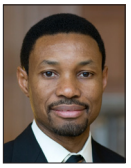
HCC IN FOCUS

Current Developments in the Management of Hepatocellular Carcinoma

Section Editor: Robert G. Gish, MD

Biomarkers for Hepatocellular Carcinoma



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G&H How has the diagnostic role of alpha-fetoprotein changed over time?

LR When hepatocellular carcinoma (HCC) was first recognized as a distinct disorder of cancer in the liver in the early 1900s, most HCCs were diagnosed at very late stages. By the time patients presented for medical attention, they would have either an obvious mass in the abdomen or evidence of compression from a growing mass in the abdomen. This was before the development of effective cross-sectional imaging or laboratory testing, so physicians would have to palpate any suspicious masses in the abdomen in order to make a clinical diagnosis of HCC. Because HCCs tend to be very vascular, physicians could often feel the thrill of blood flowing or hear it rushing through the mass, particularly when a stethoscope was placed over the liver.

In the mid-1960s, it was recognized that many HCCs, particularly larger tumors, secreted alpha-fetoprotein (AFP) into the blood; consequently, diagnosis of HCC started to involve the use of this biomarker. At the same time, there were advances in ultrasound and computed tomography (CT) scanning, so physicians could image the liver and see masses growing there. The ability to obtain imaging studies, combined with the ability to test patients' blood for elevated levels of AFP, made it possible to detect HCC at an earlier stage. Early diagnosis of HCC was preferable because more effective treatments could be used to cure the patient or substantially reduce the risk that the disease would return after treatment. Physicians also began to try to recognize which individuals were at risk for HCC so that these patients could be put into a surveillance program.

At this time, it was understood that AFP is present and measurable in the blood of a high proportion of people who have advanced HCC. However, a much smaller proportion of individuals with early HCC have high AFP levels. For example, among people with HCCs that are larger than 5 or 6 cm in size, approximately 50% to 60% have high AFP levels. In contrast, among people with HCCs that are 2 to 3 cm in size, only approximately 20% to 30% have high AFP levels.

Another issue was that, in addition to being associated with HCC, AFP seemed to be associated with liver regeneration. For example, a patient whose liver is regenerating after being damaged could also have a high AFP level. This scenario primarily occurred in patients with chronic hepatitis C virus (HCV) infection. As HCV infection increased in incidence and became more common as the cause of HCC, it became clear that an elevated AFP level was not always associated with HCC. Thus, AFP not only had a problem with sensitivity, it also had a problem with specificity. As a result, AFP began to fall out of favor for the diagnosis of early-stage HCC, and there was a need for other diagnostic methods to replace, or be used in combination with, AFP.

G&H What alternative diagnostic approaches were developed?

LR Contrast CT scans and contrast magnetic resonance imaging (MRI) became more important because they could help define distinguishing features of a mass in the liver. Many physicians began performing ultrasounds in people at risk for HCC, and when a new spot was found

in the liver, physicians would perform a CT scan or MRI to determine whether the spot was indeed HCC.

The other approach was profiling tumors using different molecular technologies. As it became possible to measure different proteins and metabolites in the blood, physicians tried to find biomarkers other than AFP. This was first done effectively in Japan, which has very high rates of HCV infection. Japanese physicians began to explore the use of Lens culinaris agglutinin-reactive AFP (AFP-L3), which is an isoform of AFP, and des-gamma-carboxy prothrombin (DCP). Tests for these 2 biomarkers were developed and have become an established part of the surveillance process, in combination with AFP, for HCC in Japan.

G&H Why are these 2 newer biomarkers helpful?

LR HCCs are not homogenous; each is different. Thus, some HCCs may have normal or only mildly elevated levels of AFP, but high levels of AFP-L3. Similarly, some of the tumors may have high levels of DCP while having normal levels of AFP. Thus, these 2 biomarkers offer alternative ways to detect early HCCs.

G&H How sensitive and specific are AFP-L3 and DCP? Can they replace the use of AFP?

LR AFP-L3 and DCP offer additional benefits beyond the use of AFP. For example, if AFP can detect 20% to 25% of early HCCs, using AFP-L3 and DCP might enable the detection of an additional 5% to 10%. In general, AFP is still one of the most widely used tests for the diagnosis of HCC, and it is still the most sensitive biomarker test. However, as mentioned above, AFP is not sensitive enough to use by itself. Likewise, AFP-L3 and DCP are not sensitive enough to be used by themselves either.

