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Identifying Hepatitis B Carriers at Low Risk for Hepatocellular Carcinoma

See "High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load," by Tseng T–C, Liu C–J, Yang H–C, et al, on page 1140.

Advances in antiviral therapy have revolutionized the treatment of chronic hepatitis B virus (HBV) infection in the last decade. Both interferon and nucleot(s)ide analogs can significantly reduce the risk of hepatocellular carcinoma (HCC) related to HBV infection.[1,2] However, interferon treatment is limited by its adverse effects and the need for subcutaneous injection. Nucleot(s)ide analogs often require long-term treatment, which leads to problems with poor drug adherence and drug resistance. Therefore, much effort has been spent to define "inactive hepatitis B surface antigen (HBsAg) carriers," a group of patients who have a low risk of developing HCC and hence may not need antiviral therapy or HCC surveillance.

The strong association between serum HBV DNA and the risk of HCC has been consistently demonstrated in large-scale, longitudinal, cohort studies.[3,4] Patients with HBV DNA >10,000 copies/mL (or 2000 IU/mL) have a greater risk of developing HCC than those with lower HBV DNA levels. In the Risk Evaluation of Viral Load Elevation and Associated Liver Disease/Cancer-Hepatitis B Virus (REVEAL-HBV) study, which included predominantly noncirrhotic hepatitis B e antigen (HBeAg)-negative persons with normal alanine aminotransferase (ALT), the incidence of HCC per 100,000 person-year follow-up was approximately 110 if HBV DNA at enrollment was $<10^4$ copies/mL, whereas it was 296, 962, and 1152 if HBV DNA at enrollment was 10^4–10^5, 10^5–10^6, and $>10^6$

copies/mL, respectively.[3] These clinical observations have formed the backbone in predicting risk of HCC. In the American Association for the Study of Liver Diseases guidelines, HBeAg-negative patients with HBV DNA <2,000 IU/mL and persistently normal ALT levels can be regarded as inactive HBsAg carriers, who may not need antiviral therapy owing to their low risk of HCC.[5]

A few groups have tried to develop risk calculators to predict the future risk of HCC. A group in Korea and 2 groups in Hong Kong have developed risk calculators using data from their respective hospital-based cohorts.[6–8] Risk scores were derived by mathematical modeling based on the clinical and laboratory factors at the first visit. In general, older age, higher HBV DNA, and the presence of liver cirrhosis increase the risk of HCC development on subsequent follow-up. In the study by Han and Ahn,[6] the 3-year risk of HCC was 0.62% for the low-risk group, compared with 4.8% in the intermediate-risk group, and 22.9% in the high-risk group.[6] In a study by Wong et al,[8] the 5-year risk of HCC was 1.7% in the low-risk group, compared with 9.5% in the intermediate-risk group, and 21.1% in the high-risk group. Patients in the low-risk group are recommended for observation and may not need antiviral therapy.

One potential problem of these models is the heavy weight assigned to liver cirrhosis in the risk score.[6–8] Because early liver cirrhosis may be missed on ultrasonography and liver biopsy is not feasible as a screening tool, the predicted risk of HCC may suffer from serious error if the presence or absence of liver cirrhosis is misclassified. Furthermore, all patients with liver cirrhosis should be considered for antiviral therapy.[5] As a result, the REVEAL-HBV study group has derived another risk calculator based on only patients who have no liver cirrhosis at study entry, and this model was validated by the hospital-based cohorts from the Korean and the 2 Hong Kong centers.[9] However, ultrasonography was used in the REVEAL-HBV cohort and inaccurate classification of liver cirrhosis was inevitable. Moreover, all patients in the REVEAL-HBV study were >30 years old, so this model might not be applicable to patients in younger age groups. In this model, different weights were given to different categories of gender, age, ALT, HBeAg, status, and HBV DNA to derive a summation score of 0–17, which corresponded with a 3- and 5-year risk of HCC of 0%–23.6% and 0%–47.4%, respectively. One difficulty of using this risk calculator is the lack of a clear cutoff value for low and high risk of HCC. For example, the 3-year risk of HCC for a score of 10–13 is 0.9%, 1.4%, 2.3%, and 3.7%, respectively. It may be more clinically useful if some of the risk scores are combined to define groups with low, intermediate, and high risk for HCC. The knowledge of the risk score cannot be easily translated to a clinical decision of antiviral therapy, because decisions about the need for and timing of antiviral therapy relies on the knowledge of HCC risk, not just in the next 3 years, but over a longer period such as in the next 10–20 years. Furthermore, levels of HBV DNA and ALT change during follow-up and patients with high levels at presentation that normalize during follow-up have lower risk of HCC than those with persistently high levels.[10]

Serum HBsAg testing has long been used qualitatively for the diagnosis of HBV infection. Recent studies have suggested that serum HBsAg level can reflect the amount and transcriptional activity of covalently closed circular DNA inside the liver.[11] Lower levels of serum HBsAg are associated with HBeAg-negative, inactive disease[12,13] and higher chance of spontaneous seroclearance of HBsAg.[14,15] HBsAg level <1000 IU/mL seems to be a reliable indicator of inactive disease and HBsAg <100 IU/mL is a good predictor of subsequent spontaneous HBsAg seroclearance. It becomes logical to hypothesize that low serum HBsAg level can be another marker of an "inactive HBsAg carrier" status with lower HCC risk.

