Biomarkers for Hepatocellular Carcinoma

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ABSTRACT: Hepatocellular carcinoma (HCC) is the third leading cause of cancer deaths worldwide. The HCC diagnosis is usually achieved by biomarkers, which can also help in prognosis prediction. Furthermore, it might represent certain therapeutic interventions through some combinations of biomarkers. Here, we review on our current understanding of HCC biomarkers.

KEYWORDS: Hepatocellular carcinoma, biomarkers, diagnosis, prognosis

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Review-Biomarkers in HCCs

Alpha-fetoprotein (AFP) is the most widely used biomarker for hepatocellular carcinoma (HCC) during the past several decades. Serum AFP level often diminishes rapidly after birth and remains low throughout adulthood.[1] The use of AFP as a diagnostic biomarker for early screening of HCC patients, in complementation to the orthodox imaging-based tools such as ultrasonography and computed tomographic (CT) scanning, has been recommended by the Asian Pacific Association for the Study of the Liver.[2] Several studies have suggested that elevated serum AFP levels are correlated with increased risks of HCC in individuals infected with hepatitis C virus (HCV).[3,4] As the measurement of serologic AFP level always yields a specific numeric value, the result, though not necessarily accurate, is generally considered to be more objective than liver images produced by ultrasound and CT technologies, the interpretation of which is often subjected to the experience and judgment of medical practitioners. In addition, there is also an economic argument for the adoption of AFP in HCC detection, especially in many developing countries where advanced imaging instruments are scarce or even unavailable.

There has been a plethora of investigations concerning the diagnostic utility of AFP, which often generate different or even contradicting conclusions. A clinical study of 309 confirmed HCC patients revealed a connection between their serum AFP concentrations and vital oncologic characteristics such as tumor size and portal vein thrombosis, particularly at levels above 400 ng/mL.[5] On the contrary, the biomarker was found not to be a significant indicator of other metrics, such as tumor differentiation and metastasis, or to possess satisfactory prognostic value. Soresi and colleagues[6] reported a sensitivity score of 65% and specificity of 89% at a cutoff value of 30 ng/mL for the ability of their serum AFP-based approach to differentiate between HCC and liver cirrhosis in a Sicilian population. A much higher cutoff value of 200 ng/mL was considered as necessary in the study by Taketa et al on a cohort of 58 patients with HCC or other liver diseases in Myanmar, above which AFP achieved a sensitivity of 70% and specificity of 100%.[7] In comparison, Marrero et al[8] performed a large case-control study involving 836 patients and identified the best cutoff value to be 10.9 ng/mL for a sensitivity of 65%. There is also debate over whether successful detection of HCC by serum AFP measurement could lead to better clinical outcome in HCC patients. According to the study by McMahon and colleagues[9] on an Alaskan native population infected with hepatitis B virus (HBV), determination of serum AFP concentrations on a semiannual basis led to both a higher probability of early diagnosis and prolonged average life expectancy of the patient cohort. In contrast, Chen et al[10] reported that serum AFP-based screening indeed contributed substantially to early HCC diagnosis in a study group of 5581 HBV carriers, showing an overall sensitivity of 55.3% and specificity of 86.5%, but generated no observable positive impact on their prognosis.

It can be seen from the above results that the current AFP-based diagnostic approaches are still far from satisfactory. In

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fact, neither the European[11] nor the American guidelines[12] for HCC screening and diagnosis included the quantification of serum AFP due to the poor sensitivity and specificity of the method. It has been shown that elevation in AFP levels is not evident in around 80% of small HCCs.[13] As seen above, a great number of studies chose cutoff values far above the conventionally accepted threshold of 20 ng/mL, above which the diagnostic performance of AFP rapidly diminishes. It should be noted that the discrepancies between different studies on the predictive value of AFP could be partly attributable to epidemiologic factors, such as the high incidence of HCV infection in Asian HCC patients, whereas fatty liver is often a more significant contributing factor in Western countries.[14]

