Review

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Perspectives on a combined test of multi serum biomarkers in China: Towards screening for and diagnosing hepatocellular carcinoma at an earlier stage

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Summary **China has 50% of the worldwide hepatocellular carcinoma (HCC) cases, and the HBV-related cases accounts for approximately 85%. Over the past few decades, although a series of standardized management methods for HCC has been implemented in China, most HCC patient in China still suffered from advanced-stage disease, in consequence, reducing the opportunity of curable treatment that can be offered to achieve long-term disease-free survival for HCC patient. Accordingly, strategies including screening and diagnose HCC at an earlier stage are urgently needed in China. In this study, the current status, challenges, and prospects of early detection of HCC in China have been analyzed. The result indicated the need for using multi serum biomarkers for early HCC detection. During the past ten years, the research on the clinical usefulness of novel serum biomarkers of des-γ-carboxy-prothrombin (DCP), Dickkopf-1 (DKK1) and Midkine (MDK) in early HCC detection for Chinese patients found that the novel serum biomarker can complete the measurement of α-fetoprotein (AFP) in the diagnosis process of HCC, particularly for the patient with negative AFP with/or at an early stage. More large-scale, multi-center studies are expected to be performed in China to provide further evidence, and using novel and reliable serum biomarkers to complement AFP as a new trend is expected to be extensively used in clinical practice to facilitate early detection for those patients with HCC in China.**

Keywords: α-fetoprotein (AFP), des-γ-carboxyprothrombin (DCP), HCC, sensitivity, tumor marker

1. Introduction

Liver cancer is the fifth most common cancer and the second leading cause of cancer-related deaths worldwide, with a reported cases of 782,000 each year (*1*). As the most common type of liver cancer, hepatocellular carcinoma (HCC) is prevalent in Asian countries, accounting for 75-80% cases reported globally (*2,3*). HCC is prevalent in males, the male incidence rates of the following countries or districts in Asia are over 25/100,000 (persons): mainland China (58/100,000), Taiwan (53/100,000), South Korea (45/100,000), Thailand (33.4/100,000), and Hong Kong (29.9/100,000) of particular note is the fact that China alone accounts for 50% of HCC cases worldwide (*4,5*). Currently, HCC become the second and third leading cause of cancer-related deaths respectively in males and in females in China (Table 1) (*6,7*), and the HCC's incidence has increased in the past few decades caused by the high prevalence of its main etiological agents, chronic hepatitis B virus (HBV) infections (*8-10*). In fact, 93 million HBV carriers are Chinese, accounting for 2/3 of such patients worldwide, and about 20 million of these people have chronic HBV infection (*11,12*).

Evidence has shown that surgical resection and liver transplantation may offer the best opportunity for treating HCC yet are only available to early-detected patients (*13-16*). The normal overall 5-year survival rate is 40%, but with a liver resection to treat early HCC, the 5-year survival rate rise to 60-70% (*17,18*). Over the past few decades, a series of standardized management methods for HCC has been implemented

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Table 1.The current status to screen for and diagnose HCC in China

Items	Current status in China
Prevalence	The second most common cancer in urban areas and most common in rural areas; with an overall prevalence rate of 26-32/10,000, even up to 70-80/10,000 in some areas (*5,11*)
Mortality	The second leading cause of cancer-related deaths in males and the third leading cause of cancer-related deaths in females; with a total mortality rate of 26.26/100,000 (*11*)
Etiological factors	85% of patients with HBV infection, 10% of patients with HCVinfection, and a small minority involve HBV and HCV (*8,9*)
Major at-risk population	People with HBV infection; 93 million HBV carriers, 20 millionof these with chronic HBV Infection (*11,12*)
Screening and surveillance	No government-funded screening and surveillance program for HCC high-risk population screening and surveillance (*10*)
Screening and Diagnostic algorithm	The test of ultrasonography and AFP every 6 months for the population ages 35-40 at risk for developing HCC (*6*)
Treatment algorithm	Comprehensive therapy predominantly in the form of surgery (*7*)
Early detection	Most patients with HCC present with advanced-stage disease (*10*)

by China Government, and the Expert Consensus on the Treatment Standards for Hepatic Carcinoma, the Chinese Guidelines on HCC, was drafted in 2009 (*19*). Currently, standard treatment for HCC in China is comprehensive therapy predominantly in the form of surgery (*7,20*). As clinical techniques have developed in China, new techniques have also become available, such as laparoscopic surgery and minimally invasive robotic surgery. However, most HCC patient in China still suffered from advanced-stage disease (*10*), thus reducing the chance of curable treatment. Accordingly, strategies to screen for and diagnose HCC at an earlier stage are urgently needed in China when curable interventions can be offered to achieve long-term disease-free survival for HCC patient (*21*).

