# Biomarkers for Hepatocellular Carcinoma in East Asia

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Peipei Song, Wei Tang, and Norihiro Kokudo

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**Abstract**

Hepatocellular carcinoma (HCC) is undoubtedly a great health threat in East Asia, with the highest incidence of an age-standardized rate of 31.9 per 100,000 in men. In terms of the absolute number of cases, almost half a million cases were reported in China, Japan, and South Korea in 2012. Worldwide, alpha-fetoprotein (AFP) testing and abdominal ultrasound (US) every 6 months are recommended

P. Song (✉) • W. Tang (✉) • N. Kokudo (✉)
Hepato-Biliary-Pancreatic Surgery Division, Department of Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
e-mail: ppsong-tky@umin.ac.jp; TANG-SUR@h.u-tokyo.ac.jp; KOKUDO-2SU@h.u-tokyo.ac.jp



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for routine surveillance of HCC in high-risk patients according to many HCC guidelines, and AFP has also been used as a diagnostic test for HCC and to evaluate prognosis and monitor recurrence following treatment. However, controversy regarding the clinical utility of AFP has arisen in the West and East in recent years. This controversy is also evident in HCC guidelines in countries in East Asia. Advances in technology and greater understanding of the pathology of HCC have led to the discovery of novel biomarkers. Data have indicated that the combined testing of AFP, the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), or des-γ-carboxyprothrombin (DCP) could help to increase the sensitivity of diagnosis of HCC, but this approach is currently used in only a few countries, such as Japan. In recent years, numerous studies have investigated the clinical usefulness of some novel biomarkers in early diagnosis of HCC, including Dickkopf-1 (DKK1), midkine (MDK), and microRNA (miRNA). Moreover, the prognostic significance of some biomarkers, such as miRNA, gamma-glutamyl transferase (GGT), and indocyanine green retention 15 min after administration (ICG-R15), has also been evaluated. However, further studies are needed to better characterize the accuracy and potential role of these approaches in clinical practice. The prevailing hope is that novel biomarkers can support clinicians in their daily practice and improve care for patients with HCC.

**Keywords**

HCC • Biomarker • Guideline • Surveillance • Diagnosis • Prognosis

**List of Abbreviations**

| | |
|---|---|
| AASLD | American Association for the Study of Liver Disease |
| AFP | Alpha-fetoprotein |
| AFP-L3 | The lens culinaris agglutinin-reactive fraction of AFP |
| ALD | Alcohol-induced liver disease |
| APASL | Asian Pacific Association for the Study of the Liver |
| CI | Confidence interval |
| CT | Computed tomography |
| DCP | Des-γ-carboxyprothrombin |
| DKK1 | Dickkopf-1 |
| EASL | European Association for the Study of the Liver |
| EBM | Evidence-based medicine |
| Gd-EOB-DTPA | Gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid |
| GGT | Gamma-glutamyl transferase |
| GP73 | Golgi protein 73 |
| GPC3 | Glypican-3 |
| HBV | Hepatitis B virus |
| HCC | Hepatocellular carcinoma |
| HCV | Hepatitis C virus |
| HR | Hazard ratio |
| ICG-R15 | Indocyanine green retention 15 min after administration |
| IL-6 | Interleukin-6 |

| | |
|---|---|
| INASL | Indian National Association for Study of the Liver |
| KLCSG | Korean Liver Cancer Study Group |
| LR+ | Positive likelihood ratio |
| MDK | Midkine |
| miRNA | MicroRNA |
| MRI | Magnetic resonance imaging |
| NAFLD | Nonalcoholic fatty liver disease |
| NCC | National Cancer Center |
| NCCN | National Comprehensive Cancer Network |
| NHFPC | National Health and Family Planning Commission |
| OR | Odds ratio |
| SCCA | Squamous cell carcinoma antigen |
| TACE | Transarterial chemoembolization |
| TARE | Transarterial radioembolization |
| US | Ultrasound |
| VEGF | Vascular endothelial growth factor |
| WHO | World Health Organization |

## Key Facts on Multiple Biomarkers for HCC Surveillance and Diagnosis

- Data have indicated that the combined testing of DCP and AFP or AFP-L3 could help to increase the sensitivity of diagnosis of HCC. The combined testing of two biomarkers had an OR of 6.29–59.81 in diagnosing HCC smaller than 5 cm in diameter, which was better than that of one biomarker alone.
- However, the combined testing of DCP and AFP or AFP-L3 is used in only a few countries, such as Japan. Although the clinical usefulness of combined testing of DCP and AFP or AFP-L3 has also been noted by several retrospective studies published in South Korea and China, it has not been recommended by HCC guidelines in South Korea and China until now.
- Data showed that the measurement of DKK1 and AFP together improved the accuracy with which HCC was diagnosed in comparison to any single test alone.
- Data showed that serum MDK had a markedly higher level of sensitivity than AFP (86.9% vs. 51.9%) but a similar level of specificity (83.9% vs. 86.3%). MDK has a significantly higher level of sensitivity than AFP (80% vs. 40%) at diagnosing very early stage HCC.
- Data showed that the combination of miRNA-21 with AFP improved the power of differentiation between HCC and chronic hepatitis, with a sensitivity of 81.0% and a specificity of 80%.

The above is a list of key facts regarding the current status of multiple biomarkers for HCC surveillance and diagnosis, including the combined testing of DCP and AFP or AFP-L3, the combined testing of DKK1 and AFP, the combined testing of MDK and AFP, and the combined testing of miRNA-21 with AFP.

## Key Facts on Alpha-Fetoprotein (AFP)

- Serum AFP has traditionally and widely been used as a tumor marker of HCC over the past two decades.
- Elevated serum AFP and a typical enhancement pattern in dynamic imaging have provided critical clues for the diagnosis of HCC.
- However, based on the high accuracy of up-to-date radiologic modalities, the importance in AFP has diminished in recent guidelines for diagnosis of HCC.
- AFP has been excluded from the surveillance criteria in the HCC guidelines published by the AASLD in 2010, and AFP is regarded as a suboptimal tool for surveillance according to the HCC guidelines published by the EASL in 2012. It is, however, still recommended by many HCC guidelines in Asia, such as guidelines in Japan, and China.
- AFP was one of the most robust predictors of death in patients with cirrhosis and HCC, and it also has significance at predicting survival after liver transplantation.
- A change in AFP levels has been found to correlate with radiologic response and overall survival after locoregional therapy, such as transarterial chemoembolization (TACE), transarterial radioembolization (TARE).

