

Review

Biomarkers: Evaluation of Screening for and Early Diagnosis of Hepatocellular Carcinoma in Japan and China

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Key Words

Tumor marker · Des- γ -carboxyprothrombin · α -Fetoprotein · *Lens culinaris* agglutinin-reactive fraction of α -fetoprotein

Abstract

Over the past few decades, the screening for and early diagnosis of hepatocellular carcinoma (HCC) has attracted attention worldwide, and especially in Asian countries such as Japan and China. Such approaches can help detecting HCC at an earlier stage when curable interventions can be offered to achieve long-term disease-free survival for patients. Biomarkers have been used to screen for and diagnose HCC in various countries. In Japan, the combined tests of des- γ -carboxyprothrombin (DCP) and α -fetoprotein (AFP) or *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) have been shown to achieve a high level of sensitivity and specificity. These tests have routinely been used to screen for HCC and are covered by Japan's national health insurance. Due to the routine practice of screening for HCC among high-risk patients, HCC nodules have been detected in the early stages in more than 60% of patients in Japan. In contrast, although several remarkable advances in the management of HCC have been made in China over the past few decades, most HCC patients still present with advanced-stage disease. AFP is the only serum biomarker that has widely been used to screen for and diagnose HCC in China. In recent years, several molecular biological studies have further investigated the clinical usefulness of DCP, and they have found that it may facilitate the screening for and diagnosis of HCC and assist with the assessment of HCC progression. DCP can serve as a biomarker to detect HCC in an early stage and facilitate definitive treatment. The wide implementation of DCP is expected, especially in China where 55% of HCC cases worldwide live.

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Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer and the third leading cause of cancer-related deaths worldwide. Asian countries account for 75–80% of the roughly 650,000 HCC cases reported globally each year. Of particular note is the fact that China alone accounts for 55% of HCC cases worldwide [1, 2]. In Japan, HCC ranks as the third leading cause of cancer-related deaths in males and the fifth leading cause of cancer-related deaths in females. More than 30,000 patients die of HCC every year [3, 4]. Over the past 10 years, HCC management in Japan has achieved remarkable results because of the wide acceptance and implementation of the ‘Japanese Evidence-Based Clinical Practice Guideline for HCC’ (J-HCC Guideline) [5] and the ‘Expert Consensus on HCC by the Japan Society of Hepatology’ (JSH Guideline) [6], with a combination of quantitative and qualitative evaluation incorporated in the guidelines [7]. Specifically, HCC nodules have been detected in the early stage in more than 60% of patients due to the routine practice of screening for HCC among high-risk patients in Japan [8]. In China, HCC ranks as the second leading cause of cancer-related deaths in males and the third leading cause of cancer-related deaths in females. The incidence of HCC is increasing in China, and HCC is the second most common cancer in urban areas and first most common in rural areas [9]. Several remarkable advances have been made in HCC management in China over the past few decades, such as ‘The Expert Consensus on the Treatment Standards for Hepatology Carcinoma’ (Chinese Guideline) published in 2009 [10]. However, most HCC patients in China still present with advanced-stage disease [11].

Currently, surgical resection and liver transplantation offer the best potential for treating HCC [12–14] but are only available to patients whose tumors are detected early. Such patients currently account for only 10–20% of cases [15]. Thus, strategies have been adopted to screen for and diagnose HCC at an earlier stage when curable interventions can be offered to achieve long-term disease-free survival for patients [16].

The Current Status of Screening for and Early Diagnosis of HCC in Japan and China

Strategies to Screen for and Diagnose HCC

There are many differences in HCC screening and early diagnosis between Japan and China (table 1). In Japan, more than 70% of patients with HCC are infected with hepatitis C virus (HCV), and approximately 15–20% of patients are infected with hepatitis B virus (HBV) [17]; these figures are similar to those reported from the United States and Europe [18, 19]. In contrast, approximately 85% of Chinese HCC cases are HBV related, 10% of cases are HCV related, and some cases involve HBV and HCV superinfection [9, 20]. Several cohort studies have shown that screening high-risk patients with HBV- or HCV-related chronic liver diseases improves the rate of early HCC detection and the rate of curative treatment [21–24]. Many guidelines for HCC treatment recommend HCC screening, including the guidelines established by the American Association for the Study of Liver Disease (AASLD) [25], the National Comprehensive Cancer Network (NCCN) [26], and the Asian Pacific Association for the Study of the Liver (APASL) [27].