In this issue of GASTROENTEROLOGY, Tseng et al[16] investigated the risk of HCC among a retrospective–prospective cohort of 2688 Taiwanese chronic hepatitis B patients followed for a mean of 14.7 years (ERADICATE-B). Liver cirrhosis was excluded by histology or ultrasonographic findings together with clinical features such as thrombocytopenia, varices, and/or ascites. In other words, only patients with advanced cirrhosis were excluded. This study confirmed the findings of the REVEAL-HBV study that male gender, older age, higher serum ALT, positive HBeAg, higher serum HBV DNA, and genotype C HBV were associated with a higher risk of HCC. The overall annual incidence of HCC among patients with HBV DNA <2000 IU/mL and 2000–19,999 IU/mL at presentation were approximately 180 and 369 per 100,000 person-years, respectively, which were comparable with that reported in the REVEAL-HBV study. On overall analysis, higher serum HBsAg level was not associated with higher risk of HCC. It was only on subanalysis of HBeAg-negative patients with HBV DNA <2000 IU/mL that serum HBsAg turned out to be an independent predictor of HCC development; the annual incidence of HCC per 100,000 person-years of follow-up was 58.2 and 326.1 for patients with HBsAg at presentation of <1000 IU/mL and ≥1000 IU/mL, respectively. Thus, HBeAg-negative patients with HBV DNA <2000 IU/mL and HBsAg <1000 IU/mL had a very low risk of HCC, whereas those with HBV DNA <2000 IU/mL and HBsAg ≥1000 IU/mL had similar risk of HCC as those with HBV DNA 2000–19,999 IU/mL (Figure 1).

In this study, the authors have not investigated whether HBsAg level can further stratify the HCC risk among patients with HBV DNA 2000–19,999 IU/mL.[16] Preliminary data from a recent analysis of the REVEAL-HBV cohort showed that the 17-year risk of HCC among 2840

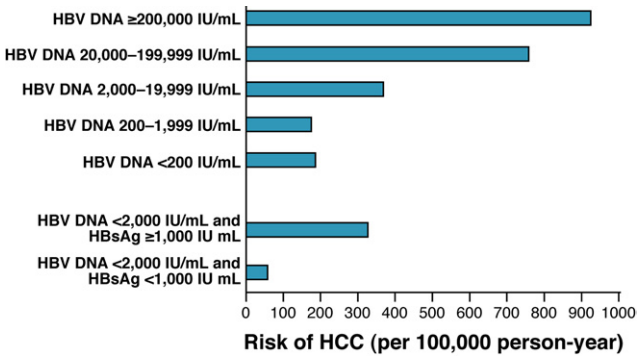

Figure 1. Risk of developing hepatocellular carcinoma with reference to the HBV DNA and HBsAg levels at study entry in the ERADICATE-B cohort.[16]

HBeAg-negative noncirrhotic persons were 5.2%, 3.4%, and 1.7% among those with HBV DNA $>10^4$–10^5copies/mL (2000–20,000 IU/mL) and HBsAg >1000, >100–1000, and <100 IU/mL, respectively.[17] On the other hand, the risk of HCC among persons with HBV DNA $<10^4$ copies/mL (<2000 IU/mL) and HBsAg >1000 IU/mL was 4.3%, which was higher than that of those with HBV DNA $>10^4$–10^5 copies/mL (2000–20,000 IU/mL) and HBsAg <1000 IU/mL. These observations suggest that HBsAg level may provide supplementary information on the risk of HCC among patients with intermediate HBV DNA levels.

With quantitative HBsAg on board, the only missing link to a good risk calculator of HCC is an accurate exclusion of liver cirrhosis and preferably advanced liver fibrosis. In previous studies, ultrasonography has been used as the primary tool to exclude early cirrhosis. In a recent study of 1130 Korean chronic hepatitis B patients followed for a median of 30.7 months, lower liver stiffness measurement by transient elastography at enrollment was associated with a lower risk of developing HCC.[18] Patients who had liver stiffness measurement ≤8 kPa had a 3-year cumulative HCC risk of 1.58%, which was much lower than those of patients with higher liver stiffness measurements.