The above is a list of key factors regarding the current status of using AFP for HCC surveillance, diagnosis, and prognosis as well as controversies over that use in the West and East.

## Definitions of Words and Terms

| | |
|---|---|
| Alpha-fetoprotein (AFP) | A host cellular protein. High levels can occur in persons with HCC. |
| Chronic HBV infection | Persistence of hepatitis B surface antigen (HBsAg) for 6 months or more after acute infection with HBV. |
| Chronic HCV infection | Continued presence of HCV 6 months or more after acquiring infection. |
| Cirrhosis | An advanced stage of liver disease characterized by extensive hepatic fibrosis, nodularity of the liver, alteration of liver architecture, and disrupted hepatic circulation. |
| Des-gamma carboxyprothrombin (DCP) | Also known as protein induced by vitamin K absence/antagonist-II (PIVKA-II), it is an abnormal prothrombin that lacks carboxylation of specific amino-terminal glutamic acid residues. |
| Gamma-glutamyl transferase (GGT) | An enzyme catalyzing hydrolysis of glutathione and transfer of gamma-glutamyl residue; a high prevalence of abnormal GGT in patients with primary or |

| | |
|---|---|
| | secondary liver cancer was showed by many clinical studies. |
| Guideline | The standardized management of care that specifies appropriate diagnoses and treatments based on scientific research evidence and collaborations between medical professionals involved in the treatment of a given condition. |
| Hepatocellular carcinoma (HCC) | Primary cancer of the liver arising in hepatocytes. |
| Sensitivity of a test | The ability of a test to correctly identify those with the infection or disease (i.e., true positives/true positives + false negatives). |
| Specificity of a test | The ability of a test to correctly identify those without the infection or disease (i.e., true negatives/true negatives + false positives). |

## Introduction

According to data from the World Health Organization (WHO), there were 14.1 million new cancer cases worldwide in 2012, 8.2 million deaths due to cancer, and 32.6 million people living with cancer (WHO 2012). Liver cancer is the fifth most common cancer in men (554,000 cases, 7.5% of the total) and the ninth in women (228,000 cases, 3.4% of the total) (WHO 2012). Hepatocellular carcinoma (HCC) accounts for more than 90% of primary liver cancers and is a major global health problem due to the high prevalence of infection with the hepatitis B virus (HBV) and/or hepatitis C virus (HCV) as risk factors. Over the past two decades, the clinical care for patients with HBV- or HCV-related liver disease has advanced considerably due to developments in diagnostic procedures and improvements in therapy and prevention (Zhang et al. 2013; Shaheen and Idrees 2015). However, the incidence of HCC worldwide is increasing, and this is likely to be associated with the often prolonged period between viral infection and the manifestation of HCC. Moreover, evidence has shown that surgical resection and liver transplantation may offer the best potential for treating HCC but are only available to patients whose tumors are detected early. The overall 5-year survival rate is 40%, but liver resection to treat early HCC could lead to a 5-year survival rate of 60–70% (Gao et al. 2012; Llovet and Bruix 2008). Thus, strategies to surveil and diagnose HCC at an earlier stage are urgently needed when curable interventions can be offered to achieve long-term disease-free survival for patients with HCC.

Serum biomarkers are striking potential tools for surveillance and early diagnosis of HCC thanks to the noninvasive, objective, and reproducible assessments they potentially enable. Worldwide, alpha-fetoprotein (AFP) testing and abdominal ultrasound (US) every 6 months are recommended for routine surveillance of HCC in

high-risk patients according to many HCC guidelines (Song et al. 2012), and AFP has also been used as a diagnostic test for HCC and to evaluate prognosis and monitor recurrence following treatment (Rich and Singal 2014). However, controversy regarding the clinical utility of AFP has arisen in the West and East in recent years. The same controversy is also evident in HCC guidelines in countries in East Asia. Other biomarkers including the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), des-γ-carboxyprothrombin (DCP), Dickkopf-1 (DKK1), midkine (MDK), microRNA (miRNA), gamma-glutamyl transferase (GGT), indocyanine green retention 15 min after administration (ICG-R15), Golgi protein 73 (GP73), interleukin-6 (IL-6), squamous cell carcinoma antigen (SCCA), glypican-3 (GPC3), osteopontin, and vascular endothelial growth factor (VEGF) are being studied in this regard. Furthermore, increasing attention has focused on the clinical utility of biomarkers as pretreatment predictors for tumor recurrence and as posttreatment monitors.

## Clinical Practice Guidelines for HCC in East Asia

Asian countries report approximately three-fourths of the cases of HCC worldwide. East Asia is the region with the highest incidence, with an age-standardized rate of 31.9 per 100,000 in men (WHO 2012). In terms of absolute numbers of cases, almost half a million cases were reported in China, Japan, and South Korea in 2012. Of particular note is the fact that there are approximately 395,000 cases of HCC in China, which accounts for 50% of HCC cases worldwide (WHO 2012).

Currently, the overall prevalence of HCC in China is 26–32 per 100,000 persons, and in some areas prevalence can be as high as 70–80 per 100,000 (Song et al. 2013b; Yuen et al. 2009). HCC is now the second most common cancer in urban areas and the first most common in rural areas. HCC ranks as 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 per 100,000 in China (Song et al. 2013a; Song 2013). 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, with more than 30,000 patients dying of HCC every year (Ikai et al. 2007; Chung et al. 2010). In South Korea, statistics published by the central cancer registry in 2013 indicated that there are 12,189 cases involving males and 4,274 cases involving females, making HCC the fourth most common cancer in males and the sixth most common cancer in females (Korean Liver Cancer Study and National Cancer Center 2015a). HCC is the top-ranked cause of death in people in their 40s and 50s, with 22.5 people (male, 33.7; female, 11.3) per 100,000 population dying annually from HCC (Korean Liver Cancer Study and National Cancer Center 2015b).

