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MINIREVIEWS

Role of biomarkers in the prediction and diagnosis of hepatocellular carcinoma

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Abstract

The prevalence of hepatocellular carcinoma (HCC) has progressively increased in recent years and is now the fifth and the second most common cancer in the World and in Egypt, respectively. Much work has focused in the development of assays for detecting hepatic carcinogensis before the observance of hepatic focal lesions. Particular attention has been directed towards HCC-specific biomarkers for use in the early diagnosis of HCC and in the confirmation of radiological studies. Although a number of biomarkers have been identified, none have been considered reliable indicators of early HCC lesions. This review presents a few of the most relevant HCC biomarkers and suggests improvements to the accuracy of diagnostic assays through their combined use. Furthermore, we present an algorithm for the biomarker-based diagnosis of HCC and highlight its important role in the early prediction of HCC.

Key words: Hepatocellular carcinoma; Epidemiology; Pathogenesis; Biomarkers; Diagnosis

Core tip: Alpha-fetoprotein (AFP) has been widely used as a reference biomarker to validate the diagnosis of hepatocellular carcinoma (HCC). However, normal physiological-levels of AFP are observed in approximately one third of HCC cases. Furthermore, a number of HCC positive patients have AFP levels less than the threshold value of 400 ng/mL. These factors make an AFP-based diagnosis of HCC far from reliable. However, high diagnostic accuracy indices have been reported when AFP is combined with other biomarkers such as midkine, golgi protein 73, des-γ-carboxyprothrombin, glypican-3, and gamma-glutamyl transferase.

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EPIDEMOLOGY

Hepatocellular carcinoma (HCC) has an annual incidence of 7.9% in men and 6.5% in women (fifth and seventh worldwide respectively)[1]. In regions with a high prevalence of hepatitis C virus (HCV) and hepatitis B virus (HBV) infections, the incidence and prevalence of HCC has progressively increased[2]. In Egypt, due to the high prevalence of HCV and HBV infections, the incidence rate of HCC has doubled in the past ten years[3].

Pathogenesis and risk factors

Important environmental risk factors for HCC include chronic hepatitis virus infections, alcohol abuse, and non-alcoholic steatohepatitis (NASH). These risk factors are also relevant aetiologic factors for cirrhosis[4]. In Eastern Asia and sub-Saharan Africa the main risk factors for HCC are chronic hepatitis B infection and exposure to aflatoxin B1. In North America, Europe, and Japan, the main risk factors are chronic hepatitis C (CHC) infection and alcohol consumption[5]. HCV increases HCC risk by promoting cirrhosis and causing specific genetic lesions to the infected liver cells[6]. Clinically relevant hepatitis viral infections has been shown to predispose individuals to HCC, similarly, occult hepatitis B infection has been associated with the development of HCC[7].

Higher viral loads with prolonged infections have been correlated with the occurrence of HCC and may be due to accumulated risks from chronic oncogenic damage[8]. Daily alcohol consumption and Aflatoxin food contamination have been associated with increased risks of HCC[9,10]. Patients with CHC, increased insulin resistance and high serum adiponectin levels are more likely to develop liver cancer[11]. Metabolic syndrome, a combination of phenomena, including obesity, dyslipidaemia, insulin resistance and type 2 diabetes mellitus (DM), is a known potential risk factor for HCC. Due to obesity or other causes, NASH has been associated with increased risks of HCC[12,13]. DM has been associated with a two to three fold increased risk of HCC. Additionally, DM has been shown to affect the prognosis of HCC after curative therapies[14]. Furthermore, DM type 2 can lead to HCC caused by carcinogenic effects on the liver and other tissues from insulin-like growth factor-1 (IGF-1) due to hyperinsulinaemia and insulin-resistance (Figure 1)[15,16]. The risk for HCC was particularly higher in diabetic patients treated with insulin[17]. However, Yamamoto *et al*[18], 2012, reported a case in which a dramatic regression of HCC was observed after four weeks of treatment with a dipeptidyl peptidase-4 enzyme (DPP-4) inhibitor in a patient with HCV-related chronic hepatitis. $CD8^+$ T-cells were shown to accumulate around the HCC tissue, indicating that a DPP-4 inhibitor may safely exert beneficial effects on HCV-related HCC through immunity modulation[19]. A 1.7-fold increase in the incidence rates of HCC was reported for individuals with hereditary haemochromatosis confirming preliminary observations in smaller studies in related populations[20]. A correlation was also observed for alcoholics presenting with liver iron overload for increased risks of HCC and C282Y mutation in haemochromatosis[21].

