The Biomarkers for Predicting Viral Hepatitis-Associated Hepatocellular Carcinoma

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ABSTRACT

Background: *Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world, and more than half of the newly diagnosed cases are chronic hepatitis B patients. Due to the lack of specific clinical manifestations, many patients are already at an advanced stage at the time of diagnosis and therefore have missed the best time for treatment. Organs in a pathological state usually secrete specific substances into the blood, which can indirectly indicate the pathological state of the organ, so some biological markers in the blood can be used as a tool to predict the incidence of HCC.*

Methods: *The Research articles related to HCC were collected by searching PubMed databases with the keywords "hepatocellular carcinoma", "serum biomarker", "hepatitis B", "prediction" and "prognosis", and Additional articles were identified by manual search of references found in the primary articles, followed by a summary and review.*

Results: *Viral hepatitis is the main cause of HCC worldwide, and this phenomenon is particularly prominent in Asian and African populations. A variety of serological markers including M2BPGi, IL-6 and COMP can be used to predict the incidence of long-term HCC in patients. The risk of HCC is dynamic rather than constant, and dynamic detection will help improve prediction accuracy. For hepatitis B patients, HBV DNA load and HBcr Ag are important predictive markers of HCC.*

Conclusion: *For a high-risk population of HCC, early risk prediction is helpful to guide clinical work, and timely adjustments of the screening frequency and treatment plan are helpful to prolong the survival time of HCC patients.*

Keywords: *Hepatocellular carcinoma, hepatitis B virus, biomarkers, predictive*

INTRODUCTION

HCC ranks fifth in the global incidence of malignant tumors, with a 5-year survival rate of only 15% due to its high mortality rate. Early HCC can be treated with surgical resection, radiofrequency ablation, and liver transplantation, while most advanced HCC can only be treated with palliative care. Therefore, timely diagnosis and early intervention are important for the prognosis of patients with HCC.[1] However, diagnosing early-stage HCC is difficult because of the lack of specific symptoms in the early stage.

Currently, the high-risk population of HCC mainly includes chronic hepatitis B (CHB) patients, chronic hepatitis C (CHC) patients, diabetes patients, alcohol misuse patients, and non-alcoholic fatty liver patients. Among these risk factors, HBV infection is the main reason for the development of HCC, especially in Asia and Africa. It has been estimated that there are approximately 248 million to 257 million HBV patients worldwide. Multiple studies have shown that the risk of HCC in CHB patients is 15-20 times higher than in a healthy population,[2] indicating that early diagnosis and timely prediction of the risk of HCC are very important for these patients.

Current guidelines recommend HCC surveillance in populations with cirrhosis or other risk factors for HCC, and ultrasound examinations are generally recommended every 6 months. Simultaneously, opinions on whether to combine alpha-fetoprotein(AFP) for screening are not uniform.[3] In early-stage HCC, morphological changes are very limited, making an ultrasound diagnosis uncertain. Previously, a meta-analysis showed that ultrasound sensitivity was only 47% in detecting early-stage HCC.

Organs in pathological conditions often secrete specific proteins or bioactive peptides into the plasma, which can indirectly indicate the pathological state of the organ. Plasma is considered an ideal source for cancer biomarkers. Some plasma markers have also proven to be useful tools for assessing the long-term risk of developing

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HCC, improving early surveillance for high-risk populations of HCC. When ultrasound was combined with AFP, the detection rate of HCC at any stage was greatly improved.[4] However, AFP tests are more frequently associated with advanced-stage disease than the early-stage disease. In addition to AFP, lens culinary agglutinin-reactive AFP (AFP-L3) and Des-γ-Carboxy Prothrombin (DCP) are two main biomarkers used in clinical practice or known to most clinicians. AFP-L3 is one of the three heterogeneous bodies associated with HCC in AFP and only produced by the tumor cells. AFP-L3 positivity indicates early vascular infiltration and intrahepatic metastasis tendency. The expression level of DCP is mainly related to abnormal prothrombin synthesis caused by hypoxia in hepatocytes and low vitamin K concentration, and it is better than AFP and AFP-L3 in diagnosis cirrhotic-associated HCC. However, these markers are mostly used in the diagnosis and prognosis of HCC and rarely in early prediction. A good predictive serological marker will guide clinicians to develop more personalized surveillance programs for populations at high risk of HCC. This article reviews the predictive serological markers of developing HCC, especially HBV-associated HCC, and discusses its future developments.