HCC is undoubtedly a great health threat in East Asia. Coping with HCC is a challenge not only for clinicians but also for health policymakers. With the development of evidence-based medicine (EBM), the concept of incorporating "current best evidence into clinical decision-making" has garnered substantial attention

worldwide. Guided by current best evidence, many clinical practice guidelines for HCC have been published worldwide (Song et al. 2014b). In East Asian countries, the Korean Liver Cancer Study Group (KLCSG) and National Cancer Center (NCC) jointly published clinical practice guideline for HCC (KLCSG-NCC Korea Guideline) in 2003 (Park et al. 2004), revised it in 2009 (Korean Liver Cancer Study and National Cancer Center 2009), and then updated it in 2014 (Korean Liver Cancer Study and National Cancer Center 2015a). With the support of the Japanese Ministry of Health, Labor, and Welfare, the first evidence-based clinical practice guideline for HCC in Japan (J-HCC Guideline) was published in 2005 (Makuuchi and Kokudo 2006), revised in 2009 (2010), and then updated in 2013 (Japan Society of Hepatology 2013). In China, "The Expert Consensus on the Treatment Standards for Hepatocellular Carcinoma (Chinese HCC Consensus)" was published in 2009 based on the consensus opinions of more than 60 experts (Chinese Anti-Cancer Association Society of Liver Cancer 2009), and the National Health and Family Planning Commission (NHFPC) of the People's Republic of China also published the updated "Guideline on Diagnosis and Treatment for Primary Liver Cancer" (NHFPC HCC Guideline) in 2011 (National Health and Family Planning Commission 2011) to promote the standardized management of HCC.

## Biomarkers for HCC Surveillance and Diagnosis in Japan, China, and South Korea

Several cohort studies have documented the cost-effectiveness and survival benefit of surveillance programs to detect HCC early (Yang et al. 2011; Stravitz et al. 2008). Worldwide, many guidelines for HCC management recommend HCC surveillance, including the guidelines established by the American Association for the Study of Liver Disease (AASLD), the National Comprehensive Cancer Network (NCCN), and the Asian Pacific Association for the Study of the Liver (APASL).

### The Current Status of Surveillance and Early Diagnosis of HCC in Japan

Approximately three-fourths of cases of HCC worldwide occurred in Asian countries due to the high prevalence of chronic infection with HBV (Yuen et al. 2009). However, chronic hepatitis C is more commonly related to liver cancer in Japan, which accounts for up to 70% of these cases (Chung et al. 2010).

In Japan, where HCV is the most significant etiological factor for developing HCC, there is a more detailed definition for high-risk patients of HCC (Table 1) – the "very-high-risk group" includes patients with HBV- or HCV-related liver cirrhosis, and the "high-risk group" includes patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes.

**Table 1** The epidemiology, surveillance, and early diagnosis of HCC in Japan

| Items | Current status |
|---|---|
| Epidemiology | The third leading cause of cancer-related deaths in males and the fifth in females |
| Etiological factors | 70% of patients with an HCV infection, 15–20% of patients with an HBV infection |
| Major at-risk population | High-risk group: patients with an HBV/HCV infection or cirrhosis due to other causes; Very-high-risk group: patients with HBV-/HCV-related cirrhosis |
| Guideline | J-HCC Guideline published in 2005, revised in 2009, updated in 2013 |
| Surveillance tools | US and combined test of DCP and AFP or AFP-L3 |
| Surveillance criteria | 3–4 months for the very-high-risk group; 6-month intervals for the high-risk group |
| Diagnosis | Dynamic CT/MRI for definitive diagnosis; DCP/AFP/AFP-L3 for adjunctive diagnosis |

Currently, AFP, AFP-L3, and DCP are widely and routinely used for HCC surveillance in Japan, and these tests are covered by Japan's national health insurance as serological biomarkers for HCC surveillance in clinical settings. According to the criteria for HCC surveillance and diagnosis in the latest version of the J-HCC Guideline published in 2013 (Fig. 1), the combined testing of AFP, AFP-L3, or DCP and US should be performed at intervals of 3–4 months for the very-high-risk group and at intervals of 6 months for the high-risk group.

If US suggests a new nodular lesion in HCC surveillance in Japan, dynamic computed tomography (CT) or dynamic magnetic resonance imaging (MRI) will be performed to make a differential diagnosis; if the AFP level continues to rise 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 (Japan Society of Hepatology 2013).

In 2009, 200 Japanese experts were surveyed regarding the use of biomarkers in HCC surveillance in clinical practice in Japan, and responses indicated that 72% of these experts simultaneously measured the biomarkers of AFP, AFP-L3, and DCP, and 44% of the experts combined these measurements with US (Kudo 2010). 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 (Song et al. 2013c).

## The Current Status of Surveillance and Early Diagnosis of HCC in China

In China, HBV is the biggest factor for developing HCC; approximately 85% of Chinese cases of HCC are HBV-related, 10% of cases are HCV-related, and some cases involve HBV and HCV superinfection (Tanaka et al. 2011). The high-risk

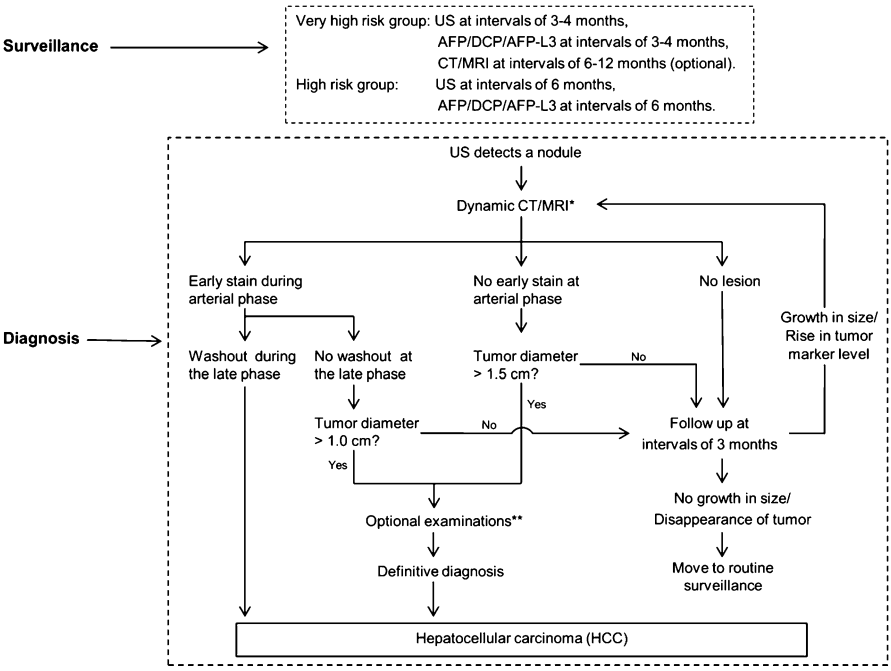


**Fig. 1 Surveillance and diagnostic algorithm for clinical management of HCC according to the latest J-HCC Guidelines published in 2013 in Japan**. *Note: If the AFP level rises continuously or has increased to 200 ng/ml or more, the DCP level is at least 40 mAU/ml, or the AFP-L3 fraction is 15% or more, dynamic CT/MRI will be considered, even if US shows no evidence of a tumor. **CT-angiography/liver-specific contrast-enhanced MRI/contrasonography/liver biopsy

group for HCC in China includes patients chronically infected with HBV, HCV, or HBV and HCV superinfection, and patients with cirrhosis, alcoholism, 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 (Chinese Anti-Cancer Association Society of Liver Cancer 2009, National Health and Family Planning Commission 2011) (Table 2). If the AFP level continues to rise or US suggests a new nodular lesion, a differential diagnosis will be made based on diagnostic imaging, serological diagnosis, or histological diagnosis (Fig. 2).