MOLECULAR PATHOGENESIS

The pathogenesis of HCC remains undetermined. Evidence exists supporting the notion that DNA damage occurs, resulting in the dysregulation of DNA methylation, chromosomal instability, proto-oncogene activation, and tumour suppressor gene inactivation. The renin angiotensin system signalling pathways have been observed to activate, which leads to cell proliferation (Figure 2)[22,23]. Major risk factors for HCC typically lead to liver cirrhosis and the accumulation of genetic and epigenetic changes, such as the activation of oncogenes and the inactivation of tumour suppressor genes. The signalling pathways (*e.g*., Raf/MEK/ERK, PI3K/AKT, and Wn/β-catenin pathways) are activated through various growth factors. Growth factor receptor signalling typically results in abnormal hepatocyte proliferation and subsequently, tumour angiogenesis (Figure 3)[24,25]. Furthermore, multiple mutations at the chromosomales, genetics and epigenetics levels have been implicated as pathogenetic mechanisms in the development of HCC[26,27]. MicroRNA has been shown to aid in the transcription of HCC oncogenes and (Wnt) signaling, and it plays important roles in the development of HCC through the activation of β catenin, the overexpression of Wnt receptors and the inactivation of E-cadherin[28].

BIOMARKERS IN DIAGNOSIS OF HCC

Alpha-fetoprotein and alpha-fetoprotein-L3

There are three forms of alpha-fetoprotein (AFP) according to electrophoresis lectin-reactivity (AFP-L1, AFP-L2, and AFP-L3). A high percentage of AFP-L3 seems to differentiate HCC from chronic liver diseases and may be an indicator of HCC when the total serum AFP levels are $\geqslant$ 200 ng/mL[29].

AFP has been traditionally used as a reference biomarker to screen and support the diagnoses of HCC. However, approximately one-third of HCC patients have normal, physiological-levels of AFP. AFP levels > 200 ng/mL are specific for HCC, and levels > 500 ng/mL correlate with tumour size. AFP-L3 levels above 10% to 15% threshold level (percentage of AFP-L3 over AFP) have been detected in approximately one-third of HCC patients[30]. The use of AFP levels to diagnose HCC patients relies on a threshold value of 200 ng/mL, a sensitivity of 0.310, a specificity of 0.960 and an area under the curve (AUC) of 0.835[31,32].

Des-γ-carboxyprothrombin

Des-γ-carboxyprothrombin (DCP) is produced in HCC cell lines, and it is found at significantly higher concentrations than normal in 50% to 60% of all HCC patients and in 15% to 30% of early HCC cases. DCP

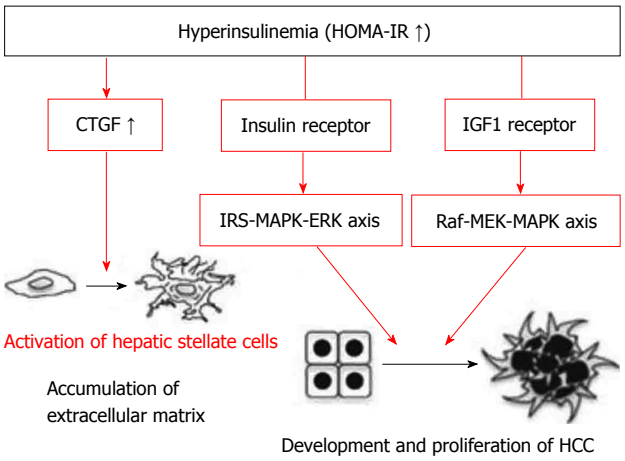


Figure 1 Insulin resistance and the development of hepatocellular carcinoma (cited from Eslam *et al*[16], 2011). HCC: Hepatocellular carcinoma; HOMA-IR: Homeostasis model of insulin resistance; IRS: Insulin receptor; MAPK: Mitogen-activated protein kinase; IGF1: Insulin like growth factor 1; CTGF: Connective tissue growth factor.