Another potential problem with serum AFP lies in the apparent lack of discriminating power. This is closely related to the fact that HCC usually occurs in a liver that has already been damaged by one or more preexisting pathologic conditions, including cirrhosis and chronic hepatitis resulting from HBV or HCV infection.[15] These etiologies were consistent with an earlier finding that HBV patients with cirrhosis also exhibited high serum AFP levels.[16] Moreover, AFP is often insufficient in differentiating between HCC and intrahepatic carcinoma (ICC), another liver cancer with a frequent origin in cirrhosis.[17] This could have a critical impact on the outcome of the misdiagnosed patients because surgical resection is generally the preferred (when applicable) therapeutic choice for HCC but not ICC.[12] These limitations highlight the necessity and urgency of identifying additional biomarkers with the potential of being used alone or complementing AFP for HCC diagnosis.[18]

AFP-L3

Three different glycoforms of AFP have so far been identified, which are distinguished by the difference in their binding affinities for Lens culinaris agglutinin (LCA). Whereas AFP-L1 and AFP-L2 represent the nonbinding and weakly binding fractions of total AFP, respectively, AFP-L3 comprises the portion that can associate effectively with LCA[19] and has recently been considered as a more specific biomarker for HCC. Sterling and colleagues[20] performed a multicenter prospective study and suggested that HCC diagnosis based on AFP-L3 could achieve a specificity of nearly 92%, albeit with a low sensitivity score of 37%, which undoubtedly hampered the method's clinical potential. Nevertheless, the subsequent development of an advanced automated immunoassay system using on-chip affinity-based electrophoresis, which was referred to as "highly sensitive AFP-L3" (hs-AFP-L3),[18] was shown to result in an improvement in the sensitivity score.[21] Another drawback for AFP-L3 is that its shows little diagnostic value in HCC patients with a total serum AFP concentration below 20 ng/mL. In this regard, the hs-AFP-L3 assay was found by Oda and colleagues[22] to show clinical value for early HCC diagnosis even at low AFP levels.

Des-γ-carboxyprothrombin

Des-γ-carboxyprothrombin (DCP), also called prothrombin induced by vitamin K absence-II (PIVKA II), is an abnormal form of prothrombin. The production of DCP stems from a defective vitamin K–dependent posttranslational carboxylation machinery, which promotes the malignant proliferation of HCC cells.[23] Upregulation of DCP has been found to correlate with the degree of malignancy of HCC, as DCP-positive tumors are characterized by increased likelihoods of intrahepatic metastasis, capsule infiltration, and portal venous invasion. Volk et al[24] suggested that DCP is a superior diagnostic biomarker to both total AFP and AFP-L3 particularly in differentiating between HCC and nonmalignant hepatic cirrhosis, with a sensitivity of 92% and specificity of 93% at a cutoff value of 150 mAU/mL. Moreover, the DCP-based method correctly identified early-stage HCC in 15 of 17 patients, none of whom was diagnosed when total AFP was used as the biomarker (using a cutoff of 20 ng/mL). In several additional studies,[25,26] however, serum DCP-based diagnosis showed suboptimal sensitivity (48%-62%) but satisfactory specificity (81%-98%) in HCC patients. To address this problem, combined application of DCP- and AFP-based biomarkers has been tested. A novel predictive model comprising AFP-L3, AFP and DCP achieved a sensitivity of 60.6% and specificity of 100% when applied to a cohort of 104 HCC patients, 43% of whom showed AFP levels below 10 ng/mL.[27] Meanwhile, a large multicenter case-control study conducted in 2010[28] suggested that DCP-based diagnostic method coupled with AFP immunoassay for HCC detection resulted in an increase in sensitivity from 65% to 87% at the expense of the specificity score, which dropped from 84% to 69%. These studies lent credence to the diagnostic value of DCP for early-stage HCC. Nevertheless, additional investigations are necessary to further evaluate the effectiveness of DCP for HCC diagnosis, especially when used in combination with other biomarkers.