Screening tools should have an acceptable rate of accuracy and should be affordable. In general, the tests used to screen for HCC in different countries depend on imaging technology and biomarkers [28, 29]. Ultrasound (US) is the imaging technique most often used to screen for HCC because it is simple, inexpensive, and noninvasive and allows real-time observation. However, the success of US depends on the expertise of the physician, the US equipment available, and the echo texture of the liver; thus, the actual sensitivity of US is difficult to assess due to the lack of a definitive standard for HCC [27, 30]. Currently, the

Table 1. The current status of HCC screening and early diagnosis in Japan and China

Variables	Japan	China	References
Leading cause of cancer-related deaths	Third in males, fifth in females	Second in males, third in females	[3, 4, 9]
Etiological factors	70% of patients with HCV infection, 15–20% of patients with HBV infection	85% of patients with HBV infection, 10% of patients with HCV infection	[9, 15, 18]
Government-funded nationwide screening program	Established in 2002 for patients over 40 years with HCV/HBV infection	No	[11, 25, 46, 47]
High-risk group	High-risk group: patients with HBV/HCV infection or cirrhosis due to other causes; Very-high-risk group: patients with HBV-/HCV-related cirrhosis	Patients infected with HBV, HCV, or HBV and HCV superinfection and patients with cirrhosis, alcohol abuse, diabetes mellitus, or a family history of HCC; Patients aged 35–40 years	[5, 10, 48, 49]
Screening tool	US and combined test of DCP and AFP or AFP-L3	US and AFP	[6, 8, 10, 49, 50]
Screening criteria	3–4 months for very-high-risk group; 6-month intervals for high-risk group	6-month intervals for high-risk group	[5, 6, 8, 10, 48, 50]
Diagnosis	Dynamic CT/MRI for definitive diagnosis; DCP/AFP/AFP-L3 for adjunctive diagnosis	US/CT/MRI, AFP, and biopsy for differential diagnosis	[5, 10, 49, 50]
Early detection (reference)	>60% of HCC detected by national screening in the early stages	Most patients with HCC present with advanced-stage disease	[8, 11]

combination of US and α -fetoprotein (AFP) measurement is widely accepted and implemented as a screening strategy. This approach offers an increased possibility to detect small HCC compared to US or AFP measurement alone [31–33].

AFP has a sensitivity and specificity for detecting HCC in the range of 41–65% and 80–90%, respectively, when an AFP cut-off of 20 ng/ml is used [34]. However, up to 50% of patients with HCC have an AFP level below 20 ng/ml [35], thus AFP cannot be used as the sole tool to screen for HCC. There are two other biomarkers besides AFP to screen for HCC, namely des- γ -carboxyprothrombin (DCP, also known as prothrombin induced by vitamin K absence-II, PIVKA-II) and *Lens culinaris* agglutinin-reactive fraction of AFP (AFP-L3) [36, 37]. Many studies have compared the clinical usefulness of these three biomarkers, but the final conclusions are still being discussed. However, multiple reports have shown that a combination of DCP and AFP or AFP-L3 is more effective in detecting HCC at present [38–41].

When a nodule is detected in screening, a differential diagnosis is made. The choice of diagnostic tools depends on the diameter of the nodule, i.e. <1, 1–2, or >2 cm. This criterion is recommended by many countries' guidelines for HCC [7]. In general, the tests used to diagnose HCC in different countries include diagnostic imaging, serological diagnosis, and histological diagnosis. Diagnostic imaging techniques include US, computed tomography (CT), and magnetic resonance imaging (MRI). According to a systematic review, US has a sensitivity of 60% and a specificity of 97%, CT has a sensitivity of 68% and a specificity of 93%, and MRI has a sensitivity of 81% and a specificity of 85% [42]. Techniques for serological diagnosis such as AFP measurement have widely varying sensitivity and specificity, and the guidelines for HCC in some countries such as the UK and South Korea recommend that these techniques should be used as an adjunct diagnostic tool [43, 44]. Histological diagnosis can be

done by a biopsy (also known as fine needle aspiration cytology, FNAC), which has an overall sensitivity of 95.2% and a specificity of 100% [45, 46]; however, biopsies should be avoided if curative surgery is planned because there is a slight chance (2.7%) of needle-track tumor seeding following a biopsy. This holds true unless a biopsy might affect patient management or imaging techniques and AFP fails to resolve doubts about the diagnosis [47, 48].

