The prognosis of patients with hepatocellular carcinoma (HCC) is dependent on the stage of the tumor at diagnosis. Irrespective of its etiology, cirrhotic patients who have a high risk of developing HCC were recommended by all professional societies worldwide for HCC surveillance, including the American Association for the Study of Liver Diseases (AASLD), European Association for the Study of the Liver (EASL), and the Asian Pacific Association for the Study of the Liver (APASL). The guidelines point out that surveillance should be performed by experienced personnel using abdominal ultrasound every six months with or without test of serum α-fetoprotein (AFP). The evidences cited in the "AASLD guidelines for the treatment of hepatocellular carcinoma" showed that improvement in HCC survival has been observed with the surveillance, which is most likely due to the higher early-stage detection and higher curative treatment rates.

Currently, hepatic resection is still the mainstay of curative treatment for HCC which is confined to the liver with satisfactory liver function. The aim of HCC surveillance is to reduce HCC-related mortality and this is usually achieved through a screening and diagnosis at its early stage that enhances the applicability and improves cost-effectiveness of therapies. Some evidences showed that the HCC surveillance is not cost-effective in the cirrhotic patients with advanced liver failure or decompensation (Child-Pugh class C cirrhosis), that prevents effective HCC therapies, and was not recommended for these patients. Therefore, the target population for HCC screening, that is the candidates at-risk, is important. The specific set of population should have a relatively higher incidence of HCC and there is a higher probability for effective therapies that are suitable for these patients once diagnosed.

Because approximately 20%-50% of HCC patients have previously undiagnosed cirrhosis in some areas of China, all individuals seropositive for hepatitis B surface antigen (HBsAg) have been proposed to be screened for HCC by ultrasonography (US) and serum AFP (US/ AFP) every 6 months. A randomized surveillance study performed in one high-risk group of chronic HBsAg carriers during the early 2000 showed a 37% reduction in mortality for those who underwent surveillance. However, the results from a demonstration screening project following the same protocol conducted very recently in another high-risk group of chronic HBsAg carriers of China did not show a reduction in liver cancer mortality within the first 4 years of follow-up. The precise detection of HCC by ultrasonography requires experienced specialists, which restricted the widespread application to all HBsAg-positive individuals particularly to those with limited acceptability of resource. Furthermore, biannual screening for all HBsAg-positive individuals is associated with more than necessary follow-up appointments and anxiety-producing procedures. Clinical practices have realized the low compliance to the repeated screening, unnecessary follow-up procedures and anxiety in the asymptomatic HBsAg-seropositive individuals. Currently, most HCC cases in China are detected on the basis of clinical symptoms rather than by HCC screenings and are at an advanced stage when diagnosed in hospitals.

Tumor biomarkers, including AFP, AFP-L3, and des-y-carboxy-prothrombin (DCP) had been examined for accurate early detection of HCC and the data available show that the biomarkers tested are suboptimal for routine surveillance. Recently, several studies developed liquid biopsy assays based on detection of the genetic alterations in circulating tumor DNA (ctDNA) in peripheral blood of the at-risk individuals and showed promising for finding curable HCC and early-stage cancers of diverse tissue types. However, most of these studies were conducted on individuals previously diagnosed with...
cancer, and no high-risk individuals were used as controls.\textsuperscript{7} The performance of these liquid biopsy assays could be compromised in high-risk populations with chronic HBV infection, because some precancerous lesions, such as cirrhosis, might also harbor driver mutations prevalent in HCCs.\textsuperscript{8}

Recently, we developed a novel liquid biopsy assay, termed HCCscreen (Figure 1), to identify HCC cases in a high-risk, asymptomatic community population.\textsuperscript{9} The HCC and non-HCC cases in the training cohort were identified from the same population with the same method, AFP/US. Such non-HCC cases, especially those with liver nodules, were useful for the identification of HCC-specific biomarkers and thresholds to precisely detect HCC cases. In the selection of biomarkers, we focused on frequently altered genetic biomarkers with a clear oncogenic mechanism, such as the mutations in the promoter of telomerase reverse transcriptase (TERT), and serum protein markers that are of clear diagnostic value, such as DCP. We included a limited number of candidate biomarkers with a clear link to HCC, to avoid the overfitting effect when studying numerous candidate biomarkers on a limited number of tumor/normal cases. With the combination of population and biomarker selections, we aimed to set up an HCC early detection model that would not only show appropriate sensitivity and specificity in the training cohort but also have a comparable diagnostic value of early-stage HCC from high-risk individuals in different populations.