PREDICTIVE BIOMARKERS OF HCC

M2BPGi

Mac-2 binding protein (M2BP) is a glycoprotein produced by hepatic stellate cells (HSCs), which is widely glycosylated to become Mac-2 binding protein glycosylation isomer (M2BPGi or WFA+-M2BP). M2BPGi is able to interact with galectin-3 (Mac-2) and other extracellular matrix molecules (ECM) and act as a messenger between HSCs and Kupffer cells; it has been shown to be useful in assessing the degree of fibrosis in chronic liver disease.[5] Recent studies have reported that M2BPGi might predict HCC development. For a cohort of CHB patients treated with entecavir (ETV), HCC cases compared with 185 matched patients without HCC, serum M2BPGi levels were higher both at baseline and after 3 years of antiviral treatment. The baseline M2BPGi level was ultimately proven to be an independent risk factor for HCC, and baseline M2BPGi > 1.15 COI indicated a 2-fold increase in HCC risk.[6] The results are consistent with a study in Japan, which included 234 patients with CHB who were treated with antiviral drugs for more than 1 year and viral replication was well controlled. Multivariate analysis showed that in males, AFP levels ≥ 9.65 ng/mL and M2BPGi levels ≥ 1.215 COI at 48 weeks of treatment both were predictors of increased risk of HCC. M2BPGi levels > 1.215 COI were associated with a 5-fold increase in the risk of HCC.[7] A follow-up study of 355 HCV patients who had achieved a sustained viral response for an average of 2.9 years showed that having serum M2BPGi level ≥ 2.80 COI increased the risk of HCC development 15 times. The five-year cumulative incidence rate of HCC development in the low M2BPGi population was significantly lower than that in the high M2BPGi population (0.4% vs 17.6%).[8]

OPN

Osteopontin (OPN) is a highly modified integrin-binding extracellular matrix glycophosphoprotein and is widely distributed in a variety of tissues and cells and is involved in tissue repair, self-metabolism, tumorigenesis, and metastasis. Overexpressed OPN has been found in several tumor organs, including the liver, stomach, lung, breast, and colon.[9] In HCC, elevated plasma OPN levels correlate with intrahepatic metastasis, early recurrence, and a worse prognosis, and OPN is also an early-stage HCC biomarker and can be used to predict HCC development.[10] A nested case–control study in Europe showed that when OPN levels were above 47.15 ng/mL, OPN levels increased linearly with the risk of HCC (per 10% increment, OR = 1.30). This association was stronger in viral hepatitis patients as well as for cases diagnosed within 2 years.[11] More studies are required to confirm the value of OPN in predicting the risk of HCC in other ethnic groups.

COMP

Cartilage oligomeric matrix protein (COMP) is a cellular matrix glycoprotein located in human cartilage that regulates cell phenotype during tissue growth and remodeling. Studies have found that COMP is not expressed in normal liver tissues, and the content of COMP in HCC tissues is significantly increased.[12] Li et al.[13] demonstrated that activated HSCs can produce COMP, which can promote the development of HCC. Elevated COMP can be used not only as a diagnostic marker for HCC but also as a predictor of the incidence of HCC. A study that included

Main Points

- *Viral hepatitis is the main cause of HCC worldwide, and this phenomenon is particularly prominent in Asian and African populations.*
- *Some specific serological markers have been found that can be used to predict the future risk of Viral-related HCC.*
- *M2BPGi and IL-6 have been identified as risk factors for HCC in many prospective experiments and can be used to predict the risk of HCC.*
- *For the HBV-related HCC, HBV DNA load and HBcr Ag are the most important predictive markers for the higher HBV DNA load and low HBV DNA-Load patients, respectively.*

187 chronic liver disease patients (mainly CHB and CHC patients) followed for an average of 8 years showed that when COMP levels were above 15 U/L, the risk of developing HCC was tripled.[14] Of course, multicenter and larger studies are needed to demonstrate further the predictive value of COMP in high-risk populations of HCC.

sPD-1

Programmed death receptor 1 (PD-1) is an immunosuppressive receptor expressed by activated T cells, B cells, and myeloid cells. Since the combination of PD-1 and its ligand (PD-L1) can lead to tumor immune escape, inhibiting the interaction between PD-1 and PD-L1 can enhance T cell response and mediate anti-tumor activity. PD-1 and PD-L1 as targets of immunosuppression have attracted increasing attention for the treatment of tumors. In CHB patients, the expression level of PD-1 is related to the viral load, and PD-1 expression on CD8+ T cells decreases after effective antiviral therapy.[15] PD-1 mRNA can form soluble PD-1 (sPD-1) by splicing, and it was found that sPD-1 could act as a signal antagonist of PD-1. The sPD-1/sPD-L1 have recently been detected in the serum of HCC patients. Previous research found that CHB patient mortality increases with increasing serum concentrations of sPD-L1.[16] A study of 2903 male CHB patients proved that sPD-1 plasma levels are positively correlated with viral load. When baseline sPD-1 levels >282 pg/mL, the risk of developing HCC was double.[17] This suggests that sPD-1 is a very promising predictor of HCC.