At present, AFP measurement and US at 6-month intervals are the standard tools for HCC surveillance in China. AFP is considered to be a useful and feasible tool for HCC surveillance and early diagnosis in China due to its convenience and particularly because of the fact that more than 60% of patients with HCC have an AFP level of >400 ng/ml (Song et al. 2012). The clinical usefulness of AFP in China has been confirmed by a randomized controlled trial involving 18,816 patients ages 35–59 years with an 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. Results showed that biannual screening

**Table 2** The epidemiology, surveillance, and early diagnosis of HCC in China

| Items | Current status |
|---|---|
| Epidemiology | The second leading cause of cancer-related deaths in males and the third in females |
| Etiological factors | 85% of patients with an HBV infection, 10% of patients with an HCV infection |
| Major at-risk population | People with an HBV infection; 93 million HBV carriers, 20 million people with a chronic HBV infection |
| Guideline | Chinese HCC Consensus published in 2009; NHFPC HCC Guideline updated in 2011 |
| Surveillance tools | AFP and US |
| Surveillance criteria | 6-month interval for HCC the high-risk population ages 35–40 |
| Diagnosis | US/CT/MRI and biopsy for differential diagnosis; AFP for adjunctive diagnosis |

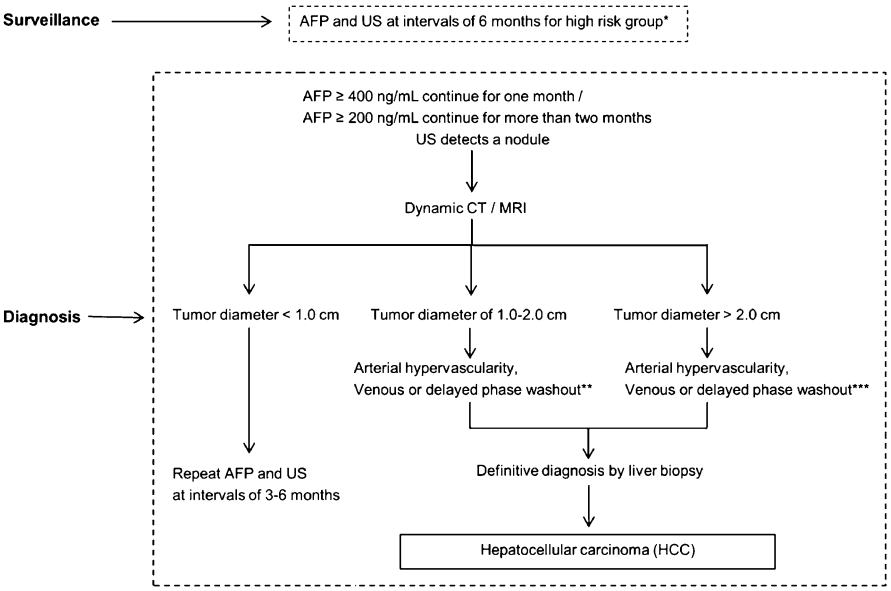


**Fig. 2 Surveillance and diagnostic algorithm for clinical management of HCC according to the latest NHFPC HCC Guideline published in 2011 in China**. NHFPC, the National Health and Family Planning Commission of the People's Republic of China. *Evidences show liver cirrhosis, HBV and/or HCV infection. ** Detected by dynamic CT and MRI. ***Detected by dynamic CT or MRI

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 unscreened patients who had a survival rate of 31.2% at 1 year, 7.2% at 3 years, and 0% at 5 years (Zhang et al. 2004).

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 in order to assess the value of DCP in surveilling and diagnosing Chinese patients with HCC (Cui et al. 2002). 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 (51.7%) or AFP (56.7%) alone. Another study assessed the clinical usefulness of DCP in Chinese patients with HCC in 2003 (Cui et al. 2003). This study involved 120 patients with HCC and 90 patients with cirrhosis. The study found no significant correlation between serum levels of DCP and AFP in the 120 patients with HCC ($r_s = 1.106$, $p = 0.249$). 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%) or AFP (58.3%) alone. In addition, many other studies have investigated the clinical utility of DCP for Chinese patients with HCC in recent years.

In 2014, a large-scale, multicenter study investigated the measurement of both AFP and DCP in differentiating Chinese patients with HCC (71.18% with an HBV infection) from patients without HCC and normal subjects. Results showed that the combined testing of DCP with a cut-off value of 86 mAU/mL and AFP with a cut-off value of 21 ng/mL resulted in a sensitivity of approximately 90% in diagnosis of HCC, which was significantly higher than that for DCP or AFP alone, and this finding held even for a tumor smaller than 2.0 cm (Song et al. 2014a). These results suggest that the measurement of both AFP and DCP may facilitate the diagnosis of patients with a broad range of HCC. However, the clinical utility of DCP in China has not been noted in Chinese guidelines on HCC, and more large-scale prospective studies should be performed to provide sufficient evidence.

## The Current Status of Surveillance and Early Diagnosis of HCC in South Korea

In South Korea, HBV is the biggest factor for developing HCC. One study of patients with HCC reported that underlying liver diseases included hepatitis B (72.3%), hepatitis C (11.6%), alcoholic liver disease (10.4%), and non-B non-C hepatitis (0.7%) (Korean Liver Cancer Study and National Cancer Center 2015b). Another study reported that 74.6% of HCC patients were positive for HBV, 9.3% were positive for HCV, 7.4% were long-term alcohol abusers, and 8.7% had unidentified causes (probably metabolic liver disease) (Kwak et al. 2014). HCC develops in 1–4% of cirrhotic patients annually and eventually develops in approximately one-third of cirrhotic patients (Ioannou et al. 2007).