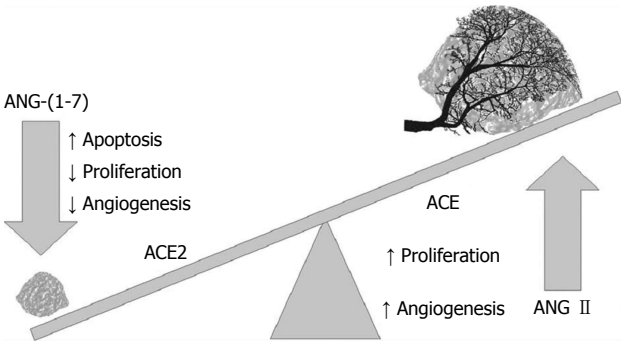


Figure 2 The balancing effects of the renin-angiotensin system on tumourigenesis (cited from Ager *et al*[23], 2008). The renin-angiotensin system can promote or inhibit angiogenesis and cellular proliferation and thereby, support or block tumour neovascularisation, growth and metastasis. ANG: Angiopoietin; ACE: Angiotensin converting enzyme.

can be used together with AFP-L3 to diagnose HCC[33]. At a 125 mAU/mL threshold, DCP has high sensitivity (89%), specificity (95%) and AUC (0.797)[34,35] in the prediction of HCC.

Midkine

AT a 654 ng/mL threshold level, midkine (MDK) serum has higher sensitivity (86.9% *vs* 51.9%), specificity (86.3% *vs* 83.9%) and AUC (0.915 *vs* 0.754) compared with AFP. Therefore, MDK can be used in the diagnosis of AFP-negative HCCs and very early-stages of HCCs. Furthermore, MDK can be used in HCC patients after curative resections to diagnose tumour recurrence[36].

Dickkopf-1

Dickkopf-1 (DKK-1) can be used together with AFP for the diagnosis of HCC and especially for HCC cases with low levels of AFP. DKK-1 can distinguish HCC from non-malignant chronic liver diseases and has sensitivity of 69.1% and a specificity of 90.6%[37].

Golgi protein 73

Golgi protein 73 (GP73) serum levels increase in patients with liver disease and HCC[38]. At a threshold value of RU 10 units, GP73 sensitivity, specificity and AUC are 69%, 75% and 0.914 respectively[39].

The combination of GP73 and AFP (with a 35 ng/mL threshold for AFP and an 8.5 RU threshold for GP73) increased the HCC diagnostic sensitivity to 89.2%, specificity to 85.2%, with an AUC of 0.914[40].

Glypican-3

Glypican-3 (GLP-3) is a 60 kDa cell surface-linked heparin sulfate proteoglycan and is not expressed in adult livers[41]. GLP-3 serum levels are higher in HCC patients than in patients with HCV-induced cirrhosis. Furthermore, GLP-3 is more sensitive than AFP for the detection of smaller HCC. The combined use of GLP-3 and AFP has been shown to provide higher sensitivity and specificity than the individual use of each marker[42].

Gamma-glutamyl transferase

In HCC and liver diseases, significant changes occur in gamma-glutamyl transferase (GGT) serum activity. GGT-Ⅱ is a hepatoma-specific GGT and an early enzyme marker of precancerous and cancerous processes[43]. At threshold levels of 100 U/L and 100 IU/mL for GGT and AFP, respectively, GGT had a higher sensitivity and a lower specificity than AFP (69.5% *vs* 43.5%) and (41.9% *vs* 96.4%), respectively, for the diagnosis of HCC. When AFP and GGT were combined, the sensitivity was 56.5% and specificity was 69.5%[44].