VWF and ADAMTS13

Von Willebrand factor (VWF) is present in endothelial cells and platelets and plays a crucial role in hemostasis and tissue injury. A study showed that because of its unique functional structure and capability, VWF could promote cancer metastasis. A previous study demonstrated that VWF plasma concentration was significantly reduced in CHB, LC, and healthy controls compared with the HCC group.[18] Therefore, VWF is considered a potential predictive biomarker for HCC development, and Takaya et al. proved this in an 8.3-year follow-up study.[19] A disintegrin-like and metalloproteinase with thrombospondin type-1 motifs 13 (ADAMTS13) is produced only in HSCs adjacent to endothelial cells. It has been previously reported that the imbalance of ADAMTS13 and VWF is related to the transformation of liver cirrhosis (LC) into HCC.[20] Therefore, ADAMTS13 is considered a predictor of HCC. A cohort of CHB and CHC patients in Japan that has been followed for 3 years proved that higher plasma ADAMTS13 activity or antigen level was accompanied by a higher risk of developing HCC.[21]

IL-6

Interleukin-6 (IL-6) is a crucial cytokine produced by macrophages and lymphocytes that can activate several pathways to play a crucial role in cell proliferation, protection from apoptosis, and increased metastatic potential. In the inflammatory response to hepatitis virus infection, IL-6 is also one of the key factors.[22] In addition to being a tumor marker of HCC,[23] IL-6 can also be used as a predictor of HCC. A 7.3-year follow-up study of CHB patients demonstrated that high serum IL-6 (defined as >7 pg/mL) was related to 3-fold increased risk of HCC.[24] Another study of HCV-associated HCC (including 150 males and 180 females) in Japan came to the same conclusion. Nakagawa H. et al. divided patients into high, medium, and low groups based on the concentration of serum IL-6 levels. They found that the higher the serum IL-6 level, the greater the risk of developing HCC. Further gender segregation analysis showed that this tendency was only significant in female patients. When the IL-6 level was above 50 pg/mL, the risk of HCC was double compared with cases when the IL-6 level was below 5 pg/mL.[25]

IGF-1

Insulin-like growth factor-1 (IGF-1) is the key regulator of energy metabolism and growth and is also involved in tissue repair and disease pathogenesis throughout life. An increasing number of studies have demonstrated that IGF-1 and its signaling pathway play a crucial role in the progression of many types of cancer.[26] IGF-1 plays a slightly different role in different tumors. Epidemiologic studies have found that serum IGF-1 levels are significantly elevated in esophageal cancer, colon cancer, and breast cancer. In contrast, in HCC patients, lower serum IGF-1 levels are significantly related to higher mortality and shorter survival.[27] A prospective study demonstrated that in patients with HCV-related cirrhosis, a yearly IGF-1 reduction > 9.3 μg/L was associated with 33-fold increased HCC risk.[28]

ICAM-1

Intercellular adhesion molecule (ICAM-1) is an important adhesion molecule that mediates cell adhesion and mediates its biological activity by binding to specific receptors on the surface of vascular endothelial cells.[29] A study found that in HCC, high sICAM-1 concentrations are always related to poor prognosis.[30] A study from Japan has shown that ICAM-1 may be a marker associated with the incidence of HCC. Researchers have found that ICAM-1 above 400 ng/mL was correlated to a 5.7-fold increase in the risk of HCC through long-term follow-up of

CHC patients. The authors divided the patients based on changes in the concentration of sICAM-1 during the follow-up period into the reduction group and the increasing group, and the analysis demonstrated that the incidence of HCC in the increasing group was 6 times that of the reduction group.[31] Another larger study validated this finding. During the 5.4-year follow-up, Chen et al. found that high sICAM-1 was associated with a significant increase in the incidence of HCC. sICAM-1 above 11861 MFI was associated with a 2.75-fold increase in HCC risk.[32]