According to the KLCSG-NCC Korea Guideline published in 2014, US was recommended for surveillance of patients with HBV/HCV or cirrhosis in the high-risk group (Table 3), and HCC is diagnosed on the basis of either pathology or clinical criteria ascertained with imaging techniques. When HCC is suspected in the

**Table 3** The epidemiology, surveillance, and early diagnosis of HCC in South Korea

| Items | Current status |
|---|---|
| Epidemiology | The top-ranked cause of death in people in their 40s and 50s; 22.5 people (male, 33.7; female, 11.3) per 100,000 population die annually from HCC |
| Etiological factors | 70–75% of patients with an HBV infection, 10% of patients with an HCV infection |
| Major at-risk population | Patients with an HBV/HCV infection or cirrhosis due to other causes |
| Guideline | KLCSG-NCC Korea Guideline published in 2003, revised in 2009, updated in 2014 |
| Surveillance tools | US |
| Surveillance criteria | 6-month interval for HCC the high-risk population |
| Diagnosis | Dynamic contrast-enhanced CT/MRI or liver-specific contrast-enhanced MRI; Pathology |

high-risk group during surveillance with US, dynamic contrast-enhanced CT/MRI or liver-specific contrast-enhanced MRI should be performed for diagnosis (Fig. 3). HCC can be diagnosed in the high-risk group if one or two of the aforementioned imaging techniques indicates that nodules $\geq$ 1 cm in diameter have typical features of HCC (including arterial phase enhancement with washout in the portal or delayed phase). Two or more imaging modalities are required to diagnose nodules 1–2 cm in diameter if a suboptimal imaging technique is used. Nodules $<$ 1 cm in diameter can be diagnosed as HCC in high-risk patients when both of the following conditions are met: typical features of HCC in two or more of the aforementioned imaging modalities and a continuous rise in serum AFP with hepatitis activity under control (Korean Liver Cancer Study and National Cancer Center 2015a).

In South Korea, AFP was not recommended for HCC surveillance but was regarded as an adjunctive diagnostic tool. Over the past 10 years, the utility of AFP has been described differently in the diagnostic criteria in three versions of the KLCSG-NCC Korea Guidelines. In the KLCSG-NCC Korea Guideline published in 2003 (Park et al. 2004), HCC was diagnosed based on imaging and AFP (AFP levels $\geq$400 ng/mL with one typical dynamic imaging technique, or AFP levels $<$400 ng/mL with two typical dynamic imaging techniques), regardless of tumor size. However, the revised KLCSG-NCC Korea Guideline published in 2009 (Korean Liver Cancer Study and National Cancer Center 2009) suggested that a tumor of 2 cm or larger in patients with liver cirrhosis that had characteristics typical of HCC in dynamic contrast enhancement CT or MRI could be diagnosed as HCC regardless of the serum AFP levels. If nodules in high-risk patients are smaller than 1 cm and diagnosis cannot be verified by a radiologic or histologic examination, a tumor marker test and US should be performed several times at an interval of 3–6 months to monitor for any increase in the size and level of tumor markers. In the latest version of the KLCSG-NCC Korea Guideline published in 2014 (Korean Liver Cancer Study and National Cancer Center 2015a), AFP was also recommended as an adjunctive diagnosis tool with two positive techniques of

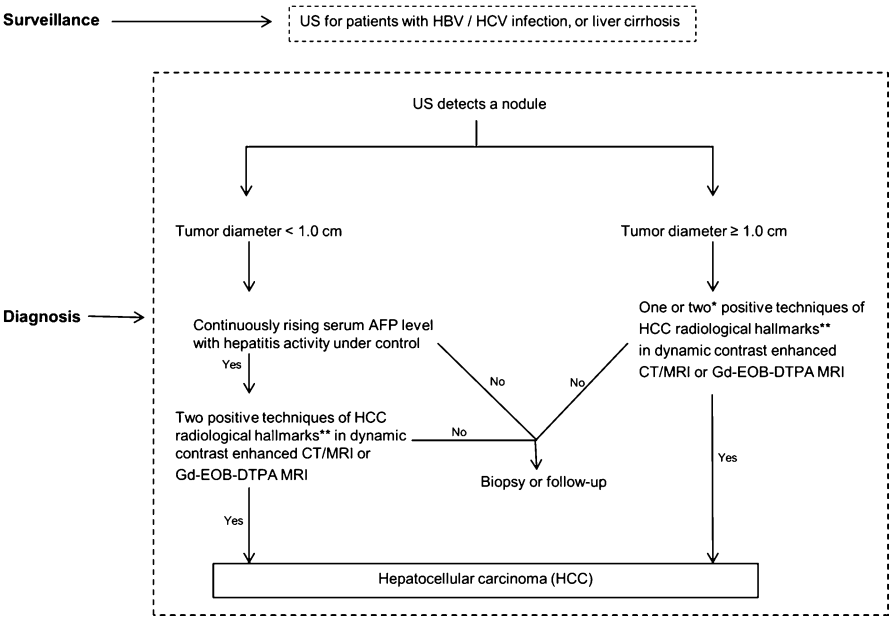


**Fig. 3 Surveillance and diagnostic algorithm for clinical management of HCC according to the latest KLCSG-NCC Korea Guideline published in 2014 in South Korea**. KLCSG, the Korean Liver Cancer Study Group; NCC, the National Cancer Center. *For diagnosis of nodules 1.0–2.0 cm in diameter, two or more imaging modalities are required if suboptimal imaging technique is used. **HCC radiological hallmarks include arterial phase enhancement with washout in portal or delayed phase

HCC radiological hallmarks in dynamic contrast-enhanced CT/MRI or gadolinium ethoxybenzyl diethylenetriamine pentaacetic acid (Gd-EOB-DTPA) MRI for cases with tumor $< 1$ cm.

Like in Japan, use of DCP has also been approved in South Korea. Several Korean retrospective studies also reported the clinical usefulness of combined testing of the tumor markers AFP, AFP-L3, and DCP (Kim et al. 2006; Yoon et al. 2002). However, further well-designed studies are warranted to confirm the role of these markers in the diagnosis of HCC.

## Controversies Regarding Use of Biomarkers in HCC Surveillance and Diagnosis in the East and West

### AFP as a Traditional Biomarker for HCC Surveillance and Diagnosis

Serum AFP has traditionally and widely been used as a tumor marker of HCC over the past two decades. However, there is increasing debate regarding the utility of AFP as a surveillance test. An analysis of recent studies has indicated that the serum

AFP level is normal in up to 35% of cases of small HCC and can be nonspecifically elevated in patients with active hepatitis or active hepatocyte regeneration (Korean Liver Cancer Study and National Cancer Center 2015b). Elevated AFP levels may also be seen in patients with cirrhosis or exacerbation of chronic hepatitis or cholangiocarcinoma (Bertino et al. 2012; Nguyen et al. 2002).