Using this HCCscreen assay, we found it possible to identify individuals with early-stage HCCs and to discriminate them from those non-HCC individuals with chronic liver disease, including cirrhosis. The assay yielded 85% sensitivity and 93% specificity in the diagnosis of HCC from the individuals with US-detected liver nodules and/or increased serum AFP. More importantly, the performance was also maintained with the AFP/US-negative validation cohort where the sensitivity and specificity were 100% and 94%, respectively. The current positive predictive value (PPV) of 17% from the validation cohort was significantly higher than previously obtained with screening for AFP levels alone.\textsuperscript{10} The PPV could be further improved if a second HCCscreen test is provided to the cases positive in the first test. A high PPV would be very helpful for routine application in the clinic, as it would reduce unnecessary anxiety and follow-up examinations of the non-HCC individuals.

The HCCscreen liquid biopsy assay enables centralized and standard processing and requires minimal expertise and equipment. Currently, we are conducting a controlled, randomized clinical trial (ChiCTR1800020233) to compare the efficacy of detecting the HCC that are <3 cm, and are <5 cm in diameter and the false negative rate by using HCCscreen or using US/AFP. This trial will be finished by Dec 31, 2020. When the efficacy is proven, this assay might be highly suitable for HCC screening as a routine test in at-risk individuals particular in the clinics with limited resource.

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**References**

1. Omata M, Cheng AL, Kokudo N, Kudo M, Lee JM, Jia J, Tateishi R, Han KH, Chawla YK, Shiina S, et al. Asia–Pacific clinical practice guidelines on the management of hepatocellular carcinoma: a 2017 update. Hepatol Int. 2017;11(4):317–370
2. Kim JH, Kim YD, Lee M, Jun BG, Kim TS, Suk KT, Kang SH, Kim MY, Cheon GJ, Kim DJ, et al.; European Association for the Study of the Liver. Electronic address, e.e.e. and L. European association for the study of the, EASL clinical practice guidelines: management of hepatocellular carcinoma. J Hepatol. 2018;69(1):182-236. doi:10.1016/j.jhep.2018.07.018.
3. Heimbach JK, Kulik LM, Finn RS, Sirlin CB, Abecassis MM, Roberts LR, Zhu AX, Murad MH, Marrero JA. AASLD guidelines for the treatment of hepatocellular carcinoma. Hepatology. 2018;67(1):358–380. doi:10.1002/hep.29086.
4. Zhang B-H, Yang B-H, Tang Z-Y. Randomized controlled trial of screening for hepatocellular carcinoma. J Cancer Res Clin Oncol. 2004;130(7):417–422. doi:10.1007/s00432-004-0552-0.
5. Ji M, Liu Z, Chang ET, Yu X, Wu B, Deng L, Feng Q, Wei K, Liang X, Lian S, et al. Mass screening for liver cancer: results from a demonstration screening project in Zhongshan City, China. Sci Rep. 2018;8(1):12787. doi:10.1038/s41598-018-31119-9.
6. Xu RH, Wei W, Krawczyk M, Wang W, Luo H, Flagg K, Yi S, Shi W, Quan Q, Li K, et al. Circulating tumour DNA methylation markers for diagnosis and prognosis of hepatocellular carcinoma. Nat Mater. 2017;16(11):1155–1161. doi:10.1038/nmat4997.
7. Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, et al. Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science. 2018;359(6378):926–930. doi:10.1126/science.aar3247.
8. Zucman-Rossi J, Villanueva A, Nault J-C, Llovet JM. Genetic landscape and biomarkers of hepatocellular carcinoma. Gastroenterology. 2015;149(5):1226–1239 e4. doi:10.1053/j.gastro.2015.05.061.
9. Qu C, Wang Y, Wang P, Chen K, Wang M, Zeng H, Lu J, Song Q, Diplas BH, Tan D, 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(13):6308–6312. doi:10.1073/pnas.1819799116.
10. Chun S, Rhie S, Ki C-S, Kim J, Park H-D. Evaluation of alpha-fetoprotein as a screening marker for hepatocellular carcinoma in hepatitis prevalent areas. Ann Hepatol. 2015;14(6):882–888. doi:10.5604/16652681.1171776.