Hepatitis B Virus-Related Predictors

Hepatitis B virus (HBV) infection is the most important risk factor of HCC. The mechanism of carcinogenesis of CHB is mainly due to the virus-induced immune counterattacks on infected liver cells, leading to chronic or recurrent inflammation, resulting in the accumulation of DNA mutations that cause hepatocellular carcinogenesis.[33] The more active the hepatitis B virus replication, the more likely it is to drive the development of HCC. Many studies have shown that biological indicators representing the degree of HBV replication including e antigen (HBe Ag) positivity, serum viral load (HBV DNA), serum surface antigen (HBs Ag) levels, and core-associated antigen (HBcr Ag) levels are closely related to the risk of HBV-associated HCC.[34-36] The predictive serum biomarkers and related information discussed in the article are shown in Table 1.

HBV DNA

The current general agreement in the field is that continuous replication of HBV DNA is an independent risk factor for CHB progression to LC and HCC. The higher the HBV DNA load, the higher the incidence of HBV-related HCC, and the two are significantly positively correlated. An 11-year follow-up study of CHB patients from Taiwan found that an elevated HBV DNA load is an important risk factor for predicting HCC, whereby HBV DNA load above 1 million copies/mL reflected an 11-fold increased risk of developing HCC compared to an HBV DNA load below 300 copies/mL.[34] Reduced or even undetectable viral load by long-term antiviral therapy could reduce but not completely eliminate the risk of HBV-related HCC, and a 10-year follow-up study in China has shown that CHB patients with virologic remission (defined as undetectable HBV DNA) under antiviral therapy have a two-fold lower risk of developing HCC compared with those without virologic remission.[37] ETV and tenofovir are recommended as

Table 1. Predictive Serum Biomarkers for Hepatocellular Carcinoma

Marker	Cut-Off	Increase Risk for HCC	Control Group	Follow-up Time	Country/Region
M2BPGi	>1.15 COI	2-fold	<1.15 COI	7.1	Hong Kong[6]
M2BPGi	>1.215 COI	5-fold	<1.215 COI	4.1	Japan[7]
M2BPGi	>2.8 COI	15-fold	<2.8 COI	2.9	Japan[8]
OPN	>47.15 ng/mL	1.33-fold (10% increase)	<47.15 ng/mL	4.8	European countries[11]
COMP	Positive (>15 U/mL)	3-fold	Negative	8	Greece[14]
sPD-1	>282 pg/mL	2-fold	<282 pg/mL	19.3	Tai Wan[17]
IL-6	>7 pg/mL	3.2-fold	<7 pg/mL	7.3	Hong Kong[24]
IL-6	>50 pg/mL	2-fold	<5 pg/mL	9	Japan[25]
ICAM-1	>400 ng/mL	5.7-fold	<400 ng/mL	6	Japan[31]
ICAM-1	>11861 MFI	2.75-fold	<11861 MFI	5.4	America[32]
IGF-1	-	33-fold (yearly reduction > 9.3 ug/L)	-	4.7	Italy[28]
VWF	-	1.007-fold (per 1% increase)	-	8.3	Japan[19]
ADAMST 13	-	1.2-fold (per 10% increase)	-	3	Japan[21]
HBV DNA	>1 million copies/mL	11-fold	<300 copies/mL	10	Tai Wan[34]
HBsAg	>1000 IU/mL	6.5-fold in men and 11-fold in women	5-9 IU/mL	15	China[42]
HBsAg	>1000 IU /mL	8-fold	<100 IU/mL	14	Tai Wan[43]
HBe Ag	Positive	4.5-fold	Negative	9	Tai Wan[43]
HBcr Ag	≥7.8 kU/mL	3.27-fold	<7.8 kU/mL	7	Hong Kong[36]

first-line antiviral treatments for CHB patients by practice guidelines.[38] However, they seem to be slightly different in reducing the risk of HBV-related HCC, as a recent study in South Korea showed that CHB patients treated with ETV had a higher risk of developing HCC than those treated with tenofovir.[39]