Glypican-3

Glypican-3 (GPC3) belongs to the glypican family that consists of various glycosylphosphatidylinositol-anchored cell-surface heparin-sulfate proteoglycans.[29] Glypican-3 has been established to play important roles in cell proliferation and tumor suppression. A study conducted by Sung et al[30] found GPC3 was upregulated in HCC tissues obtained from patients and subsequently confirmed its secretion by HCC-derived cell lines. The N-terminal soluble fraction of GPC3 was proposed as a complementary serologic biomarker with better diagnostic performance than AFP due to its ability to accurately distinguish between patients with small, well-differentiated HCC tumors and those with cirrhosis.[31] Often, the increase in the serum level of GPC3 displayed no correlation to that of AFP, as was evidenced by the results of Tangkijvanich et al,[32] which also found utility in combining the 2 markers for better

detection sensitivity on small HCC tumors. GPC3 was also chosen to be applied alone or in combination with HSP70 and glutamine synthase for the differential detection of early and grade 1 HCC from cirrhosis, with similar sensitivity and specificity scores in both cases (69% and 91%, respectively, when used alone; 72% and 100%, respectively, when in combination).[33] This was in fact recommended in the clinical practice guidelines jointly published by European Association for the Study of the Liver and European Organization for Research and Treatment of Cancer.[11] In particular, a recently conducted phase I clinical trial showed promising evidence that supported the therapeutic potential of a GPC3 peptide-based vaccine against advanced HCC.[34]

Cytokeratin 19

Cytokeratin 19 (CK19) is a novel HCC biomarker that has been consistently linked to a poor clinical prognosis in patients. The simultaneous detection of CK19 and GPC3 expression in HCC patients was shown to be a predictive indicator of higher risks of cancer invasion and metastasis, as well as worse treatment outcome.[35] Furthermore, the combination of CK19 and GPC3 demonstrated better diagnostic sensitivity (90.6%) compared with the use of GP3 alone (54.2%) when applied for the detection of HCC in a study cohort of 518 patients.[36] Consistently, several other studies also confirmed a correlation between increased CK19 expression and a lower survival rate and/or a shorter remission period in HCC patients.[37,38] It is worth noting that CK19 expression was found to coincide with an increase in tumorigenic potential in preneoplastic hepatocytes,[39] which could offer a mechanistic rationale for its use as a potential HCC biomarker.

Golgi protein 73

Golgi protein 73 (GP73), a transmembrane protein localized in the Golgi complex, is absent in normal hepatocytes but can be found in the sera obtained from patients with liver diseases, particularly HCC.[40] Marrero et al[41] reported significantly elevated concentrations of serum GP73 in HCC patients in comparison with those afflicted with cirrhosis. It is worth noting that the GP73-based diagnostic model exhibited a sensitivity score of 62%, which was much higher than that of AFP at 25%, for the diagnosis of early-stage HCC. This was, at least in part, attributable to the finding that upregulation of GP73 could still be detected in most of the HCC patients whose serum AFP levels were below the diagnostic threshold of 20 ng/mL. In contrast, Tian and colleagues[42] found little evidence supporting the superiority of serum GP73 over AFP for detecting early-stage HCC, although the combined use of both biomarkers could lead to an improvement in the discriminating ability of the diagnosis. GP73 was also coupled to AFP-L3 to achieve better diagnostic accuracy and reliability for HCC patients showing low levels of serum AFP.[43] These examples, therefore, suggested that GP73 could be well suited for diagnosing patients with small, early-stage, and/or low-AFP HCC.

Midkine

Midkine (MDK) is a heparin-binding growth factor that has been associated with tumor migration and proliferation.[44] Not surprisingly, MDK is often overexpressed in various human tumors, making it an attractive target in tumor detection and treatment.[45] A clinical study on a cohort of 388 HCC patients and 545 hospital enrollees diagnosed with other diseases identified MDK as a discriminating tissue and serum biomarker with better sensitivity (86.9%, serum MDK) than AFP (51.9%).[46] The distinguishing power of MDK remained evident even for very early-stage HCC. These results were echoed in another study that confirmed that the MDK-based predictive model was dramatically more sensitive than its AFP counterpart (90% vs 40%) in differentiating between patients with early-stage HCC and those with cirrhosis.[47]