Alpha-l-fucosidase

Alpha-l-fucosidase (AFU) is a lysosomal enzyme. Its serum levels have been shown to increase in patients with cirrhosis and HCC[45]. At a threshold of 2.3005 μmol/L per minute, AFU yielded a sensitivity and specificity of 90% and 97.5%, respectively[46].

Transforming growth factor beta-1

Transforming growth factor beta-1 (TGF-beta-1) is a cytokine with multiple biological functions. It has a role in cell growth and extracellular matrix formation[47]. With a threshold of 64.33 ng/mL, TGF-beta-1 has a sensitivity of 78.3% and a specificity of 29.5% for the diagnosis of HCC. The combined use of AFP and TGF-beta-1 altered the specificity and the sensitivity to 86.6% and 30.4%, respectively[48].

IGF

IGFs Ⅰ and Ⅱ are polypeptides that play important roles in hepatic carcinogenesis. The serum levels of IGF Ⅰ are significantly lower in patients with HCC compared with patients without HCC[49]. At a threshold of 4.1 mg/g IGF Ⅰ has a 63% sensitivity and a 90% specificity for diagnosings of HCC. The combination of IGF Ⅰ and AFP increased the sensitivity to 80% and the specificity to 90%[50].

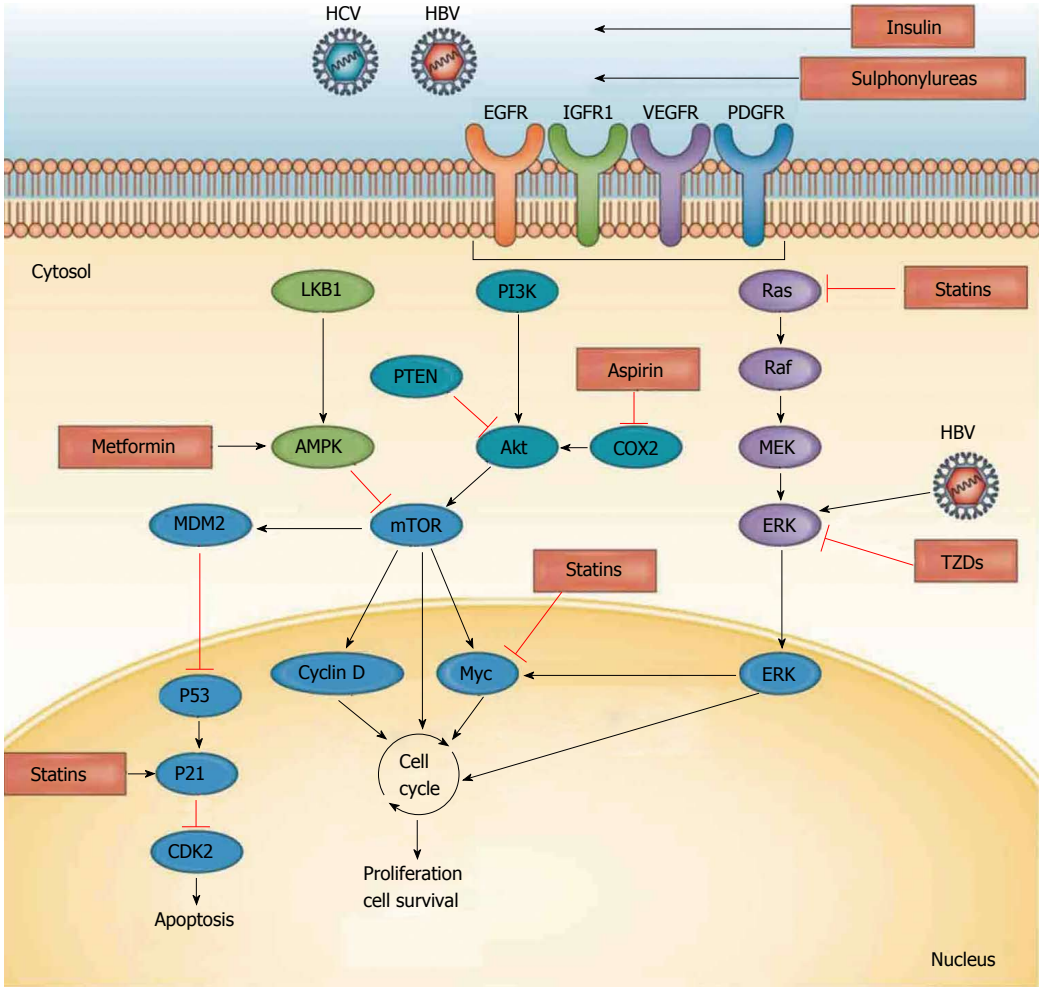