Given these findings, US is regarded as a more appropriate test for surveillance with an acceptable diagnostic accuracy (sensitivity ranging from 58% to 89%, and specificity greater than 90%) (Singal et al. 2009; Bolondi 2003). Currently, US is recommended as the only tool for HCC surveillance in some Western countries. AFP has been excluded from the surveillance criteria in the HCC guidelines published by the AASLD in 2010 (Bruix et al. 2011), and AFP is regarded as a suboptimal tool for surveillance according to the HCC guidelines published by the European Association for the Study of the Liver (EASL) in 2012 (European Association for the Study of the Liver et al. 2012). Nevertheless, the results of US in the early detection of HCC are highly dependent on the expertise of the examiner and the quality of the equipment. Currently, the combination of AFP and US at approximately 6-month intervals is still recommended by many HCC guidelines in Asia, such as guidelines in Japan (Japan Society of Hepatology 2013), China (National Health and Family Planning Commission 2011), and guidelines published by the APASL (Omata et al. 2010). Thus, whether AFP should be excluded from surveillance criteria needs to be investigated in more large, randomized controlled trials.

Over the past few decades, elevated serum AFP and a typical enhancement pattern in dynamic imaging have provided critical clues for the diagnosis of HCC. AFP was recommended as an adjunctive diagnostic tool in HCC guidelines published in Western countries, including the guideline published by the EASL in 2000, that published by the AASLD in 2005, and that published by the NCCN in 2009. Nevertheless, the importance in AFP has diminished in recent guidelines for diagnosis of HCC and the importance of imaging has increased based on the high accuracy of up-to-date radiologic modalities.

According to updated HCC guidelines published by the AASLD in 2010 (Bruix et al. 2011), nodules larger than 1 cm found during US surveillance of a cirrhotic liver should be investigated further with either a 4-phase multidetector CT scan or dynamic contrast-enhanced MRI. If the appearance of the nodule is typical of HCC, the lesion should be treated as HCC; if the findings are not characteristic or the vascular profile is not typical, a second contrast-enhanced study involving another imaging modality should be performed, or the lesion should be biopsied. In agreement with updated guidelines from the AASLD, the panel that drafted the HCC guidelines of the NCCN in 2014 (Benson et al. 2014) also considered an imaging finding of classic enhancement to be more definitive in this instance since the level of serum AFP may be elevated in persons with certain nonmalignant conditions or it may be within normal limits in a substantial percentage of patients with HCC.

In Asian countries, according to the HCC guidelines published by the APASL in 2010 (Omata et al. 2010), typical HCC can be diagnosed based on imaging

regardless of tumor size if a typical vascular pattern, i.e., arterial enhancement with portal venous washout, is obtained on dynamic CT/MRI or contrast-enhanced US, AFP was recommended as an adjunctive diagnostic tool and AFP alone was not recommended for diagnosis of HCC. Similar recommendations were made by HCC guidelines published in Japan (Japan Society of Hepatology 2013), China (National Health and Family Planning Commission 2011), and South Korea (Korean Liver Cancer Study and National Cancer Center 2015a).

A continuously rising level of AFP or a level of 200 ng/mL or more was recommended as the cut-off value for AFP according to the versions of the J-HCC Guideline published in 2005, 2009, and 2013 (Japan Society of Hepatology 2013; Makuuchi and Kokudo 2006; The Japan Society of Hepatology 2010). A cut-off value of AFP $\geq$ 400 ng/mL was recommended by the Chinese HCC Consensus published in 2009 (Chinese Anti-Cancer Association Society of Liver Cancer 2009). A cut-off value of AFP $\geq$ 400 ng/mL for more than 1 month or AFP $\geq$ 200 ng/mL for more than 2 months was recommended by the NHFPC HCC Guideline updated in 2011 (National Health and Family Planning Commission 2011). The 2003 version of the KLCSG-NCC Korea Guideline published in South Korea recommended a cut-off value of AFP $\geq$ 400 ng/mL (Park et al. 2004), while the 2009 version recommended a cut-off value of AFP $\geq$ 200 ng/mL (Korean Liver Cancer Study and National Cancer Center 2009). When a tumor $\geq$ 2 cm in patients with liver cirrhosis has typical characteristics of HCC in dynamic contrast enhancement CT or MRI, it can be diagnosed as HCC regardless of the serum AFP levels. The latest version of the KLCSG-NCC Korea Guideline published in 2014 (Korean Liver Cancer Study and National Cancer Center 2015a) recommends a continuous rise in serum AFP with hepatitis activity under control as an adjunctive diagnostic tool when a nodule $<$ 1 cm in diameter has typical features of HCC according to two or more dynamic imaging modalities.

## The Combined Testing of AFP, AFP-L3, and DCP for HCC Surveillance and Diagnosis

Current expert opinion from Western countries has been rather critical of the clinical value of biomarkers. Imaging-based surveillance criteria were recommended by guidelines from Western countries, such as the updated HCC guidelines published by the AASLD in 2000 (Bruix et al. 2011) and similar guidelines published by the EASL in 2012 (European Association for the Study of the Liver et al. 2012). In Asian countries, as typified by Japan's HCC guidelines (Japan Society of Hepatology 2013), US and measurement of AFP, AFP-L3, or DCP should be performed at intervals of 3–4 months for the very-high-risk group (patients with HBV- or HCV-related liver cirrhosis) and at intervals of 6 months for the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes). The testing of AFP, AFP-L3, and DCP levels is covered by Japan's national health insurance as serological biomarkers for HCC surveillance in clinical settings.

When diagnosing HCC in cases of a tumor smaller than 5 cm in diameter (Tateishi et al. 2008), AFP with a cut-off value of 20 ng/mL had a sensitivity of 49–71%, a specificity of 49–86%, an odds ratio (OR) of 4.06, and a positive likelihood ratio (LR+) of 2.45. AFP with a cut-off value of 200 ng/mL had a sensitivity of 8–32%, a specificity of 76–100%, an OR of 6.99, and an LR+ of 5.85. AFP-L3 can differentiate an increase in AFP due to HCC from that due to benign liver disease. AFP-L3 with a cut-off value of 10% had a sensitivity of 22–33%, a specificity of 93–99%, an OR of 6.43, and an LR+ of 4.89 in diagnosing HCC smaller than 5 cm in diameter. AFP-L3 with a cut-off value of 15% had a sensitivity of 21–49%, a specificity of 94–100%, an OR of 10.50, and an LR+ of 13.10. DCP has also been recognized as a highly specific marker for HCC. DCP with a cut-off value of 40 mAU/mL had a sensitivity of 14–54%, a specificity of 95–99%, an OR of 21.31, and an LR+ of 12.60 in diagnosing HCC smaller than 5 cm in diameter. DCP with a cut-off value of 100 mAU/mL had a sensitivity of 7–56%, a specificity of 72–100%, an OR of 6.70, and an LR+ of 4.91.