Osteopontin

Osteopontin (OPN) is a secreted and highly phosphorylated extracellular matrix (ECM) protein functionally implicated in a diverse range of biological processes, such as bone remodeling, chemotaxis, ECM degradation, and inflammation.[48] Like many other oncogenic factors, OPN is upregulated in various types of malignancies.[49] A meta-analysis study compared the diagnostic power of OPN with that of AFP and found the former to be more sensitive (86% vs 66%) to but less specific (86% vs 95%) for HCC.[50] This was in good agreement with the investigation of Fouad et al[51] suggesting OPN and AFP to have comparable diagnostic performance at a cutoff value of 280 ng/mL. The diagnostic potential of OPN also consisted in its ability to provide better discrimination for HCC from cirrhosis compared to AFP according to the clinical study of Shang and colleagues.[52] It is worth emphasizing that the upregulation of plasma OPN level could be detected well in advance of the eventual diagnosis.[52] Because OPN is an extracellular protein with a role in angiogenesis, it has also been proposed as a therapeutic target for inhibiting HCC metastasis with the possibility of being more accessible to drug molecules than the other cytoplasmic proteins.[48] One of the obvious disadvantages of using OPN for HCC diagnosis is, however, that its elevation could be linked to more than 30 types of cancers.[53] Therefore, it should best be used in combination with one or more HCC-specific biomarkers to enhance the overall reliability and accuracy of the screening approach.

Squamous cell carcinoma antigen

Squamous cell carcinoma antigen (SCCA) is a serine protease inhibitor present in squamous epithelium and has demonstrated clinical value particularly in identifying patients with

progressive liver diseases at higher risk of hepatocellular carcinoma development.[54] Based on serum samples collected from a total of 327 HCC or cirrhosis patients and healthy volunteers, the predictive model based on SCCA complexed with IgM (SCCA-IgM) achieved a higher sensitivity score (89%) than AFP (48%), but a lower specificity (50% vs 85%), in HCC diagnosis.[55] Moreover, SCCA-IgM level in the serum was verified by multivariate analysis to be an independent prognostic indicator, exhibiting an inverse correlation with treatment response. In another study, the use of serologic AFP and SCCA levels in conjunction could correctly identify 90.83% of all HCC patients in the cohort,[56] lending strong evidence to the use of SCCA as a supplementary diagnostic marker for HCC.

Annexin A2

Annexin A2 is a calcium-dependent, phospholipid-binding protein commonly found in the cell surface.[57] Many biological functions of Annexin A2 are related to cell mobility and protein interaction with the actin cytoskeleton, as well as endocytosis. Due to these roles, Annexin A2 has also been implicated in the development and metastasis of HCC. Not surprisingly, the overexpression of Annexin A2 was revealed to be an indicator of the general degree of HCC tumor malignancy in patients and showed an inverse correlation with their survival rates.[58] Annexin A2 also demonstrated higher sensitivity and specificity than AFP (83.2% and 67.5% for Annexin A2, compared to 54.7% and 81.3% for AFP) for the detection of early-stage HCC.[59] In conclusion, Annexin A2 might serve as a serologic candidate for diagnosing and determining the prognostic outcome of early-stage HCC patients.

Circulating microRNAs

Circulating microRNAs (miRNAs) were first proposed as potential cancer biomarkers in 2008.[60] There is evidence indicating that these noncoding nucleotide sequences are resistant to RNase degradation, boiling, repeated freeze-thaw cycles, as well as acid/base treatment.[61,62] The remarkable stability of circulating miRNAs has attracted significant attention from clinical researchers, who seek to investigate their diagnostic utility for a wide range of diseases including HCC.