Figure 3 Pathogenesis of hepatocellular carcinoma and targets for chemopreventive agents (cited from Singh *et al*[25], 2014). AMPK: Adenosine monophosphate-activated protein kinase; IGFR1: Insulin-like growth factor receptor 1; MAPK: Ras mitogen-activated protein kinase; mTOR: Mammalian target of rapamycin; PI3K: Phosphatidylinositol 3-kinase; HBV: Hepatitis B virus; HCV: Hepatitis C virus; PTEN: Phosphatase/Tensin homolog deleted on chromosome 10; P53: A tumor suppressor protein; P21: Cyclin-dependent kinase 2 inhibitor; CDK2: Cyclin-dependent kinase 2; ERK: Extracellular signal-regulated kinase protein; MEK: Minase that phosphorylate mitogen activated protein (MAP); PDGFR: Platelet derived growth factor receptor; VEGFR: Vascular endothelial growth factor receptor; IGFR1: Insulin like growth factor receptor 1; EGFR: Epidermal growth factor receptor; Ras: Prototypical member of the RAS superfamily of proteins; Raf: A MAP kinase kinase kinase (a serine/threonine specific kinase); Akt: A protein kinase family of genes involved in regulating cell survival.

Squamous cell carcinoma antigen

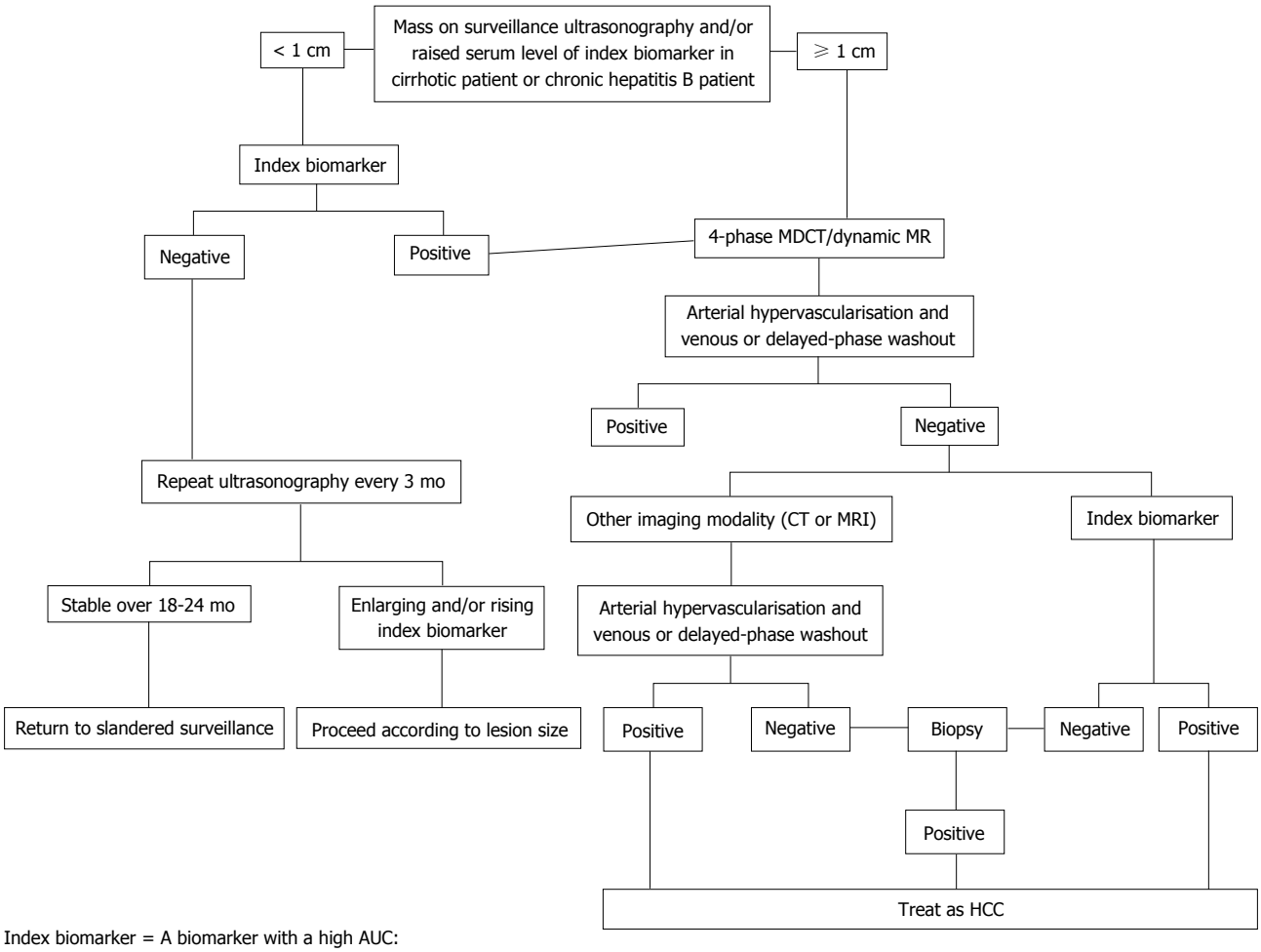
Squamous cell carcinoma antigen (SCCA) levels are persistently elevated in patients with HCC displaying normal, physiological-AFP levels. This property is useful in the early detection and follow-up diagnoses for patients treated for HCC[51]. At a threshold of 0.368 ng/mL, SCCA has an AUC of 0.705, a sensitivity of 84.2% and a specificity of 48.9%[52].