Data have indicated that the combined testing of DCP and AFP or AFP-L3 could help to increase the sensitivity of diagnosis of HCC. The combined testing of two biomarkers had a OR of 6.29–59.81 in diagnosing HCC smaller than 5 cm in diameter (Tateishi et al. 2008), which was better than that of one biomarker alone. However, the combined testing of DCP and AFP or AFP-L3 is used in only a few countries, such as Japan. Although the clinical usefulness of combined testing of DCP and AFP or AFP-L3 has also been noted by several retrospective studies published in South Korea and China, this testing has not been recommended by HCC guidelines in South Korea and China until now. Further well-designed studies are warranted to confirm the roles of these biomarkers in the diagnosis of HCC.

## The Clinical Utility of AFP, AFP-L3, and DCP in HCC Prognosis

The biomarkers AFP, AFP-L3, and DCP have been evaluated for their power in diagnosing HCC, and they have also been studied for their prognostic significance. A high level of AFP expression in serum correlates with profound cell proliferation, profound angiogenesis, and limited apoptosis and is associated with a poor prognosis (Mitsuhashi et al. 2008; Llovet et al. 2012). AFP was one of the most robust predictors of death in patients with cirrhosis and HCC (Tandon and Garcia-Tsao 2009), and it also has significance at predicting survival after liver transplantation (Mailey et al. 2011). Changes in AFP while on the waitlist also predicted posttransplant survival, and identifying these changes could facilitate better patient selection to optimize organ allocation and posttransplant outcomes (Rich and Singal 2014). A change in AFP levels has been found to correlate with radiologic response and overall survival after locoregional therapy. As an example, a 50% decrease in AFP levels resulted in a better time-to-progression [hazard ratio (HR): 2.8, 95% confidence interval (CI): 1.5–5.1] and overall survival (HR: 2.7, 95% CI: 1.6–4.6) in

comparison to patients whose AFP levels failed to respond to treatment with transarterial chemoembolization (TACE) or transarterial radioembolization (TARE) (Riaz et al. 2009). Although the question of whether AFP is useful at predicting the response to sorafenib is controversial (Llovet et al. 2012; Nakazawa et al. 2013), several studies have indicated that AFP response was correlated with time-to-progression (7.9 vs. 2.4 months, $p = 0.004$) and overall survival (13.3 vs. 8.2 months, $p = 0.022$) (Personeni et al. 2012).

AFP-L3 and DCP were also identified as prognostic biomarkers for survival after resection of HCC. Patients who have undergone resection of HCC and who had elevated levels of AFP, AFP-L3, and DCP at the baseline had a worse prognosis than patients who tested positive for just one or two of the markers before surgery (Kiriyama et al. 2011; Nakagawa et al. 2014). Furthermore, several studies have also recently investigated the potential clinical usefulness of DCP in assessing HCC progression. These studies found that (Song et al. 2013c) (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.

Among the current guidelines for HCC management worldwide, the guidelines of the NCCN published in 2014 (Benson et al. 2014) recommend high-sectional imaging every 3–6 months for 2 years and then every 6–12 months for posttreatment monitoring. If AFP levels are initially elevated, the guidelines recommend that monitoring be performed every 3 months for 2 years and then every 6–12 months. The Indian National Association for Study of the Liver (INASL) published the first guidelines in India in 2014 (Kumar et al. 2014), and these guidelines make similar recommendations. The guidelines recommend that posttreatment monitoring be performed with dynamic CT or MRI studies every 3 months for the first 2 years and then routine surveillance every 6 months thereafter. The guidelines also note that the serum tumor markers AFP and DCP may help to evaluate the response to treatment or evaluate follow-up when AFP or DCP is elevated at diagnosis and when AFP or DCP decreases after treatment but rises again. The guidelines do note, however, that tumor markers cannot replace imaging modalities. According to the HCC guidelines published in Japan in 2013 (Japan Society of Hepatology 2013), follow-up using the serum biomarkers AFP, AFP-L3, and DCP and imaging should be performed every 3–4 months after treatment. According to NHFPC HCC Guideline published in China in 2011 (National Health and Family Planning Commission 2011), posttreatment monitoring with AFP and imaging should be performed every 3–4 months for 3 years, every 4–6 months for 3–5 years, and then every 6–12 months thereafter if no abnormal findings are detected.

## Novel Biomarkers: Potential Applications to Surveillance, Diagnosis, and Prognosis

AFP, AFP-L3, and DCP have been recommended by HCC guidelines in East Asia and elsewhere around the world, but biomarkers such as DKK1, MDK, miRNA, GGT, ICG-R16, GP73, IL-6, SCCA, GPC3, osteopontin, and VEGF are currently being studied to investigate their clinical utility in HCC surveillance, diagnosis, or prognosis.

Of these novel biomarkers, a retrospective, cross-sectional study involving 424 patients with HCC and 407 controls without HCC (213 were healthy, 98 had chronic HBV infection, and 96 had liver cirrhosis) was published in 2012 (Shen et al. 2012); results showed that DKK1 was highly accurate at diagnosing AFP-negative patients with HCC, including patients with early stage HCC. The results also showed that the measurement of DKK1 and AFP together improved the accuracy with which HCC was diagnosed in comparison to any single test alone. These findings add a new piece to the puzzle of diagnosing HCC and they open the door for further investigation of this promising tumor biomarker in independent, prospective studies (Forner and Bruix 2012).

In 2013, a study involving 388 patients with HCC and 545 different controls (Zhu et al. 2013) found that serum MDK had a markedly higher level of sensitivity than AFP (86.9% vs. 51.9%) but a similar level of specificity (83.9% vs. 86.3%). MDK has a significantly higher sensitivity than AFP (80% vs. 40%) at diagnosing very early stage HCC, and its sensitivity could be as high as 89.2% when diagnosing cases of AFP-negative HCC. Serum MDK levels decreased significantly in patients with HCC after curative resection and rose again when the cancer recurred.

