Hepatocellular Carcinoma Column

Serum biomarkers for early diagnosis of hepatocellular carcinoma

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Abstract: Globally, many guidelines for hepatocellular carcinoma (HCC) treatment recommend HCC screening and surveillance. Serum biomarkers are striking potential tools to screen for and diagnose HCC early thanks to the non-invasive, objective, and reproducible assessments they can potentially enable. α-fetoprotein (AFP) is the biomarker most widely used to test for HCC, but the sensitivity and specificity of AFP vary widely, and total AFP is not always specific, especially when HCC is in its early stages. Recent years, research into multiple serum biomarkers to detect HCC early has garnered attention around the world. Multiple reports have found that combined testing with des-γ-carboxyprothrombin (DCP) and AFP has a sensitivity of 47.5-94.0% and a specificity of 53.3-98.5% in detecting HCC early. Most recently, using new reliable serum biomarkers, such as Dickkopf-1 (DKK1) and Midkine (MDK), to complement AFP as a new trend is expected to be used to facilitate screening for and diagnosing HCC at an earlier stage. However, more such studies are needed before they can be included as valid biomarkers in programs to screen for HCC and in strategies to diagnose patients who present with liver masses.

Keywords: Liver cancer; early detection; tumor marker; sensitivity; specificity

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Hepatocellular carcinoma (HCC) is the major histologic subtype of liver cancer, which is largely a problem of the less developed regions where 83% (50% in China alone) of the estimated 782,000 new liver cancer cases worldwide occurred in 2012 (1). HCC is the fifth most common cancer in men and is most prevalent in Eastern and South-Eastern Asia (31.9/100,000 and 22.2/100,000, respectively), it is the second most common cause of death from cancer worldwide and is estimated to be responsible for nearly 746,000 deaths in 2012 (1). Currently, surgical resection and liver transplantation offer the best potential for treating HCC but are only feasible when tumors are detected early (2,3). The overall 5-year survival rate for patients with HCC is about 40%, but liver resection of early HCC could result in a 5-year survival rate of 60-70% (4). Screening programs in many Asian countries have improved the early detection of HCC and have had a positive impact on survival, but most Asian patients with HCC still present with advanced stage disease (5,6). Thus, a well-considered strategy to screen for and diagnose HCC at an earlier stage is urgently needed when curable interventions can be offered to achieve long-term disease-free survival for patients (7).

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), the National Comprehensive Cancer Network (NCCN), and the Asian Pacific Association for the Study of the Liver (APASL) (8). In general, imaging tools have been widely used in the US and Europe 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 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% (9). Ultrasound is the most common imaging tool used to screen for HCC thanks to its features such as simplicity, low cost, minimal invasiveness, and the fact that it allows real-time observation. However, successful

ultrasound detection relies on the expertise of the physician, the availability of ultrasound equipment, and the echo texture of the liver. Thus, evaluating the actual sensitivity and specificity of ultrasound detection is difficult because of the lack of standards (10,11).

Serum biomarkers are striking potential tools to screen for and diagnose HCC early thanks to the non-invasive, objective, and reproducible assessments they can potentially enable. α-fetoprotein (AFP) is the biomarker most widely used to test for HCC, but the sensitivity and specificity of AFP vary widely, and total AFP is not always specific, especially when HCC is in its early stages. AFP has been found to have a sensitivity of 41-65% and a specificity of 80-90% when detecting HCC given an AFP cut-off of 20 ng/mL (12). However, up to 50% of patients with HCC have an AFP level below 20 ng/mL (13), and elevated levels of AFP can also be found in patients with non-malignant chronic liver disease, including 15-58% with chronic hepatitis and 11-47% with liver cirrhosis (12,14). Thus, AFP cannot be used as a sole tool to screen for and diagnose HCC. New reliable serum biomarkers need to be identified soon to complement AFP in order to improve clinical outcomes for patients.

Two other serum biomarkers besides AFP—the *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)—have been studied around the world to explore their clinical usefulness in screening for and diagnosing HCC. 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 six months in the high-risk group (patients with HBV- or HCV-related chronic liver disease or liver cirrhosis due to other causes) (15,16). Currently, AFP, AFP-L3, and DCP are used widely and routinely as a tool to screen for HCC in Japan, and these tests are covered by Japan's national health insurance as serum biomarkers to screen for HCC in clinical settings. Due to the routine practice of screening for HCC among high-risk patients, HCC nodules have been detected in the early stage in more than 60% of patients in Japan (17).

Since Liebman *et al*. first reported DCP in the plasma of 90% of patients with HCC in 1984 (18), 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 HCC. Multiple reports have found that combined testing with DCP and AFP has a sensitivity of 47.5-94.0% and a specificity of 53.3-98.5% in detecting HCC early (5).

Recent years have also seen many studies on the clinical usefulness of other serum biomarkers in detecting HCC early, including Golgi protein-73 (GP73), glypican-3 (GPC3) and gamma-glutamyltransferase (GGTII). Most recently, research on Dickkopf-1 (DKK1) and Midkine (MDK) as diagnostic serum biomarkers has garnered interest.

In *Lancet Oncology*, Shen *et al*. published a retrospective, cross-sectional study in 2012 that assessed whether measurement of DKK1 concentrations in serum could enhance its accuracy at diagnosing HCC. Shen *et al*. used receiver operating characteristic analysis to calculate the optimum cut-off concentration in a test cohort of 424 patients with HCC and 407 controls without HCC (213 were healthy, 98 had chronic HBV infection, and 96 had liver cirrhosis) (19). They found that serum levels of DKK1 were significantly higher in patients with HCC than in all of the controls; that DKK1 was highly accurate at diagnosing AFP-negative patients with HCC, including patients with early-stage HCC; and that measurement of DKK1 and AFP together improved the accuracy with which HCC was diagnosed in comparison to any 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 (20).

In *Clinical Cancer Research*, Zhu *et al*. published a study in 2013 involving three independent cohorts with a total of 933 participants (388 patients with HCC and 545 different controls). Zhu *et al*. evaluated the value of serum MDK as a diagnostic biomarker of HCC, and particularly for patients who were negative for AFP and who had HCC in an early stage (21). They found that MDK levels were significantly elevated in HCC tissues as well as in serum samples; that serum MDK had a markedly higher sensitivity than AFP (86.9% *vs*. 51.9%) at diagnosing HCC but similar specificities (83.9% *vs*. 86.3%); that MDK has a significantly higher sensitivity than AFP (80% *vs*. 40%) even at diagnosing very early-stage HCC; that its sensitivity could be as high as 89.2% when diagnosing cases of AFP-negative HCC; and that serum MDK levels decreased significantly in patients with HCC after curative resection and rose again when the cancer recurred.

These two studies 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 an early detection biomarker for cancer developed by the National Cancer Institute's Early Detection Research Network (22), more prospective, randomized controlled trials need to be conducted at multiple centers to provide further validation using a 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).

In conclusion, research into multiple serum biomarkers to detect HCC early has garnered attention around the world. Using new reliable serum biomarkers, such as DKK1 and MDK, to complement AFP as a new trend is expected to be used to facilitate screening for and diagnosing HCC at an earlier stage. However, more studies of serum DKK1 and MDK are needed before they can be included as valid biomarkers in programs to screen for HCC and in strategies to diagnose patients who present with liver masses.

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