Osteopontin

Osteopontin (OPN) is an integrin-binding glycophosphoprotein involved in many cellular functions, such as regulating the survival, migration, invasion, and metastasis of tumour cells[53]. When compared with cirrhosis, CHC, chronic hepatitis B or healthy controls, OPN plasma levels were significantly elevated in HCC patients[54]. At a serum of 557 ng/mL, OPN has sensitivity, specificity and AUC of 26%, 92.5% and 51%, respectively, for the diagnosis of HCC[55,56]. Furthermore, OPN was more valuable when combined AFP-L3[57].

Heat shock protein

Heat shock proteins (HSP) are stress-induced proteins and belong to the glucose-regulated proteins (GRPs) family. In neoplasms, the expression of HSP has been associated with apoptosis regulation and tumour immune response. The expressions of HSP27, HSP70, HSP90, GRP78, and GRP94 increased in a stepwise pattern as HCC developed from a dysplastic nodules to early HCC and, finally to advanced HCC[58]. In HBV-infected patients, the expressions of GRP78, GRP94, or HSP90 have been significantly correlated with vascular invasion and intrahepatic metastasis. HSP27 has been detected in 90% HCC patients sera and two HBV patients sera, but in none of normal sera[59]. The optimal diagnostic threshold for HSP27 was 456.5 pg/mL. This yielded a sensitivity of 70% and a specificity of 73%, with an AUC



Index biomarker = A biomarker with a high AUC:
GP73 AUC = 0.914
AFP + DCP AUC = 0.874
AFP + GP73 AUC = 0.932
AFP + AFPL3 AUC = 0.748

Figure 4 Diagnostic algorithm for hepatocellular carcinoma. MDCT: Multidetector CT; MR: Magnetic resonance; AFP: Alpha-fetoprotein; DCP: Des-γ-carboxyprothrombin; GP73: Golgi protein 73; AUC: Area under the curve; MRI: MR imaging; CT: Computed tomography; HCC: Hepatocellular carcinoma.

of 0.749[60].

Interleukin-6 and syndecan-1

Syndican: The serum level of syndecan-1 significantly increased in HCC patients when compared with cirrhotic and control groups. Syndecan-1 levels significantly increased with progressive stages of Barcelona-Clinic Liver Cancer Group[61]. The diagnostic value of the test was significantly increased when combined with AFP.