Xu et al[63] analyzed the serum levels of miR-21, miR-122 and miR-223 in a cohort consisting of 101 patients with HCC, 48 with chronic type B hepatitis, and 89 healthy participants as controls. They found that all 3 miRNAs were significantly upregulated in HCC patients compared to controls. However, similar increase was also observed in patients with chronic hepatitis. Receiver-operator characteristic (ROC) curve analysis confirmed the feasibility of using these serum miRNAs to distinguish HCC or chronic hepatitis patients from healthy individuals, but not to differentiate between the 2 liver pathologies. Similarly, circulatory miR-122 and miR-223 were shown to undergo upregulation in the sera of HCC patients and also those afflicted with HBV, with miR-122 suggested as having the highest diagnostic value.[64] No significant differences in the levels of these miRNAs were detected between HBV patients who were diagnosed with HCC and those who were not. Furthermore, miR-122 was dramatically downregulated in the sera obtained from patients after surgery compared with the preoperative samples. In contrast to the above findings, Tomimaru and colleagues[65] suggested that plasma microRNA-21 level in HCC patients they examined was actually higher than in patients with chronic hepatitis ($P < .0001$) and healthy volunteers ($P < .0001$). Overall, the sensitivity and specificity scores of the miRNA-21 model were determined by ROC analysis to be 61.1% and 83.3%, respectively, for differentiating between HCC and chronic hepatitis. For discriminating HCC patients from healthy individuals, the same analysis yielded a sensitivity of 87.3% and specificity of 92.0%. Two meta-analyses conducted by Huang et al[66] and Li et al[67] also found circulating miR-21 to be better at detecting HCC than miR-122 and miR-223. Increased serum miR-221 was also observed in HCC patients and showed an evident correlation with the occurrence of cirrhosis and tumor size as well as stage.[68] In addition, the overall survival rate in HCC patients with high miRNA levels in the blood was 27.6%, which was significantly lower than that in those showing comparatively low miR-221 expression levels (62.3%, $P < .05$). These results demonstrated the predictive power of serum miR-221 for HCC prognosis. Qu and colleagues[69] probed the serum levels of several miRNAs, including miR-16, miR-195, and miR-199a, in a cohort comprising 105 HCC and 107 chronic liver disease (CLD) patients, together with 71 healthy volunteers. The study revealed that both miR-16 and miR-199a were suppressed in subjects diagnosed with HCC than those with CLD or the controls ($P < .01$). It is noteworthy that the use of miR-16 alone led to the correct prediction of HCC in 18 of the 26 (69.2%) patients for whom none of the 3 conventional diagnostic markers was tested positive because of the small tumor sizes (< 3 cm). The 3 miRNAs were also compared to and ranked together with the conventional HCC biomarkers in terms of their sensitivities, the results of which demonstrated that both miR-16 and miR-199a were more sensitive than AFP, DCP and AFP-L3%, with miR-16 being the most sensitive. Further analysis suggested that a diagnostic model coupling miR-16 with the abovementioned 3 conventional markers could achieve the optimal combination of sensitivity (92.4%) and specificity (78.5%) for detecting HCC tumors with sizes below 3 cm. Taken together, it was proposed that serum miR-16 be tested further in clinical studies for HCC diagnosis, possibly applied together with other common and/or novel biomarkers. Shigoka et al[70] have found that plasma level of miR-92a declined in HCC patients in comparison with healthy individuals, but was boosted following the operation. These findings implied a link between the dysregulation of miR-92a level in blood and the pathogenesis and progression of HCC. It

is worth emphasizing that the miRNA expression profiles in HCC patients could vary significantly according to the tumor stages. As a consequence, studies that fail to distinguish between patients with different tumor stages often diverge on the diagnostic value of a specific miRNA candidate.

With the advances in miRNA screening techniques and the development of new bioinformatics tools, a growing number of research groups also embarked on probing the feasibility of using a panel of circulating miRNAs to achieve higher sensitivity and specificity in HCC diagnosis. Circulating miRNA profiling of 513 subjects, consisting of healthy individuals and patients with HBV, HCV, or HCC, revealed a panel of 13 miRNAs with altered expression patterns between HBV patients and the controls. A diagnostic model constructed based on these miRNAs was found to be HBV-specific, even when the patients were also afflicted with HCC.[71] However, the serum levels of 6 miRNAs rose significantly in patients with both HBV and HCC compared with other groups. It was later established that among them, miR-25, miR-375, and let-7f combined could discriminate HCC cases from controls, highlighting the clinical utility of serum miRNA profiles for noninvasive diagnosis of HBV-positive HCC. In another study,[72] a panel of circulating miRNAs comprising miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801 was generated that achieved high area under the curve scores in the prediction of HCC, regardless of the tumor stages. The miRNA panel could also distinguish HCC cases from other common liver pathologies, such as chronic hepatitis B and cirrhosis. Recently, Lin and colleagues[73] developed a serum miRNA-based model composed of miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505, which they demonstrated could achieve better diagnostic sensitivity (cut-off, 20 ng/mL) than and similar specificity to AFP, particularly when it came to the detection of small and/or early-stage tumors. The results suggested that the miRNA panel could be used as a preclinical parameter to improve the treatment outcome of HCC patients.