In summary, Tseng et al[16] have demonstrated that noncirrhotic chronic hepatitis B patients with HBV DNA <2000 IU/mL and HBsAg <1000 IU/mL have very low risk of HCC development in a large-scale, long-term follow-up study. Serum HBsAg level can supplement, but not substitute for, HBV DNA in the definition of "inactive HBsAg carrier." In the development of future management guidelines and risk calculators, one should consider integrating serum HBsAg level into the known disease indicators, particularly among patients with low and intermediate HBV DNA levels. The remaining question is how high a risk of HCC can one accept for deferring antiviral therapy. The HCC risk of inactive HBsAg carriers will remain higher than non-HBV infected persons no matter how we define it, but the benefits of treating low-risk carriers must be balanced against risks of side effects and drug resistance as well as costs.[19]

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Conflicts of interest

H.L.Y. Chan is a consultant and speaker for Abbott, Bristol-Myers Squibb, Gilead, Merck, Novartis Pharmaceutical, and Roche; has served as a speaker for Fu Rui and Glaxo-Smith-Kline; and has received an unrestricted grant from Roche for hepatitis B research.

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Variants in Autophagy Genes Affect Susceptibility to Both Crohn's Disease and *Helicobacter pylori* Infection

See "Vacuolating cytotoxin and variants in *Atg16L1* that disrupt autophagy promote *Helicobacter pylori* infection in humans" by Raju D, Hussey S, Ang M, et al, on page 1160.

Helicobacter pylori (H pylori) is a Gram-negative, microaerophilic bacterium that selectively colonizes the stomachs of half the world's human population. *H pylori* is the etiologic agent of several gastric diseases, including chronic gastritis and peptic ulcers. Long-term, chronic inflammation subsequently increases the risk for the development of mucosa-associated lymphoid tissue lymphoma and gastric cancer.[1,2] During a well-choreographed interaction between the bacteria and the host gastric epithelium, *H pylori* infection initiates an immune response that leads to a massive infiltration of inflammatory cells.[1] An evolutionarily conserved cellular mechanism, autophagy, functions as an innate defense lysosomal pathway in response to infection to degrade intracellular micro-organisms attempting to establish a replicative niche in the host epithelial cell cytoplasm.[3] Unfortunately, *H pylori* utilizes a novel escape mechanism to evade lysosomal destruction in host epithelial cells that subsequently supports chronic infection. A study in this month's issue of GASTROENTEROLOGY identifies, for the first time, an autophagy gene as a candidate for host susceptibility to *H pylori* infection and disease progression.[4]

The *H pylori*–Vacuolating Cytotoxin

Vacuolating cytotoxin (VacA) is an important virulence factor for *H pylori* disease pathogenesis. The VacA gene encodes a 96-kDa precursor protein that is secreted and cleaved into an 88-kDa mature protein and a 10.5-kDa passenger domain.[1] The mature 88-kDa toxin can undergo further proteolytic cleavage to yield the p33 and p50 fragments which represent the VacA functional domains that are required for toxin activity.[5] The most well recognized effect of VacA intoxication of mammalian cells is the induction of vacuolation.[6] The toxin generates the formation of membrane channels by being secreted as monomers that oligomerize at the host plasma membrane.[6] Glycosylphosphatidylinositol anchored proteins enriched early endosomal compartments are involved in endocytosis of VacA.[7] VacA then traffics to lysosomal compartments where the toxin induces vacuolation via a mechanism dependent on GTPase Rab7,[8,9] dynamin,[10] and syntaxin 7.[11] In addition to vacuolation, VacA intoxication has detrimental effects that include inhibition of T-cell proliferation[12] and the induction of apoptosis,[6] increased cellular permeability,[8] and autophagy within gastric epithelial cells.[3,9] Initial studies demonstrated that *H pylori* invades gastric epithelial cells and resides within vacuoles.[13] Later experiments then showed that these vacuoles were associated with the autophagy pathway and attributed to bacterial intracellular survival of *H pylori* within gastric epithelial cells.[3,9]

Autophagy in Response to *H pylori* VacA and Persistent Bacterial Infection

In recent years, autophagy has been recognized as being of central importance for the maintenance of cell homeostasis and survival but also for the regulation of inflammation and for bacterial defense at body surfaces.[14] Although *H pylori* is generally considered to be a noninvasive pathogen, emerging evidence demonstrates that the bacteria also invade and replicate within autophagasomes of macrophages, dendritic, and epithelial cells.[3,9,12,15] In view of the observation that *H pylori* infection induces autophagy within epithelial cells,[3,9] the question then remains as to how *H pylori* are allowed to replicate within the autophagasome and evade lysosomal-induced degradation.

Raju et al[4] recently reinforced that VacA, independent of the bacteria, alters the degradative capacity of the endocytic pathway. Using gastric cancer epithelial AGS cells treated with culture supernatants from VacA-positive *H pylori* the investigators show that although VacA intoxication induces autophagasome–lysosomal fusion cathepsin D levels were negligible.[4] Cathepsin D is a key hydrolase necessary for lysosomal-induced degradation. Therefore, lack of cathepsin D alternately disrupts autophagic degradative