Interleukin-6: At a threshold of 7.9 pg/mL, interleukin-6 (IL-6) has a sensitivity of 0.83, a specificity of 0.83, and an AUC of 0.810[62].

Higher C-reactive protein and IL-6 levels correlated well with larger tumour sizes, poorer Child-Pugh functions, shorter survival times and more predictable outcomes in patients with HCC receiving loco-regional therapy[63].

Soluble CD25

In a study by Cabrera *et al*[64], 2012, sCD25 was found be elevated in HCC patients when compared with those with cirrhosis. At a threshold of 2180 pg/mL, sCD25 based diagnoses of HCC had a sensitivity of 92.3% and a specificity of 37.7%; for the early detection of HCC. sCD25 based diagnoses had a sensitivity of 89.6% and specificity of 39.3%[64].

Vascular endothelial growth factor

Vascular endothelial growth factor (VEGF), especially VEGF-A, was found to be elevated in HCC, particularly in advanced tumour stages and metastasis[65]. High serum levels of VEGF indicate poor HCC prognosis[66].

Glucoregulatory enzymes

In experimental studies, the sera and tissue levels of the alpha feto protein and glucoregulatory enzymes, HK, GAPDH and G6PD of hexose monophosphate shunt were significantly higher in HCC-bearing animals when compared with normal controls[67]. Glucoregulatory enzymes are promising targets as biomarkers for the prediction of HCC in humans (Table 1).

Table 1 Threshold values, sensitivity, specificity and area under the curve of a few biomarkers

Marker	Threshold	Sensitivity	Specificity	AUC	Ref.
AFP	200 ng/mL	0.310	0.960	0.835	[31,32]
DCP	7.5 ng/mL	0.600	0.940	0.797	[34,35]
GP73	10 RU	0.69	0.75	0.914	[39]
AFP-L3	10%	0.410	0.990	0.710	[30]
MDK	654 ng/mL	86.9%	86.3%	0.915	[36]
AFU	2.3005 μmol/L	90%	0.975		[46]
AFP + GP73	7.4 RU	0.770	0.840	0.932	[40]
GGT + AFP	100 U/L + 100 IU/mL	0.57	0.70	-	[44]
SCCA	0.368 ng/mL	0.84	0.49	0.705	[52]
HSP27	456.5 pg/mL	0.70	0.73	0.649	[70]
IL-6	7.9 pg/mL	0.83	0.83	0.810	[62]

AUC: Area under the curve; AFP: Alpha-fetoprotein; DCP: Des-γ-carboxyprothrombin; GP73: Golgi protein 73; MDK: Midkine; AFU: Alpha-l-fucosidase; GGT: Gamma-glutamyl transferase; SCCA: Squamous cell carcinoma antigen; IL-6: Interleukin-6.

Guideline diagnostic work-up for HCC

HCC nodules 1 cm or smaller are difficult to diagnose by imaging and require further tested. Nodules exceeding 1 cm can be diagnosed by imaging computed tomography (CT) or magnetic resonance imaging (MRI) with contrast. The uptake during the arterial phase and contrast washout during the venous or delayed phases provides diagnostic clues for HCC[68]. However, the conventional practices and methods for diagnosing HCC are cumbersome and invasive. Nodular lesions showing an atypical imaging patterns indicative of HCC on one of the dynamic scans (CT or MRI) are validated with the other dynamic scan (CT or MRI). Any liver nodules $\geqslant$ 2 cm showing atypical imaging pattern on both dynamic scans (CT and MRI) subsequently require histological confirmation[69]. However, the use of biomarkers with high HCC diagnoses accuracy indices can preclude the need for these invasive and hazardous liver biopsies (Figure 4).

CONCLUSION

Multiple biological markers are available to aid in the diagnosis of HCC. However, their individual use does not provide sufficient sensitivity and specificity. As presented in this review, the combined use of more than one biomarker may increase the predictive accuracy of HCC diagnoses in cirrhotic patients.

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