Cell-free DNA

Dysregulated levels of cell-free DNA (cfDNA) were first associated with oncogenesis and cancer progression in the pioneering study of Leon et al,[74] in which cfDNA was found to be upregulated in pancreatic cancer patients but suppressed after chemotherapy. Since then, there is mounting evidence that advocates for a more prominent role of cfDNA in cancer diagnosis, monitoring of treatment progress, and even outcome prediction.[75,76] Single-nucleotide polymorphism of cfDNA is an important feature that could provide the key to early cancer detection. One of the most scrutinized mutations in HCC patients is the Ser249 p53 mutation, which was detected from the plasma DNA in several studies.[77-82] The same mutation, however, can also be identified, albeit much more sporadically, in the plasma DNA collected from non-HCC patients or healthy individuals.[81] It is possible that the development of this mutation long precedes the occurrence of HCC, making it a viable preclinical biomarker worthy of further examination. Differential methylation signatures identified in cfDNA can also serve as critical clues to nascent tumorigenesis due to their early occurrence. Wang and colleagues[83] reported an anomalous methylation pattern in the promoter region of the glutathione S-transferase gene GSTP1 in both tissue and serum samples from HCC patients. Methylation in p15 and p16 genes, which was absent in noncancerous tissues, was found concurrently in the sera of a predominant majority of the HCC patients enrolled in the studies by Wong et al.[84,85] Hypermethylation of the ras association domain family 1A (RASSF1A) promoter was shown to be detectable in cfDNA among 42.5% of the HCC patients whose tumor tissues demonstrated the same oncogenic anomaly.[86] It is worth mentioning that RASSF1A hypermethylation in cfDNA was also observed in individuals infected with HBV, albeit at a significantly lower concentration compared with that in HCC patients, which implied that its early manifestation could facilitate the prompt identification of malignancy and premalignancy.[87] However, a recent meta-analysis conducted by Liao et al[88] recommended against the use of cfDNA-based assay as the sole analytic method in HCC diagnosis due to its lack of robustness. Instead, the authors argued that cfDNA should be applied in combination with the conventional HCC biomarker AFP.[88] This was echoed by Shi et al,[89] who also found that a predictive model that comprised both cfDNA and AFP could improve the accuracy of HCC diagnosis (Tables 1 and 2).

Identification of Novel HCC Biomarkers Through Proteomics-Based Approaches

A continuing trend in biomarker discovery is the increasing adoption of high-throughput proteomics-based approaches.[90,91] These methods are capable of rapidly examining thousands of hypothetical candidates in very large study groups and, coupled with bioinformatics analysis, can allow researchers to accurately pinpoint the global and local discrepancies in protein profiles among different populations. These features could in theory provide enormous benefits particularly for the identification of cancer biomarkers due to the heterogeneity and mechanistic complexity of these diseases.[92] Recently, there has been noticeable progress in applying proteomics to the identification of potential HCC biomarkers. Yin et al screened the sera of patients with different liver diseases for core-fucosylated (CF) proteins using a mass spectroscopic approach, which revealed 3 CF peptides from fibronectin at site 1007 with a sensitivity score of 85.7% and specificity of 92.9% for discriminating HCC from cirrhosis in patients with alcohol liver diseases.[93] Moreover, they found CF cadherin-5 at site 61 to be the best candidate for distinguishing between HCV-linked HCC tissues and their cirrhosis counterparts, albeit with less

Table 1. The clinical applications, sensitivities and specificities of individual biomarkers mentioned in this review.

