HCC IN FOCUS

Current Developments in the Management of Hepatocellular Carcinoma

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Update on Methylation Markers as Risk Predictors of Hepatocellular Carcinoma

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G&H How do methylation markers work for risk prediction of hepatocellular carcinoma?

JD Methylation markers are expressions of methylated, or modified, DNA. Genomic DNA can undergo epigenetic changes such as methylation. Some of these methylation changes can lead to further mutations in genes that result in tumorigenesis and cancer. Thus, the rationale is that as normal cells transform into tumor cells, their DNA undergoes more methylation changes. Therefore, if it is possible to measure methylation changes in different genes, this can potentially be used to predict the risk of hepatocellular carcinoma (HCC) or to detect it.

G&H Which methylation markers have been studied for HCC risk prediction, and what are their sensitivities and specificities?

JD Methylation has been studied in many genes. *SEPT9* and *HOXA1* are two examples of genes that have been evaluated in multiple studies. The key to understand is that methylation in one gene marker is very unlikely to have prognostic value. Usually, what has been done is to look at multiple sets of genes. Some of the most successful studies have looked at multiple sets of genes sometimes combined with protein markers, age, and/or sex. The sensitivities and specificities vary quite a bit. One of the best studies that has been performed so far is a phase 3 study that was published in *Clinical Gastroenterology and Hepatology* by Chalasani and colleagues. The authors looked at panels with 3 different genes that were methylated plus 1 protein marker (alpha-fetoprotein [AFP]) plus age, which had a sensitivity of 72% and a specificity of 88%. Although these are promising findings, it should be noted that many studies in this area have been performed in small numbers of HCC patients, not in large sample sizes.

G&H How do these markers compare with methods traditionally used for HCC risk prediction such as AFP, ultrasound, or risk scores?

JD Current methods include ultrasound with AFP or scores such as the GALAD score (based on gender, age, Lens culinaris agglutinin-reactive AFP [AFP-L3], total AFP, and des-gamma-carboxy prothrombin [DCP]) and the ASAP score (based on age, sex, AFP, and DCP). Depending on the study, some of the methylation markers perform the same as traditional methods, some perform worse, and some perform better. Markers such as AFP and scores such as GALAD have sensitivities of only approximately 60%. However, they have been studied in very large populations by different groups multiple times in different parts of the world, so these methods are relatively reliable. Even though their sensitivities may be better, a lot of the methylation markers being developed right now have been studied in only one population or in small groups. Interestingly, my colleagues and I looked at methylation markers in a large cohort of patients from Europe as well as Latin America and did not find that any of the markers led to early detection of HCC.

G&H Overall, what are the advantages of using methylation markers?

JD The main advantage is that they are blood-based markers. Methylation markers are usually expressed in liver tissue. However, for HCC screening or risk stratification, researchers are trying to measure these markers in blood. In order to do that, one has to detect cell-free DNA (cfDNA) in blood, often referred to as *liquid biopsy*. This involves detection of small pieces of DNA that are thought to be secreted by the tumor into the blood. If a particular gene is known to be methylated in a tumor and that methylated gene can be detected in blood when amplifying cfDNA, this can theoretically be used as a marker instead of relying on ultrasound or any other imaging technique. Another advantage is that there is no need to rely on proteins produced by the tumor. Historically, the majority of the markers that have been used in blood, either for HCC or for other cancers, are usually proteins that are produced by the tumor. Methylation markers represent methylated mutations of DNA from the tumor that are detected in blood, which are a little more specific.

G&H What are the main disadvantages of using these markers?

JD One of the disadvantages so far is that no particular panel has been validated for HCC in multiple large prospective or cross-sectional cohorts. The majority of studies have been small. What applies to one population might not apply to another. Another disadvantage is that in order to detect these methylated markers in blood, we need to be able to detect DNA from the tumor in blood. Many studies have shown that tumors have to be quite large to secrete enough DNA into blood to be detected. Also, sometimes a large amount of blood has to be extracted in order to detect this type of DNA. Another issue is that if methylated markers are detected in a patient who does not have HCC, we might know that this patient has a higher risk of developing HCC, but not when that will occur—for example, in 3 months, 6 months, 1 year, 5 years, or even never. It is sometimes unclear what to do with the data obtained from methylated markers. Some of the information may induce anxiety in patients, rather than help them.