In addition to using these 3 biomarkers in combination, some physicians have created models that include other patient features. For example, Dr Philip Johnson from the United Kingdom has developed the GALAD model, which is named for the patient features it includes (gender, age, AFP-L3, AFP, and DCP). In several recent publications, this model was better than any of the biomarkers alone at detecting HCC.

G&H Currently, how are each of these 3 biomarkers being used in the clinical setting?

LR Most commonly, these biomarkers are being used to estimate a person's future risk of developing HCC. Currently, AFP-L3 and DCP have been approved by the US Food and Drug Administration as risk predictors. In other words, if a patient is at risk of developing HCC and has a high AFP, AFP-L3, or DCP level, that person is more likely to develop HCC in the future than a person who has a normal level of AFP, AFP-L3, or DCP.

As discussed above, AFP can also be used to help diagnose patients with HCC. A very high AFP level of 400 or 500 ng/mL in the absence of other causes of elevation is strongly predictive of HCC.

In addition, these biomarkers can be used to predict the outcomes of patients with HCC. For example, it is clear that a high AFP level is associated with worse outcomes and more aggressive forms of HCC. In some countries, such as Canada, a very high AFP level excludes patients from receiving a liver transplant because the risk of recurrence after transplant has been shown to be higher.

The biomarkers can also be used to follow patients who are receiving treatment. If a patient has HCC and a high level of AFP, AFP-L3, or DCP, the level of the biomarker is expected to fall if treatment is effective. On the other hand, if the biomarker level continues to rise, either the treatment is not working or there is more active disease that the physician needs to look for and treat.

G&H Is there a relationship between AFP elevation and high alanine aminotransferase levels?

LR High alanine aminotransferase (ALT) levels are associated with inflammation of the liver, and individuals who have high ALT levels are more likely to have damage to the liver that is ongoing and causing regeneration. This is a situation in which AFP is less specific for a diagnosis of HCC. Knowing a patient's ALT level can be quite useful because it helps the physician know whether an AFP measurement is more or less reliable.

G&H What are the advantages of using HCC biomarkers?

LR Most physicians understand that surveillance of the liver for HCC requires imaging via ultrasound, but this tool may not be effective in some patients. For example, an ultrasound may not be able to visualize the entire liver of an obese patient with a high body mass index. Another example is when the HCC is very subtle and, thus, difficult to see as a distinct mass on an ultrasound or even a CT scan or MRI. In these scenarios, it is helpful to measure the patient's AFP level. Therefore, many physicians use these tests in combination and consider biomarkers and ultrasound as being complementary in the surveillance and diagnosis of HCC. In countries such as Japan where there is a high prevalence of chronic HCV infection, it is standard to use AFP-L3 and DCP along with AFP and ultrasound.

G&H Are there challenges with using any of these biomarkers in clinical practice?

LR When using biomarkers, it is important to consider the clinical context of the patient and look at trends in the levels of the biomarkers. For example, the normal limits of AFP, depending upon the test, may be 6 or 10 ng/mL. If a patient has an AFP level of 20 ng/mL but does not have an obvious mass on an ultrasound or CT scan, that finding should serve as an alert that the patient might be at higher risk for HCC. The finding might not trigger anything other than a request to repeat AFP measurement in a month or 2 to establish whether there is a rising trend of AFP. Even though 1 measurement by itself may not be highly sensitive, the observation of how AFP changes over time can add value. It is important for physicians to gain expertise and familiarity with how these biomarkers can change over time so that they can make the most informed decisions based upon the trends and levels of the biomarkers.

G&H Will biomarkers replace imaging in the future?

LR Such an outcome is unlikely because imaging is extremely important in helping physicians know where to perform treatment, how effective the treatment is likely to be, and which types of treatment should be used. The location of the tumor in the liver is quite important. Even if biomarkers were developed that could detect with 100% certainty which patients have HCC, physicians would still need to perform imaging studies to be able to develop the most effective treatment plan for each patient.

G&H Do any other biomarkers, such as osteopontin, show promise for HCC?

LR Initial research suggested that osteopontin could be an effective biomarker for HCC in combination with other biomarkers such as AFP. However, more research is needed on this biomarker.

There are also a number of other potential biomarkers, such as GCP3 and latent-transforming growth factor β binding-protein 2, that have been shown to be useful for early diagnosis of HCC, either alone or in combination with other biomarkers. Additional studies are needed on these molecules, as well as on other potential non-protein biomarkers, such as DNA methylation markers, microRNAs, and long noncoding RNAs. There are also exciting studies being performed using urine and stool as the biofluids for surveillance testing.