BIOMARKER	CLINICAL APPLICATION	SENSITIVITY	SPECIFICITY	CUTOFF VALUE	REFERENCE
AFP	HCC from cirrhosis	65%	89%	30 ng/mL	7
	HCC	70%	100%	200 ng/mL	7
	Early-stage HCC	65%	82%	10.9 ng/mL	8
	HCC among HBV carriers	55.30%	86.50%	20 ng/mL	9
	HCC among cirrhotic HBV	61%	71%	20 ng/mL	20
	Early-stage HCC from cirrhotic HCV	47%	75%	20 ng/mL	27
	HCC among cirrhosis	55%	90%	20 ng/mL	30
	HCC from nonmalignant liver diseases	73%	77%	20 ng/mL	31
	HCC from other liver cancers	73%	84%	20 ng/mL	31
	HCC from chronic cirrhosis	95.20%	47.10%	13.6 ng/mL	41
	HCC	51.90%	86.30%	20 ng/mL	45
	Early-stage HCC	40%	NA	20 ng/mL	45
	HCC	62.50%	53.30%	20 ng/mL	46
	Early-stage HCC	40%	NA	20 ng/mL	46
	HCC from cirrhosis	53.00%	93.00%	20 ng/mL	51
	HCV-HCC from cirrhotic HCV	46%	88%	20 ng/mL	51
	Early-stage HCC from cirrhosis	46.00%	93%	20 ng/mL	51
	HCC	45%	87.60%	13.7 IU/ml	55
	HCC	63.40%	79.70%	14.88 ng/mL	58
	Early-stage HCC	55%	81.30%	15.64 ng/ml	58
	HCC from healthy control	58.70%	86.70%	19 ng/mL	64
	HCC from chronic hepatitis	77.80%	96%	6 ng/mL	64
AFP-L3	HCC among cirrhotic HBV	37%	92%	10 ng/mL	20
hs-AFP-L3	HCC from benign liver disease	57%	63.50%	5%	22
DCP	HCC among cirrhotic HBV	39%	90%	7.5 ng/mL	20
	HCC from nonmalignant hepatic cirrhosis	92%	93%	150 mAU/mL	24
	HCC from cirrhosis	48-62%	81-98%	125 mAU/mL	26
	Early-stage HCC from cirrhotic HCV	43.00%	94%	40 mAU/mL	27
sGPC3	HCC among cirrhosis	51%	90%	2 ng/mL	30
GPC3	HCC from nonmalignant liver diseases	53%	99%	Undetectable in the control group (except 2)	31
	HCC from other liver cancers	73%	84%	Undetectable in the control group (except 2)	31
	HCC	73.58%	96.15%	5%-10% immunoreactive cells	32
	HCC	54.20%	99.40%	30 ng/mL	35
GP73	HCC from cirrhosis	69%	75%	10 relative units	40
	Early-stage HCC from cirrhosis	62%	88%	10 relative units	40
	HCC from chronic cirrhosis	75%	51.80%	13.8 μg/L	41
MDK	HCC	87%	84%	0.654 ng/mL	45
	Early-stage HCC	80%			45
	HCC	92.50%	83.30%	0.387 ng/mL	46
	Early-stage HCC	90%		0.387 ng/mL	46
OPN	HCC	100%	98%	280 ng/mL	50
	HCC from cirrhosis	74%	66%	91 ng/mL	51
	HCV-HCC from cirrhotic HCV	82%	65%	91 ng/mL	51

Table 1. *(Continued)*

BIOMARKER	CLINICAL APPLICATION	SENSITIVITY	SPECIFICITY	CUTOFF VALUE	REFERENCE
	Early-stage HCC from cirrhosis	75%	62%	91 ng/mL	51
SCCA	HCC	89%	50%	89 AU/mL	54
	HCC	84.20%	48.90%	0.368 ng/ml	55
Annexin A2	HCC	81.70%	68.30%	17.43 ng/μl	58
	Early-stage HCC	83%	67.50%	17.3 ng/μl	58
miR-21	HCC from healthy control	84%	73.50%	Relative level of 0.46	62
miR-122	HCC from healthy control	70.70%	69.10%	Relative level of 0.70	62
miR-223	HCC from healthy control	80.00%	76.50%	Relative level of 1.91	62
miR-122	HCC from healthy control	81.60%	83.30%	Relative level of 0.475	63
miR-21	HCC from healthy control	87.30%	96%	Relative level of −0.108	64
	HCC from chronic hepatitis	61.10%	86.70%	Relative level of 0.754	64
miR-16	HCC	72.10%	88.80%	ΔCt Cutoff = 6	68
miR-199	HCC	78.10%	64.50%	ΔCt Cutoff = 10	68

Abbreviations: AFP, alpha-fetoprotein; DCP, des-γ-carboxyprothrombin; GP73, Golgi protein 73; GPC3, glypican-3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; hs-AFP-L3, highly sensitive AFP-L3; MDK, midkine; OPN, osteopontin; SCCA, squamous cell carcinoma antigen; sGPC3, soluble glypican-3.