G&H Could you discuss the potential use of these markers in different types of HCC, including early-stage disease, HCC of any etiology, and noncirrhotic HCC?

JD It would be ideal to try to use these markers in early-stage HCC. That is when these markers would be most useful. There is not much sense in using them to detect late-stage HCC. However, right now it is not yet clear that these markers can be used in early-stage HCC. As for HCC of different etiologies, I think methylation markers will work. However, we do not yet know whether the same markers could be used for every etiology or if etiology-specific markers would be needed. Whether methylated markers can be used in noncirrhotic HCC is also an interesting issue. A lot of patients with liver disease are noncirrhotic, but only a very small proportion of these patients will develop HCC, so I do not think it is justifiable at this point to screen all of these patients. However, one could envision running a methylation profile in some of these patients with liver disease to identify those at much higher risk of HCC and follow those patients more closely.

It should also be noted that some of these markers might work better in cancers that occur in healthy individuals (eg, colon or breast cancer that occurs in people who are healthy and all of a sudden develop a tumor). In such cancers, methylated DNA might be more specific. In the case of HCC, the overwhelming majority of patients have underlying cirrhosis, and cirrhosis of the liver already leads to methylated DNA changes. It is important to understand this difference so we can make sure our studies are appropriate and not get overexcited or come up with markers that are not good enough.

G&H What challenges remain regarding the use and validation of methylation markers for risk prediction in HCC?

JD I touched on this before, but one of the biggest challenges involves early-stage small tumors. In order to detect methylated DNA in blood, cfDNA is needed and a lot of studies have shown that it is difficult to obtain cfDNA in blood with small tumors. Some of the other challenges are that more and different populations are needed. Many studies have been relatively small and sometimes in only one population, so we need studies that involve a large number of patients with different underlying liver diseases and populations, for example, Latin Americans, Africans, and so on. In other words, generalization and validation of any marker are critical. Another challenge is to understand which methylation markers help in cirrhosis-related HCC vs which help in noncirrhotic HCC, which I think will differ.

G&H What other applications do methylation markers have in HCC?

JD One of the biggest applications is risk stratification for early HCC detection or screening. The goal is to draw

blood from a patient and perform a methylation assay. If the patient has a positive array, that suggests that they probably have a small tumor somewhere. That is what a lot of people are looking at methylation markers for. I think risk stratification is where these markers would have a bigger role. If methylated arrays of a patient show high risk, it might be necessary to screen this patient more often. Thus, I think that understanding which patients are at higher risk is one of the main applications. There are other applications that could be considered as well, such as response to therapy. In this case, providers could look if a patient has methylation of genes that are immune-related and then understand whether the patient has a different response to, for example, immune therapy. Studies are currently underway on this issue but are very early on.

> I believe that these markers have a lot of potential, but we are still quite early in the development process.

G&H Are there any upcoming or ongoing studies that you would like to mention?

JD There are multiple studies going on in the field of early HCC detection from different parts of the world. One that is not directly related but will be very important is the TRACER study. This study, which our institution is part of, is looking at the GALAD score in multiple different centers in the United States and is aiming to recruit more than 5000 patients who are going to be followed prospectively over many years. Although this study is focusing on the GALAD score, one of the things it will be doing is collecting blood samples that are going to be stored from the study participants. At the end of the study, there will be a large bank of samples, from which we can examine methylation markers as well as other markers so as to discover or validate markers.

G&H Do you have any final comments regarding methylation markers?

JD I think a lot of clinicians do not understand how these markers work or how the studies thus far have been performed. Coupled with a lot of interest from companies as well as from patients and providers, I think people are getting a little overexcited for the development of these blood-based biomarkers. I believe that these markers have a lot of potential, but we are still quite early in the development process. Although multiple companies are trying to obtain US Food and Drug Administration clearance, none of them have received it as of yet for HCC as far as I am aware.

Nevertheless, this is a very exciting area. For years, we have looked at either proteins produced by tumors or at somatic mutations in DNA that can predict HCC. This is one of the first times we are looking at a different type of marker related to epigenetic changes, and this is a major change in the field.

Disclosures

Dr Debes has no relevant conflicts of interest to disclose.

Suggested Reading

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