Risk stratification of HBV infection in Asia-Pacific region

Jia-Horng Kao

Department of Internal Medicine, National Taiwan University Hospital, Graduate Institute of Clinical Medicine, Hepatitis Research Center, and Department of Medical Research, National Taiwan University College of Medicine and National Taiwan University Hospital, Taipei, Taiwan

Hepatitis B virus (HBV) infection is prevalent in Asia, Africa, Southern Europe and Latin America, where the prevalence of HBsAg in the general population ranges from 2% to 20%. The long-term outcomes of chronic HBV infection vary widely. Literature review indicates that two-thirds of Asian HBV carriers have favorable clinical outcomes including inactive carrier state and loss of hepatitis B surface antigen (HBsAg), whereas one-third of them develop adverse outcomes including hepatic decompensation, cirrhosis and even hepatocellular carcinoma (HCC) in their lifetime. Because the lifetime risk of Asian HBV carriers to develop end-stage liver disease is as high as 15% to 40%. The identification of risk factors for the development of advanced liver disease, especially cirrhosis and HCC, in Asian HBV carriers is thus important for the implementation of effective antiviral treatment as early as possible. Recently, several qualitative and quantitative hepatitis B viral factors affecting the natural course and treatment outcome of HBV-DNA, HBsAg, and ALT levels, ERADICATE-B study proposed an algorithm to predict disease progression and categorize risk levels of HCC as well as corresponding management in Asian HBV carriers. The introduction of transient elastography may further enhance the predictive power. In conclusion, HBsAg level can complement HBV-DNA level for the risk stratification of disease progression in Asian adult patients with chronic HBV infection. (Clin Mol Hepatol 2014;20:223-227)

Keywords: Hepatitis B virus (HBV); Hepatocellular carcinoma; HBV-DNA; HBsAg; Risk stratification

INTRODUCTION

Hepatitis B virus (HBV) infection is prevalent in Asia, Africa, Southern Europe and Latin America, where the prevalence of HBsAg in the general population ranges from 2% to 20%. The long-term outcomes of chronic HBV infection vary widely. Literature review indicates that two-thirds of Asian HBV carriers have favorable clinical outcomes including inactive carrier state and loss of hepatitis B surface antigen (HBsAg), whereas one-third of them develop adverse outcomes including hepatic decompensation, cirrhosis and even hepatocellular carcinoma (HCC) in their lifetime. Because the lifetime risk of Asian HBV carriers to develop end-stage liver disease is as high as 15% to 40%. The identification of risk factors for the development of advanced liver disease, especially cirrhosis and HCC, in Asian HBV carriers is thus important for the implementation of effective antiviral treatment as early as possible. Recently, several qualitative and quantitative hepatitis B viral factors affecting the natural course and treatment outcome of HBV-DNA, HBsAg, and ALT levels, ERADICATE-B study proposed an algorithm to predict disease progression and categorize risk levels of HCC as well as corresponding management in Asian HBV carriers. The introduction of transient elastography may further enhance the predictive power. In conclusion, HBsAg level can complement HBV-DNA level for the risk stratification of disease progression in Asian adult patients with chronic HBV infection. (Clin Mol Hepatol 2014;20:223-227)

Keywords: Hepatitis B virus (HBV); Hepatocellular carcinoma; HBV-DNA; HBsAg; Risk stratification

Abbreviations:
BERADICATE-B, Elucidation of risk factors for disease control or advancement in Taiwanese hepatitis B carriers; cccDNA, covalently closed circular DNA; HBeAg, Hepatitis B e antigen; HBsAg, Hepatitis B surface antigen; HBV, Hepatitis B virus; HCC, Hepatocellular carcinoma; REACH-B, Risk estimation for hepatocellular carcinoma in chronic hepatitis B; REVEAL-HBV, Risk evaluation of viral load elevation and associated liver disease/cancer-hepatitis B virus; SEARCH-B, Study of E antigen seroClearance of hepatitis