The Current Status of Screening for and Diagnosis of HCC in Japan

In 2002, Japan's Ministry of Health, Labor, and Welfare started a national 5-year program to screen for HCV and HBV infection among people over 40 years given the high prevalence of HCV infection in this age group [49, 50]. By the end of 2006, 9 million people had been screened. Of these, 112,000 were found to have HCV infection and 110,000 were found to have HBV infection [27]. Since most high-risk patients were closely followed before developing HCC, HCC nodules were detected in the early stage in more than 60% of patients in Japan [8].

Currently, AFP, AFP-L3, and DCP are widely and routinely used to screen for HCC in Japan, and these tests are covered by Japan's national health insurance as serological biomarkers to screen for HCC in clinical settings [29]. According to the J-HCC Guideline, the first evidence-based clinical practice guidelines for HCC in Japan, AFP, AFP-L3, and DCP should be measured at intervals of 3–4 months in the very-high-risk group (patients with HBV- or HCV-related liver cirrhosis) and at 6-month intervals in the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes) [5, 51, 52]. The J-HCC Guideline and JSH Guideline strongly recommend the periodic and simultaneous measurement of DCP and AFP or AFP-L3 levels to screen for patients with HCC and to detect small HCC with a high level of sensitivity and specificity [6, 8, 52, 53]. A total of 200 Japanese experts were surveyed in 2009 to determine the nature of HCC screening in Japan. The survey found that 72% of these experts simultaneously measured the tumor markers of AFP, AFP-L3, and DCP, and 44% of the experts combined this measurement with US [54].

Many Western guidelines for diagnosing HCC recommend a definitive diagnosis reached by using different diagnostic tools to determine the nodular diameter [7]. In contrast, the J-HCC Guideline and JSH Guideline in Japan recommend that HCC should be diagnosed by characteristic features (hypervascularity in the arterial phase and washing out in the portal venous phase) on dynamic CT or dynamic MRI, regardless of the tumor size [52, 53]. This approach has a sensitivity of 68–91% [55, 56] and a specificity of 77–100% [57, 58].

If US suggests a new nodular lesion in screening in Japan, dynamic CT or dynamic MRI will be performed to make a differential diagnosis; if the AFP level rises continuously or has increased to 200 ng/ml or more, the DCP level is at least 40 mAU/ml, or the AFP-L3 fraction is 15% or more, dynamic CT/MRI will be considered, even if US shows no evidence of a tumor [5, 52].

The Current Status of Screening for and Early Diagnosis of HCC in China

Like Japan, Taiwan has established a screening program that screens patients with cirrhosis every 3–6 months and patients with no cirrhosis every 6–12 months; there is no age limit for screening of HBV carriers in Taiwan. However, there is no government-funded screening program for HCC in Hong Kong or other parts of China [11].

In China, the high-risk group for HCC includes patients chronically infected with HBV, HCV, or HBV and HCV superinfection and patients with cirrhosis, alcohol abuse, diabetes mellitus, or a family history of HCC. For patients aged 35–40 years, AFP and US should be performed every 6 months according to the Chinese Guideline [10]. If the AFP level rises continuously or US suggests a new nodular lesion, a differential diagnosis will be made based on diagnostic imaging, serological diagnosis, or histological diagnosis.

At present, AFP measurement and US at 6-month intervals are the standard tools to screen for HCC in China. AFP is considered to be a useful and feasible tool for screening and early diagnosis in China due to its convenience and especially due to the fact that more than 60% of patients with HCC have an AFP level of >400 ng/ml [10]. The clinical usefulness of AFP in China has been confirmed by a randomized controlled trial involving 18,816 patients aged 35–59 years with HBV infection or a history of chronic hepatitis. The patients were randomly assigned to a screening (9,373) or control (9,443) group undergoing AFP measurement and US every 6 months. The results showed that biannual screening with AFP and US significantly reduced mortality. Screened patients had a survival rate of 65.9% at 1 year, 52.6% at 3 years, and 46.4% at 5 years versus 31.2% at 1 year, 7.2% at 3 years, and 0% at 5 years for unscreened patients [59].