2. The current strategies to screen for and diagnose HCC in China

2.1. *Screening high-risk population for developing HCC*

Evidence showed that screening high-risk HBV- or HCV-related chronic liver diseases population may improve the rate of early HCC detection and curative treatment. It has been found by a systematic literature review involving over three thousand papers included in PubMed database between 2001 and 2011 (*22*), and it has also been shown by several cohort studies (*23-25*).

Unlike in the USA, European countries, and other Asian countries such as Japan where HCV is the most significant etiological factor for developing HCC (*26*), the HBV-related cases accounts for approximately 85% while only 10% are HCV-related and a small minority involve HBV and HCV in China (*8,9*). Thus, people with HBV are the largest risk population for developing HCC in China.

Globally, many guidelines for HCC treatment recommend HCC screening and surveillance, including the guidelines established by the American Association for the Study of Liver Disease (AASLD) (*27*), the National Comprehensive Cancer Network (NCCN) (*28*), and the Asian Pacific Association for the Study of the Liver (APASL) (*29*). In Asia, Japan and South Korea have implemented their own nationwide screening and surveillance program for the HCC high-risk population. In Japan, as early as 2002, the Japanese Ministry of Health, Labor, and Welfare started a national 5-year program to screen for HCV and HBV infection among people over 40 years of age, given the high prevalence of HCV infection in this age group (*30*). With the support of this program, 9 million people had been screened until the end of 2006, 112,000 scanning objects were found infected HCV and 110,000 were found infected HBV (*31*). Since most high-risk patients were closely followed, more than 60% of cases had detected HCC nodules in the early stage in Japan (*32,33*).

Similarly, a screening and surveillance program has also established in Taiwan. The program focuses on screening patients with cirrhosis every 3-6 months and patients with no cirrhosis every 6-12 months (*10,34*). However, there is no such program funded by government for HCC high-risk population screening in China, including Hong Kong (*10*). As a result, a well thought-out strategy for screening high-risk populations with HBV-related chronic liver disease is urgently needed in China to enhance the early detection of HCC.

2.2. *Serum biomarkers for screening and diagnosis of HCC*

Screening and diagnosis tools should have an acceptable accuracy and cost. In general, imaging tools have been

widely used in the USA and European countries, while serum biomarkers are widely used in HCC screening and diagnosis in Asia. Diagnostic imaging techniques include ultrasonography, computed tomography (CT), and magnetic resonance imaging (MRI). According to a systematic review, ultrasonography has a 60% sensitivity and a 97% specificity, CT has a 68% sensitivity and a 93% specificity, and MRI has a 81% sensitivity and a 85% specificity (*35*). Ultrasound is the most common imaging tool used in the screening process for HCC thanks to is the features such as simple, inexpensive, non-invasive, and allows real-time observation. However, a successful ultrasound-detection relies on the expertise of the physician, the available of ultrasound equipment, and the echo texture of the liver. So the actual sensitivity and specificity of ultrasound-detection is difficult to evaluate due to the lack of standard in China (*36,37*).

Serum biomarkers are striking prospective alternative tools for screening and early diagnosis of HCC thanks to the non-invasive, objective, and reproducible assessments they would enabled (*38*). According to the Chinese Guidelines on HCC, ultrasonography and α-fetoprotein (AFP) measurement are recommended to be performed every 6 months for the people ages between 35 and 40 at risk for developing HCC (*6*). Currently, the serum biomarker AFP is considered as a useful and practicable tool for the screening and early diagnosis of HCC in China. The clinical usefulness of AFP in China has been ascertained by a trial that with a randomized control in 2004 which involved 18,816 Chinese patients aged between 35 and 59 with HBV infection or a history of chronic hepatitis (*39*). However, the sensitivity of AFP is unsatisfactory (25-65%) at the commonly used cut-off (20 ng/mL), especially in the detection of early-stage HCC (*40,41*), up to 50% of HCC patients have an AFP level below 20 ng/mL. Elevated levels of AFP could also be found in non-malignant chronic liver disease patients, including 15-58% with chronic hepatitis and 11-47% with liver cirrhosis (*42-44*).

