrics 2000;56: 1082– 1087.