G&H Is there a need for liver cancer biopsy tissue biomarkers to guide therapy?

LR Absolutely. We are learning that HCCs can be quite heterogeneous; there are different subtypes of HCC that are being driven by different signaling pathways that cause cells to proliferate and tumors to grow. One of the benefits of having different biomarkers is the potential to identify which pathways are active in particular HCCs. If we can identify biomarkers for active pathways and develop drugs that will suppress them, it will be possible to identify the best treatment for a particular patient. In the future, one of our goals is to be better able to tie biomarkers that can be measured to the most effective treatment for that type of tumor.

G&H Has there been any research on this particular use of biomarkers?

LR A fair bit of research has been conducted. Some evidence suggests that patients who have amplification of the fibroblast growth factor (FGF) 3 or 4 gene are much more likely to have a profound response to sorafenib (Nexavar, Bayer HealthCare), which is the only drug currently approved by the US Food and Drug Administration for advanced HCC. This suggests that it might be possible, in the future, to predict which patients will have a good response to sorafenib by measuring *FGF3* or *FGF4* in the tumor. The same finding has been shown with the vascular endothelial growth factor A gene. Tumors with amplification of this gene seem to be more sensitive to sorafenib therapy than tumors without such amplification.

G&H What other research is currently being conducted on HCC biomarkers?

LR The National Cancer Institute has an Early Detection Research Network (EDRN), and participants have been building tools for validation studies of new biomarkers. A phase 2 sample set was collected from centers around the United States a few years ago. The researchers identified approximately 200 patients with early-stage HCC and approximately 200 patients with later-stage HCC. The researchers also collected samples from 400 patients with cirrhosis who did not have HCC, who received follow-up 6 months later to confirm that they had not developed HCC during the interim period. When a new biomarker is developed, the investigator or research company can request these samples to see how well the biomarker performs independently.

Currently, a group of 7 medical centers across the United States is recruiting participants for a phase 3 biomarker study to improve HCC diagnosis. This study, called the Hepatocellular Carcinoma Early Detection Strategy study, is also funded by the National Cancer Institute EDRN. The group of researchers, led by Dr Jorge Marrero of the University of Texas Southwestern Medical Center,

is prospectively identifying people who are at risk of HCC because they have cirrhosis of the liver and is following them every 6 months. Thus far, we have recruited approximately 1100 individuals with cirrhosis, and the current plan is to enroll a total of 1500 participants. Every 6 months, the patients undergo imaging and blood tests, and we obtain a blood sample that is then stored at the National Cancer Institute. We are planning to follow these individuals over a 5-year period. During this time, we expect that approximately 150 to 200 of these individuals may develop HCC. We will then be able to test the blood samples of these patients using the new biomarkers not only to determine how often the test is positive when a patient has the disease (ie, sensitivity), but also to determine how often the test is negative when a patient does not have the disease (ie, specificity). We will also be able to see how far in advance of the clinical diagnosis the biomarker was elevated and, thus, how far in advance the biomarker could tell us which people will develop HCC.

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Suggested Reading

Chaiteerakij R, Addissie BD, Roberts LR. Update on biomarkers of hepatocellular carcinoma. *Clin Gastroenterol Hepatol*. 2015;13(2):237-245.

Chaiteerakij R, Zhang X, Addissie BD, et al. Combinations of biomarkers and Milan criteria for predicting hepatocellular carcinoma recurrence after liver transplantation. *Liver Transpl*. 2015;21(5):599-606.

Leerapun A, Suravarapu SV, Bida JP, et al. The utility of Lens culinaris agglutinin-reactive alpha-fetoprotein in the diagnosis of hepatocellular carcinoma: evaluation in a United States referral population. *Clin Gastroenterol Hepatol*. 2007;5(3):394-402; quiz 267.

Lozada ME, Chaiteerakij R, Roberts LR. Screening for hepatocellular carcinoma and cholangiocarcinoma: can biomarkers replace imaging? *Curr Hepatol Rep*. 2015;14(2):128-138.

Marrero JA, Feng Z, Wang Y, et al. Alpha-fetoprotein, des-gamma carboxyprothrombin, and lectin-bound alpha-fetoprotein in early hepatocellular carcinoma. *Gastroenterology*. 2009;137(1):110-118.

A copy of this interview is appearing in the April 2016 issue of *Clinical Advances in Hematology & Oncology.*