Besides, there are many diagnostic difficulties in clinical practice, such as cases with high AFP level, but no space occupying lesion by imaging finding, cases with negative AFP, less than 1 cm or no HCC featured lesion by imaging finding (*7*). Thus, AFP cannot be used as a sole tool to screening and diagnose HCC. The novel and dependable serum biomarkers to complement AFP are urgently needed to be discovered in order to improve the clinical outcomes.

3. The future perspective on using multi serum biomarkers in early HCC detection in China

3.1. *The combined test of AFP, AFP-L3, and DCP*

Besides AFP, there are two other serum biomarkers – lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), and des-γ-carboxyprothrombin (DCP, also known as prothrombin induced by vitamin K absence-II, PIVKA-II) – that have been studied worldwide to explore for clinical usefulness in HCC screening and diagnosis, and has been used in some countries (*45-48*). According to HCC Guidelines in Japan, ultrasonography and measurement of AFP, AFP-L3, or DCP should be performed every 3-4 months in the highest-risk group (HBV- or HCV-related liver cirrhosis patients) and every 6-month in the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes) (*49,50*). Currently, AFP, AFP-L3, and DCP are used widely and routinely as a tool for screening HCC in Japan, and these tests are covered by Japan's national health insurance as the serum biomarkers to screen for HCC in clinical settings.

Worldwide, a number of researches have carried out at DCP. In 1984, Liebman *et al*.(*51*) first reported DCP in the plasma of 90% of patients with HCC. Since then, substantial evidence has been assembled through numerous clinical trials, and studies have demonstrated the clinical usefulness of serum DCP levels to screen for and diagnose patients with HCC (*48,52,53*). Multiple reports have found that the combined testing of DCP and AFP have a sensitivity of 47.5-94.0% and specificity of 53.3-98.5% in HCC early detection (Table 2) (*45,46,48,53-67*).

Meanwhile, many researchers recommend that DCP could also be used in assessing the progression of HCC, including serving as an HCC recurrence indicator after curative therapy, a good predictor of the vascular invasion presence and could be used to select liver transplants' recipients, and could facilitate the research of new chemotherapeutic strategies for treating HCC (*68-74*). However, currently, DCP is approved in Japan, Korea and Indonesia (*75*), yet has not been approved in China until now.

3.2. *Evidence for exploration of using DCP in early HCC detection in China*

Evidence has shown that the test that combine DCP and AFP could achieve a better sensitivity and specificity in HCC early detection, and the testing of DCP has been widely used for many HCV-related HCC cases, such as in Japan. But in China, 85% of HCC cases have HBV infection. So, is DCP applicable as a screening and diagnostic tool in China? What about its sensitivity and specificity in Chinese HCC cases? Furthermore, what is its clinical usefulness in assessing HCC progression? According to evidence-based medicine (EBM), systematic evaluation needs to be performed to assess the screening and diagnostic value of DCP in Chinese patients with HCC.

In China, such a study was conducted in 2002 to

Table 2.The exploration of clinical usefulness of using serum biomarker DCP to complement AFP in HCC early detection*