Many studies have strongly recommended the combined testing of DCP and AFP or AFP-L3 to screen for patients with HCC and to detect small HCC with a high level of sensitivity and specificity, but DCP testing is currently approved only in Japan, South Korea, and Indonesia [60]. In order to assess the screening and diagnostic value of DCP in Chinese patients with HCC, a study was conducted in China in 2002 to determine DCP and AFP levels in 60 patients with HCC and 30 patients with cirrhosis but no HCC [61]. This study found no significant correlation between serum levels of DCP and AFP in the 60 patients with HCC ($r_s = 1.101$, $p = 0.247$). DCP had a sensitivity of about 51.7% and a specificity of about 86.7%, while the combined tests of DCP and AFP had 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 in Chinese patients with HCC was reported in 2003 [62]. This study involved 120 patients with HCC and 90 patients with cirrhosis. No significant correlation between serum levels of DCP and AFP in the 120 patients with HCC was found ($r_s = 1.106$, $p = 0.249$). DCP had a sensitivity of 53.3% and a specificity of 85.6%, while 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%).

Two studies found DCP to be a useful biomarker to screen for and diagnose HCC, and these studies found that sensitivity may improve when DCP is combined with AFP in Chinese patients. Such work provides a better perspective on the use of DCP to detect HCC in the early stage. However, these studies were small in scale and involved few Chinese patients. Large-scale and multicenter studies of Chinese patients are needed to provide more data and corroborate earlier findings. The good news is that a program was launched by the Japan-China Joint Team for Medical Research and Cooperation on HCC in 2012 to assess the clinical usefulness of DCP in Chinese patients through a large-scale, multicenter study. This study found no significant correlation between serum levels of DCP and AFP. DCP may have a specificity as high as 90%, and the combined tests of DCP and AFP have a significantly higher sensitivity compared to AFP alone in Chinese patients.

Prospects of Biomarkers for HCC in Japan and China

As noted earlier, biomarkers have been widely used to screen for and diagnose HCC in both Japan and China. The difference is that the combined tests of DCP and AFP or AFP-L3 have been routinely used to screen for HCC in Japan and are covered by Japan's national health insurance. In contrast, some studies have been conducted to assess the clinical usefulness of DCP in Chinese patients, but AFP is the only serum biomarker that has been widely used to screen for and diagnose HCC in China until now.

Although AFP is the most widely used biomarker of HCC, total AFP is not always specific for HCC [42, 63], and elevated levels of AFP are also found in patients with liver diseases other than HCC, including viral hepatitis, at a rate of 10–42% [64–66]. In contrast, AFP-L3

and DCP are very specific for HCC, compared to AFP alone [67, 68]. In 1984, Liebman et al. [69] 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 in screening for patients with HCC [70–73]. Some studies have found that the combined test of DCP and AFP has a sensitivity of 84% and a specificity of 83%. The combined test of DCP and AFP-L3 has a sensitivity of 89.5–89.8% and a specificity of 41.7–66.7% when detecting small HCC with a tumor diameter of ≤ 3 cm [41, 74–76].

Furthermore, Tang's research group [77–85] has recently investigated the potential clinical usefulness of DCP in assessing HCC progression. They found that: (i) positivity for serum DCP was significantly related to the presence of vascular invasion, intrahepatic metastasis, tumor size, and TNM stage as well as a high frequency of tumor recurrence, indicating that DCP could serve as an indicator of HCC recurrence after curative therapy; (ii) a high level of DCP is a good predictor of the presence of vascular invasion and could be used to select recipients of liver transplants, and (iii) the use of an inhibitor of DCP in multidrug chemotherapy may induce antiproliferative and antiangiogenic action, indicating that DCP may facilitate the development of new chemotherapeutic strategies for treating HCC.

Conclusion

DCP is considered to be a useful serum biomarker to screen for and diagnose HCC. Many studies have strongly recommended the combined testing of DCP and AFP or AFP-L3, and these tests have been routinely used to screen individuals for HCC in Japan and to sensitively and specifically detect HCC in the early stages. Until now, AFP is the only serum biomarker that has been widely used to screen for and diagnose HCC in China. Currently, more large-scale and multicenter studies are desperately needed to assess the clinical usefulness of DCP in Chinese patients. The potential clinical usefulness of DCP needs to be investigated in terms of screening for and early diagnosis of HCC and also in terms of its treatment. DCP can help to detect HCC in the early stages and facilitate definitive treatment. The wide implementation of DCP is expected, especially for China that accounts for 55% of HCC cases worldwide.

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Conflict of Interest

No conflicts of interest to disclose.

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