Liquid biopsies for Hepatocellular cancer and their potential in clinical practice

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Clinicians need to be aware of the powerful new technologies that are being directed toward early diagnosis of hepatocellular carcinoma (HCC). A recent article by **Qu C, Wang Y, Wang P, et al. Detection of early-stage hepatocellular carcinoma in asymptomatic HBsAg-seropositive individuals by liquid biopsy. Proc Natl Acad Sci U S A. 2019;116:6308-6312** reported that the presence of somatic mutations and HBV integrations in circulating cell free DNA (cfDNA), the concentration of cfDNA and concentration of protein tumor markers in blood were combined with age and gender to develop a "liquid biopsy" to identify early stage HCC. Mutations in TP53, CTNNB1, AXIN1 and the TERT promoter, and HBV integrations were detected using a newly developed parallel assay. The two protein markers were serum α-fetoprotein (AFP) and des γ carboxy prothrombin (DCP). The assay (HCCscreen) successfully identified four early-stage HCC cases (<3 cm) in 331 HBsAg (+) individuals who were negative for HCC on both AFP and ultrasonography screening. Although the diagnostic positive predictive value was low, the test panel has significant promise for the early detection of HCC in asymptomatic community based HBsAg-seropositive individuals.

This report adds to the small but increasing number of studies that have addressed this important new technology. Cell-free DNA (average length 120–160 bp) in plasma is derived from dying cells and in individuals without cancer most is released from hematopoietic cells. In patients with cancer, a variable fraction of cfDNA is derived from tumors and is thought to result from cancer cell apoptosis or necrosis. A study in 2018 by Labgaa et al, demonstrated the ability of ultra-deep sequencing to detect cfDNA mutations in known HCC oncogenes and tumor suppressor genes using plasma from patients with HCC[2]. A critical additional observation was that the mutations were present in HCC derived cfDNA and not in non-HCC cfDNA. Another study by Cohen et al, used tumor cfDNA mutations and protein tumor makers in a diagnostic "liquid biopsy" (CancerSEEK) for seven common cancers - colorectal, ovary, pancreas, breast, upper GI, lung and liver[3]. CancerSEEK utilizes a 61-amplicon panel that analyses 16 genes that are commonly mutated in these cancers and a single immunoassay platform that measures eight protein markers for these cancers. The assay reliably distinguished cancer patients from normal controls. Interestingly, in the 44 patients with liver cancer (89% HCC) the test had a sensitivity of 60% and a specificity of 97% to detect liver cancers and importantly the sensitivity for the five early-stage cancers (stage I) was 100%. The eight protein markers that were included in the assay were chosen because they were most useful in discriminating all cancers and not specifically HCC. The estimated cost of the assay was $500.

The Qu et al, paper has extended these findings. The addition of known protein biomarkers for HCC (AFP and DCP) to genetic mutations and insertions in HCC cfDNA has increased the sensitivity for detecting HCC to 98% and the specificity to 100% using a low risk HBsAg (-) healthy control training population. For the first time however, they used high risk individuals without HCC in the development of a different assay (HCCscreen). These high-risk individuals were HBsAg (+), AFP and/or ultrasound scan positive but had no HCC on diagnostic CT or MRI scans. A concern regarding the use of "liquid biopsy" assays is that HCC related cfDNA

alterations may also be present in non-tumor cell-free DNA from individuals with chronic hepatitis (e.g. HBV, HCV) or cirrhosis without HCC. This could result in increased numbers of false positive results. As predicted, there was a reduction in the sensitivity of HCCscreen to 85% and specificity to 93% in the high-risk training population but the AUROC was very good at 0.93. Finally, HCCscreen was tested in a lower risk community-based group of HBsAg (+) patients who were negative for HCC using AFP and ultrasound scan. In this group the assay identified HCC only with a 17% positive predictive value, 100% (4/4) sensitivity and 94% specificity (307/327). Limitations of the study were the short follow up time of 6 to 8 months and the loss of performance of HCCscreen in healthy controls that limits its use to HBsAg (+) individuals. The estimated cost of the assay was $150.

HCC is an unusual cancer where the diagnosis can be made either by histopathology or more commonly by dynamic CT or MRI liver scans[4]. Histopathological diagnosis is not affected by the pre-test probability for HCC, whereas a radiological diagnosis using LIRADS criteria can only be reliably made in those with cirrhosis, i.e. with a higher pretest probability for HCC. The advantage of an imaging diagnosis is that the stage of HCC is also obtained. One barrier that needs to be addressed before HCC “liquid biopsies” can be used for diagnosis in clinical practice is to increase the accuracy of the test regardless of the population in which it is used. Several types of promising biomarkers have been reported, and a multi-marker panel is anticipated to provide the improved sensitivity and specificity required in view of disease heterogeneity and fibrosis stage. Early studies of cfDNA in cancer patients found cancer associated methylation changes and more recent studies of HCC patients detailed the presence of methylated tumor suppression genes that could be used to potentially diagnose HCC[5]. Technologies to isolate circulating tumor cells from patients with HCC have improved and their presence has been associated with decreased survival and decreased disease-free survival[5]. Mass spectrometry techniques have allowed analysis of many proteins and post translational modifications of proteins e.g. glycosylation and metabolites and has identified a number associated with HCC. One study utilized the well documented Reveal-HBV Taiwan community cohort of untreated HBsAg (+) patients to show that glycosylated M2BP was a strong and independent short-term predictor of the development of HCC[6]. Similarly, the reported utility of serum metabolites CDCA, LPC20:5, succinyladenosine and uridine in HCC diagnosis awaits confirmation through independent validation[7]. Circulating noncoding RNAs are thought to function as post transcriptional modifiers of gene expression. One form of these, microRNA (miRNA) may be potentially useful for HCC detection. In a cohort of 60 HCV subjects a panel using miRNA-122-5p+ miRNA-486-5p + miRNA-142-3p distinguished HCC from cirrhosis (AUROC = 0.94; sensitivity = 80%, specificity = 95%) but this requires further validation[8].

Finally, the cost effectiveness of “liquid biopsies” needs to be established and this depends on the clinical context in which they will be used. Currently, these tests can stratify HCC risk in cirrhotic patients and therefore direct screening ultrasound scan ±AFP to those at highest risk. Cost-effectiveness analysis assesses the costs and patient outcomes of new healthcare options compared with current practice or other alternatives. In a screening context, over a long period these analyses predict the aggregated resources used in screening and all downstream events, diagnostic accuracy and the consequences of false results, patient-reported quality of life and likely survival. One cost-effectiveness analysis in the US setting used a 30-year Markov model to demonstrate that the use of a biomarker-based risk stratification test would be cost effective

compared to routine bi-annual ultrasound for moderate and high-risk individuals[9]. Depending on the screening protocol, in most cases the incremental cost-effectiveness ratio was under the acceptable $50,000 per quality-adjusted life year gain ratio and this was consistent across a wide range of HCC incidences (annual incidence 0.5%-7.0%). The cost-effectiveness results are predominantly governed by the values of ultrasound specificity, the abbreviated MRI specificity and cost, and the HCC risk biomarker cost. Importantly, uptake of screening is key to an effective program rollout which may be enhanced with a targeted approach to patients most likely to benefit.

It will be fascinating to observe the development of “liquid biopsies” for HCC and see how these new tests evolve from risk stratification tools to diagnostic and treatment response tests.

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