Marker	Cut-off value	Sensitivity[a]	Specificity[a]	PPV[a]	NPV[a]
DCP + AFP (*54*)	8 mAU/mL, 20 ng/mL	90.0% (90/100)	*N*	*N*	*N*
DCP + AFP (*55*)	16 mAU/mL, 20 ng/mL	87.3% (55/63)	84.0% (158/188)	64.7% (55/85)	95.2% (158/166)
DCP + AFP (*56*)	40 mAU/mL, 20 ng/mL	83.5% (76/91)	*N*	*N*	*N*
DCP + AFP (*57*)	40 mAU/mL, 20 ng/mL	86.7% (52/60)	*N*	*N*	*N*
DCP + AFP (*58*)	40 mAU/mL, 20 ng/mL	78.3% (94/120)	58.9% (53/90)	71.8% (94/131)	67.1% (53/79)
DCP + AFP (*62*)	40 mAU/mL, 20 ng/mL	83.6% (51/61)	68.2% (45/66)	70.8% (51/72)	81.8% (45/55)
DCP + AFP (*63*)	40 mAU/mL, 20 ng/mL	83.3% (204/245)	77.2% (206/267)	77.0% (204/265)	83.4% (206/247)
DCP + AFP (*59*)	40 mAU/mL, 200 ng/mL	78.3% (83/106)	*N*	*N*	*N*
DCP + AFP (*45*)	80 mAU/mL, 40 ng/mL	65.5% (19/29)	84.5% (596/705)	14.8% (19/128)	98.3% (596/606)
DCP + AFP (*64*)	90 mAU/mL, 45 ng/mL	84.4% (130/154)	*N*	*N*	*N*
DCP + AFP (*60*)	100 mAU/mL, 100 ng/mL	72.4% (55/76)	*N*	*N*	*N*
DCP + AFP (*60*)	100 mAU/mL, 300 ng/mL	63.2% (48/76)	*N*	*N*	*N*
DCP + AFP (*48*)	150 mAU/mL, 20 ng/mL	86% (-/-)[b]	63% (-/-)[b]	*N*	*N*
DCP + AFP (*48*)	619 mAU/mL, 27 ng/mL	74% (-/-)[b]	87% (-/-)[b]	*N*	*N*
DCP + AFP (*64*)	0.8 ng/mL, 45 ng/mL	88.3% (136/154)	*N*	*N*	*N*
DCP + AFP (*53*)	20.24 ng/mL, 15 ng/mL	94.0% (47/50)	80.5% (33/41)	85.5% (47/55)	91.7% (33/36)
DCP + AFP (*65*)	0.1 μg/mL, 20 ng/mL	92.9% (65/70)	53.3% (24/45)	75.6% (65/86)	82.8% (24/29)
DCP + AFP (*65*)	0.1 mg/mL, 400 ng/mL	85.7% (60/70)	82.2% (37/45)	88.2% (60/68)	78.7% (37/47)
DCP + AFP (*66*)	40 mAU/ml, 20 ng/ml	78.3% (47/60)	56.7% (17/30)	*N*	*N*
DCP + AFP (*54*)	8 mAU/mL, 20 ng/mL	66.7% (18/27)	*N*	*N*	*N*
DCP + AFP (*55*)	16 mAU/mL, 20 ng/mL	82.9% (29/35)	84.0% (158/188)	49.2% (29/59)	96.3% (158/164)
DCP + AFP (*58*)	40 mAU/mL, 20 ng/mL	59.4% (-/-) b	58.9% (53/90)	*N*	*N*
DCP + AFP (*48*)	150 mAU/mL, 20 ng/mL	78% (-/-)[b]	62% (-/-)[b]	*N*	*N*
DCP + AFP (*48*)	598 mAU/mL, 11 ng/mL	70% (-/-)[b]	80% (-/-)[b]	*N*	*N*
DCP + AFP (*55*)	16 mAU/mL, 20 ng/mL	61.5% (8/13)	84.0% (158/188)	21.1% (8/38)	96.9% (158/163)
DCP + AFP (*56*)	40 mAU/mL, 20 ng/mL	83.7% (36/43)	*N*	*N*	*N*
DCP + AFP (*67*)	40 mAU/mL, 200 ng/mL	47.5% (29/61)	98.5% (132/134)	93.5% (29/31)	80.5% (132/164)
DCP + AFP-L3 (*61*)	40 mAU/mL, 10%	41.7% (15/36)	89.8% (44/49)	75.0% (15/20)	67.7% (44/65)
DCP + AFP-L3 (*46*)	40 mAU/mL, 10%	66.7% (14/21)	89.5% (51/57)	70.0% (14/20)	87.9% (51/58)
DCP + AFP-L3 (*67*)	40 mAU/mL, 10%	54.1% (33/61)	97.8% (131/134)	91.7% (33/36)	82.4% (131/159)

* In all studies indicated, patients with chronic hepatitis and/or liver cirrhosis were designated as the comparative non-HCC patient group. [a] Sensitivity = True positive (TP) / (TP + Falsenegative (FN)), Specificity = True negative (TN) / (TN + False positive (FP)), Positive predictive value (PPV) = TP / TP + FP, Negative predictive value (NPV) = TN / TN + FN. [b] The patient distribution was not noted. *N*, Not noted or not investigated.

determine DCP and AFP levels in 60 patients with HCC and 30 patients with cirrhosis but no HCC (*66*), results showed that the combined testing of DCP and AFP could achieve a sensitivity of 78.3%, which is higher than that of DCP alone (51.7%) and AFP alone (56.7%). Another study to assess the clinical usefulness of DCP involving 120 Chinese patients with HCC and 90 patients with cirrhosis was reported in 2003 (*58*), and results also showed that the combined tests of DCP and AFP had a sensitivity of 78.3%, which is higher than that of DCP (53.3%) and AFP alone (58.3%).