HBs Ag

Hepatitis B surface antigen (HBs Ag) is used to diagnose HBV infection, and HBsAg serum clearance is currently considered to be the endpoint of antiviral therapy for HBV.[40] The complex interaction between HBV and the immune system is reflected in the serum HBsAg levels, while the level of HBsAg can also provide supplementary information for the determination of HBV DNA viral load.[41] A study in China with a follow-up time of 15 years found that HBs Ag levels greater than 1000 IU/mL were related to a 6.5-fold increase in HBV-related HCC risk in males and an 11-fold increase in risk in females compared to HBs Ag levels below 9 IU/mL, and the HCC risk increased more among males than females as HBs Ag levels rose.[42] Another 14-year follow-up study from Taiwan showed that HBsAg levels were positively correlated with HCC risk when the HBV DNA load ranged from 2000 to 19999 IU/ml. Compared with HBs Ag levels < 100 IU/mL, the risk of HBV-related HCC increased by 8 times when HBs Ag levels > 1000 IU/mL; however, when HBV DNA levels > 20000 IU/mL, no correlation was found between HBs Ag and HCC development in either cohort.[43] Interestingly, Cheung et al. found that in CHB patients with good virus suppression, HBsAg could not be used to predict the risk of HBV-related HCC.[36] This suggests that the value of HBs Ag in predicting the risk of HBV-related HCC development is correlated with the HBV DNA load.

HBe Ag

Hepatitis B e antigen (HBe Ag) seropositivity increases during active HBV infection, indicating that the liver cells are seriously damaged and that the patient is highly contagious.[44] A prospective study with a follow-up time of 9 years from Taiwan showed that men who were positive for both HBs Ag and HBe Ag were associated with a 4.5-fold higher incidence of HBV-related HCC than those who were positive only for HBs Ag.[45] A recent observational cohort study in Hong Kong found that older age at e antigen serological clearance was related to higher rates of HCC. For patients who achieved HBe Ag serological clearance at an age over 45 years, the cumulative rate of HBV-related HCC was avered 9-fold higher than that of patients aged 30-40 years in the years post HBe Ag serological clearance.[46]

HBcr Ag

Hepatitis B core-related antigen (HBcr Ag) is a newly discovered HBV serological marker that has been proposed as a diagnostic and predictive marker for CHB.[47] After antiviral treatment, the serum HBV DNA of some patients is below the detection threshold, and HBcr Ag can be used as a substitute for HBV DNA to reflect the replication of intrahepatic cccDNA.[48] HBcr Ag has been confirmed to be related to HBV-related HCC development. A study from Hong Kong confirmed this finding, and 76 patients with undetectable HBV DNA after antiviral therapy were included in the study. The results showed that pretreatment HBcr Ag levels ≥ 47.1 kU/mL were an independent risk factor for HCC and that post-treatment HBcr Ag levels ≥ 7.8 kU/mL were associated with a 3.27-fold increase in HBV-related HCC risk. When only LC patients were considered, HBcr Ag levels > 7.9 kU/mL were correlated with a 5-fold increase in the risk of developing HCC.[36] As a new serological marker, HBcr Ag has an irreplaceable advantage in predicting the risk of HBV-related HCC in populations with low HBV DNA load.

DISCUSSION

At present, several predictive scoring models have been established for predicting HBV-associated HCC, most of which were developed based on untreated Asian CHB patients and have been verified internally. They mainly combine gender, age, cirrhosis, hepatitis B virus load, AFP, transaminase, and other factors, and the specificity and sensitivity of each model are also different. Compared to a single predictor, the scoring models are more accurate but require more parameters and are not suitable for early surveillance of patients.

From the perspective of guiding clinical work, predictive biomarkers are superior to diagnostic biomarkers, and HCC monitoring programs can be customized according to individual needs. Positive predictive results can be used to evaluate the predictive model further, increase the frequency of surveillance, and use CT for imaging monitoring. CHB patients can be treated with antiviral therapy in advance.[40]

AFP is currently the most widely used HCC serological biomarker in the clinic. It has been reported that AFP can also be used as a predictive biomarker,[49] but because it is mainly used as a diagnostic marker, it was not included in this review. Due to the heterogeneity of HCC, it is difficult to find a single biomarker with 100% sensitivity and

specificity. A more reasonable method to improve the diagnostic accuracy may be a combination of different biomarkers.

Worldwide liver disease patients can differ in terms of etiology and ethnicity. Just as most scoring models are mostly confined to the same ethnic group, predictive markers between different ethnic groups may have different predictive effects. A study on the use of OPN and LTBP2 as diagnostic markers for HCC was performed in four different cohorts in Thailand, Gambia, France, and Korea. The results showed that the sensitivity and specificity of the two markers differed more than 10% between the different cohorts.[50] Therefore, the identification and characterization of a new biomarker require verification in multiple ethnic groups.

Compared to diagnostic markers, discovering new predictive markers often requires years of follow-up verification time, limiting new findings in this field. Most of the predictive biomarkers mentioned in this article have not undergone large external validation studies, so more experiments are needed to verify their accuracy.

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