The two studies mentioned earlier suggested that the novel serum biomarkers DKK1 and MDK can augment the measurement of AFP when diagnosing HCC, and particularly when diagnosing patients who are negative for AFP and/or who have HCC in an early stage. However, these studies were small in scale and involved few patients. According to the guidelines on phases of evaluating a biomarker for early detection of cancer developed by the National Cancer Institute's Early Detection Research Network (Pepe et al. 2001), more prospective, randomized controlled trials need to be conducted at multiple centers to provide further validation using a larger cohort of serum samples from patients with HCC and hepatitis B and hepatitis C infectious liver disease, nonalcoholic fatty liver disease (NAFLD), and alcohol-induced liver disease (ALD).

Noncoding RNA and microRNA (miRNA) in particular have received considerable attention as novel potential biomarkers over the past few years. Li et al. found that three miRNAs (miR-25, miR-375, and let-7f) could provide a sensitivity of 97.9% and a specificity of 99.1% in diagnosing HCC (Li et al. 2010). Zhou et al. found that a panel of seven microRNAs (miR-122, miR-192, miR-21, miR-223, miR-26a, miR-27a, and miR-801) could provide a high level of diagnostic accuracy for identification of HBV-related HCC (Zhou et al. 2011). Tomimaru et al. found that the combination of miRNA-21 with AFP improved the power of

differentiation between HCC and chronic hepatitis with a sensitivity of 81.0% and a specificity of 80% (Tomimaru et al. 2012).

In addition to their diagnostic potential, miRNAs may help to predict the prognosis for HCC. Tomimaru et al. found that the level of miR-21 expression was high in Asian patients with HCC and that level declined after surgery (Tomimaru et al. 2012). They also found that a high level of miR-21 expression in plasma correlated with a shorter cumulative survival following treatment. Köberle et al. found that in European patients with HCC higher levels of miR-1 and miR-122 expression were associated with longer overall survival compared to lower levels of expression of those miRNAs. They concluded that miR-1 may be a predictive biomarker of HCC independent of liver function. A 31-miRNA signature correlates with the stage of disease (Ura et al. 2009), and a distinct 20-miRNA signature associated with metastasis of HCC has also been identified (Budhu et al. 2008). These findings constitute mounting evidence that miRNA signature profiling can be of use in prognostic stratification. However, the potential for miRNA to serve as a biomarker has not been equally analyzed in all conditions potentially leading to HCC.

Gamma-glutamyl transpeptidase (GGT) has been identified as a prognostic marker in studies of different subgroups of patients published over the past 5 years. Sheen et al. found that patients who had HCC with type B GGT mRNA had worse outcomes, earlier recurrence, and more postrecurrence deaths (Sheen et al. 2003). Several studies of patients with HCC undergoing hepatic resection have revealed a correlation between elevated levels of GGT and worse survival for patients with HBV-related HCC, Child-Pugh A liver function, or multinodular tumors (Ju et al. 2009; Zhao et al. 2013; Zhao et al. 2012). In addition, several studies have also revealed the predictive value of GGT in patients with unresectable HCC who were treated with TACE or chemotherapy (Carr et al. 2010; Guiu et al. 2012; Nishikawa et al. 2013; Zhang et al. 2011). Furthermore, a study published in 2015 examined 384 consecutive cases of curative hepatic resection for single primary HCC to investigate the preoperative predictors of postoperative survival and recurrence (Song et al. 2015). Results showed that GGT $>$ 100 U/L was a preoperative independent risk factor associated with survival, and GGT $>$ 50 U/L and ICG-R15 $>$ 10% were identified as preoperative independent risk factors associated with tumor recurrence. Patients with GGT $>$ 50 U/L and ICG-R15 $>$ 10% had a worse 1-, 3-, and 5-year recurrence-free survival, and this was also true for patients with a tumor $<$ 5 cm in size.

## Conclusion

HCC is undoubtedly a great health threat in East Asia since the region has the highest incidence of that cancer, with an age-standardized rate of 31.9 per 100,000 in men. The biomarker AFP has been widely used for routine surveillance and noninvasive

diagnosis of HCC and to evaluate prognosis and monitor recurrence after treatment. In recent years, however, the role of AFP in HCC surveillance and diagnosis has diminished due to advances in imaging modalities. AFP has been excluded from the surveillance and/or diagnostic criteria in HCC guidelines published by the AASLD in 2010, HCC guidelines published by the EASL in 2012, and HCC guidelines published by the NCCN in 2014. Nonetheless, AFP is still regarded as a useful surveillance tool in HCC guidelines in Japan and China and is recommended as an adjunctive tool by HCC guidelines in Japan, China, and South Korea. If the serum AFP level increases steadily over time, the development of HCC should be suspected, and the usual dynamic imaging techniques should be used to make a differential diagnosis.

Advances in technology and an increased understanding of the pathology of HCC have led to the discovery of novel biomarkers. Data have indicated that the combined testing of AFP, AFP-L3, or DCP could help to increase the sensitivity of diagnosis of HCC, but this approach is currently used in only a few countries, such as Japan. In recent years, numerous studies have investigated the clinical usefulness of some novel biomarkers in the early diagnosis of HCC, including DKK1, MDK, and miRNA. Moreover, the prognostic significance of some biomarkers, such as miRNA, GGT, and ICG-R15, has also been evaluated. However, further studies are needed to better characterize the accuracy and potential role of these approaches in clinical practice. The prevailing hope is that novel biomarkers can support clinicians in their daily practice and improve care for patients with HCC.

## Summary Points

- This chapter has focused on the clinical utility of biomarkers for hepatocellular carcinoma (HCC) in East Asia, including Japan, China, and South Korea.
- Alpha-fetoprotein (AFP) testing and abdominal ultrasound (US) every 6 months are recommended for routine surveillance of HCC in high-risk patients, and AFP has also been used as a diagnostic test for HCC and to evaluate prognosis and monitor recurrence following treatment.
- However, controversy regarding the clinical utility of AFP has arisen in the West and the East in recent years. This controversy is also evident in HCC guidelines in countries in East Asia
- Data have indicated that the combined testing of AFP, the lens culinaris agglutinin-reactive fraction of AFP (AFP-L3), or des-γ-carboxyprothrombin (DCP) could help to increase the sensitivity of diagnosis of HCC, but this approach is currently used in only a few countries, such as Japan.
- Numerous studies have investigated the clinical usefulness of some novel biomarkers in early diagnosis of HCC, including Dickkopf-1 (DKK1), midkine (MDK), and microRNA (miRNA).
- The prognostic significance of some biomarkers, such as miRNA, gamma-glutamyl transferase (GGT), and indocyanine green retention 15 min after administration (ICG-R15), has also been evaluated.

- However, further studies are needed to better characterize the accuracy and potential role of these approaches in clinical practice.

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