Moreover, a large-scale, multi-center study of DCP's usefulness in early HCC detection was also launched in Chongqing, Beijing, and Tianjin of China in 2012 (*11*). As part of the study, the test was conducted in one of the centers - the Southwest Hospital, Third Military Medical University in Chongqing - involving 336 patients with HCC (80% have HBV infection) and 252 patients with liver diseases other than HCC. Results showed that there is no significant correlation between serum levels of DCP and AFP (R^2 = 0.0179); DCP had a total sensitivity of 74% while a combination of DCP and AFP had a sensitivity of 84%, which is higher than either DCP or AFP alone (*7,11*). Besides, DCP resulted in a specificity of 56% with a cut-off value of 40 mAU/mL and 94% with a cut-off value of 100 mAU/mL (*32*).

These studies found that the combined tests of DCP and AFP could improve sensitivity for detecting Chinese HCC cases, thus suggesting that DCP is a useful serum biomarker in Chinese patients for HCC screening and early diagnosis. Such evidence provides a better perspective for using DCP in HCC early detection in China. However, more large-scale, multi-center studies are expected to be performed in China to provide further evidence of the clinical usefulness of serum biomarker DCP in early HCC detection, especially with long-term surveillance and follow-up to provide strong data-support and verification.

3.3. *Research advances in other serum biomarkers for early HCC detection in China*

In recent years, there are also many studies on the clinical usefulness of other serum biomarkers in early HCC detection, including Golgi protein-73 (GP73), glypican-3 (GPC3), gamma-glutamyltransferase (GGTII), and so on. Most recently, research on

Dickkopf-1 (DKK1) and Midkine (MDK) as diagnostic serum biomarkers has raised concern in China.

Serum DKK1 as a biomarker in HCC diagnosis Published in 2012, a large-scale, multi-centre study assessed serum DKK1 for HCC diagnosis in 1,284 participants (831 in the test cohort and 453 in the validation cohort) in China (*76,77*). Results showed that serum's levels of DKK1 were significantly higher in HCC patients than in all controls; serum DKK1 had greater AUC, sensitivity, and specificity values than did AFP in patients with HCC compared with chronic HBV infection and cirrhosis controls; DKK1 maintained high diagnostic accuracy for AFP negative HCC patients, including early-stage HCC patients; raised concentrations of DKK1 in serum could distinguish HCC from chronic HBV infection and cirrhosis; measurement of DKK1 and AFP together improved diagnostic accuracy against HCC versus all controls compared with any test alone.

Serum MDK as a biomarker in HCC diagnosis Published in 2013, a study that involved three independent cohorts with a total of 933 participants including 388 HCC cases and 545 different controls enrolled from different medical centers (*78*). Results showed that MDK levels were significantly elevated in HCC tissues as well as serum samples; serum MDK for HCC diagnosis showed an obviously higher sensitivity compared with AFP (86.9% *vs*.51.9%) with similar specificities (83.9% *vs*.86.3%); even in very early-stage HCC, the sensitivity of MDK is significant higher than AFP (80% *vs*. 40%); in those AFP-negative HCC cases, the sensitivity could reach as high as 89.2%; and serum MDK level was significantly decreased in HCC patients after curative resection and re-elevated when tumor relapsed.

Both of the two studies suggested that the novel serum biomarkers of DKK1 and MDK can complete the measurement of AFP in the diagnosis process of HCC, particularly for those negative AFP patients and/or at an early stage. However, these studies were small-scale. According to the guidelines on phases of evaluating an early detection biomarker for cancer developed by the National Cancer Institute's Early Detection Research Network (*79*), further validation using larger cohort of serum HCC samples with hepatitis B and hepatitis C infectious liver disease, nonalcoholic fatty liver disease (NAFLD), and alcohol-induced liver disease (ALD) from multiple centers in a prospective, randomized controlled trial is needed to provide further evidence in China.

In conclusion, research and exploration for using multi serum biomarkers in early HCC detection has raised concern in China. Using novel and reliable serum biomarkers to complement AFP as a new trend is expected to be extensively used in China to facilitate screening for and diagnosing HCC at an earlier stage and improve clinical outcomes.

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