Table 2. The clinical applications, sensitivities and specificities of combined biomarkers mentioned in this review.

BIOMARKER	CLINICAL APPLICATION	SENSITIVITY	SPECIFICITY	REFERENCE
sGPC3 + AFP	HCC among cirrhosis	72.00%	90.00%	30
GPC3 + AFP	HCC from nonmalignant liver diseases	88.00%	76.00%	31
	HCC from other liver cancers	88.00%	84.00%	31
CK19 + GPC3 + AFP	HCC	90.60%	NA	35
AFP + GP73	HCC from chronic cirrhosis	75.80%	79.70%	41
OPN + AFP	HCC from cirrhosis	85.00%	63.00%	51
	HCV-HCC from cirrhotic HCV	86.00%	60.00%	51
	Early-stage HCC from cirrhosis	83.00%	63.00%	51
SCCA + AFP	HCC	90.83%	44.44%	55
Annexin + AFP	HCC	76.00%	80.50%	58
	Early-stage HCC	87.40%	68.30%	58
miR-21 + AFP	HCC from healthy control	92.90%	94.00%	64
	HCC from chronic hepatitis	81.00%	80.00%	64
miR-16, AFP, AFP-L3%, and DCP	HCC	92.40%	78.50%	68
miR-23b, miR-423, miR-375, miR-23a, and miR-342-3p	HBV-HCC	96.90%	99.40%	70
miR-10a and miR-125b	HBV-HCC from HBV	98.50%	98.50%	70
miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801	HCC	68.60%	90.10%	71
	HCC from healthy control	83.20%	93.90%	71
	HCC from HBV	79.10%	76.40%	71
	HCC from cirrhosis	75.00%	91.10%	71
miR-29a, miR-29c, miR-133a, miR-143, miR-145, miR-192, and miR-505	HCC	80.60%	84.60%	72
CXCR2, CCR2, and EP400	HCC	93.00%	89.00%	88
CXCR2, CCR2, EP400, and AFP	HCC	93.00%	95.00%	88

Abbreviations: AFP, alpha-fetoprotein; GP73, Golgi protein 73; GPC3, glypican-3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; OPN, osteopontin; SCCA, squamous cell carcinoma antigen; sGPC3, soluble glypican-3.

effectiveness. These results suggested that the CF peptides could also have a role in the detection and diagnosis of HCC. Based on comparative proteomic profiling, Gray et al[94] identified 4 differentially expressed apolipoprotein isoform proteins with the ability to differentiate among nonalcoholic fatty liver disease (NAFLD) without cirrhosis, NAFLD with cirrhosis, and cirrhotic NAFLD accompanied by HCC. An additional fifth protein, revealed to be CD5 antigen like, could discriminate NAFLD cirrhosis from simple NAFLD but demonstrated no diagnostic capability for HCC. Zinkin et al[95] developed a 11-peak algorithm based on analysis of serum proteins using surface-enhanced laser desorption/ionization time of flight mass spectrometry, which was shown to be more accurate than several conventional biomarkers in small-sized HCC tumors. Overall, proteomics-enabled HCC biomarker identification is still at a nascent phase and further research is needed to overcome the various challenges that it faces. One of the imminent needs is to detect low-abundance proteins with better accuracy. It is also intrinsically difficult for mass spectrometry to distinguish highly homologous proteins from each other or, in many cases, to provide potentially crucial information on posttranslational modification signatures. These hurdles will likely be addressed be further advances in proteomics methodology and instrumentation, as well as the development of better bioinformatic algorithms for data analysis.

Outlook

As HCC is raised through multiple risk factors, it is hard to characterize personal HCC using only one single biomarker. The investigation of biomarker combinations might provide more accurate and valuable information for the future personal HCC diagnosis and/or prognosis. Although more and more research is under development of novel biomarkers, further work on whether certain biomarkers can be utilized in clinical are still in real worldwide demand. We trust that identifying novel cost-efficient biomarker or high-efficient biomarker combinations for the HCC early diagnosis will be promising.

Author Contributions

All the authors conceived, organized, drafted, reviewed, and approved the manuscript.

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