Corresponding author: Jia-Horng Kao
Director and Distinguished Professor, Graduate Institute of Clinical Medicine, National Taiwan University College of Medicine, 1 Chang-Te St., Taipei 10002, Taiwan
Tel: +886-2-23123456 ext. 67307, Fax: +886-2-23825962
E-mail: kaojh@ntu.edu.tw

Received: May 14, 2014 / Accepted: Jul. 31, 2014
of HBV carriers have been identified. Among these viral factors, baseline serum HBV-DNA level is the main driving force for cirrhosis and HCC development in adult HBV carriers. Recently, quantitative hepatitis B surface antigen (qHBsAg) has been increasingly recognized to be a biomarker to predict both favorable and adverse outcomes of HBV carriers. In this review, qualitative and quantitative HBV factors affecting disease progression and the risk stratification for disease progression in Asian HBV carriers will be discussed.

**Qualitative HBV factors**

**HBV genotype**

At least 10 HBV genotypes (A to J) have been defined, and HBV genotype has been documented to have specific geographic distribution and influence the long-term outcomes of HBV infection. In Asia-Pacific region, HBV genotype B and C are the predominant strains and HBV genotype C patients, compared to genotype B patients, have late or absent HBeAg seroconversion after multiple hepatitis flares that accelerate the progression of chronic hepatitis. Most previous studies indicated that patients with HBV genotype C infection have a higher risk of cirrhosis and HCC than those with genotype B infection.

**HBV mutant**

Mutations in precore, core promoter and deletion mutation in pre-S/S genes have been reported to be associated with the progression of liver disease, including cirrhosis and HCC. Previous studies revealed that dual mutations in basal core promoter (BCP) A1762T/G1764A were strongly associated with the risk of HCC development. Deletion mutations in the pre-S gene of HBV genome frequently occur in chronic HBV infection. The deletion over pre-S gene may cause accumulation of large surface protein in the endoplasmic reticulum (ER), resulting in ER stress and hepatocarcinogenesis.

**Quantitative HBV factors**

During HBV replication, pregenomic RNA can be transcribed from covalently closed circular DNA (cccDNA) to serve as the template of negative-strand DNA through reverse transcription, and then fully double-stranded DNA through DNA polymerase within the nucleocapsid, finally with the assembly of envelope protein to form mature HBV virions. Several mRNAs can also be transcribed from cccDNA and then translate to viral proteins. Therefore, several qualitative viral factors can be clinically useful, including HBV-DNA from the infectious particles and circulating viral proteins such as HBsAg and HBeAg.

**HBV-DNA level**

Several commercial real-time PCR assays have been developed to detect and quantify HBV DNA. Nowadays, quantification of serum HBV-DNA is recommended by several international HBV guidelines to diagnose HBV infection, establish the indication for therapy, and to monitor antiviral treatment responses and emergence of drug resistance. HBV-DNA quantification also provides valuable prognostic information. The impact of viral load on the risk of HCC was assessed in a population-based prospective cohort of untreated CHB Taiwanese patients (REVEAL-HBV study). The data showed that the cumulative incidence of HCC increased with serum HBV DNA level. It ranged from 1.3% to 14.9% for patients with an HBV DNA level of less than 300 copies/ml (~60 IU/mL) and 10 copies/ml (~200,000 IU/mL) or more, respectively (P<0.001). A recent hospital-based cohort study further validated the HCC risk started to increase when HBV-DNA level was higher than 2,000 IU/mL. All these data confirm that HBV-DNA level is the trigger of hepatitis activity and the strongest risk factor associated with HCC development in adult Asian patients with chronic HBV infection.

**HBSAg quantification**

In addition to HBV-DNA level, the clinical significance of quantitative HBsAg has become increasingly recognized. This old biomarker has a new role in current management of chronic HBV infection. In the natural course of HBV infection, HBV-DNA is only from infectious particles and levels reflect viral replication. Accordingly, a decline of HBV-DNA means reduction of HBV replication. In contrast, HBsAg can be derived from both mature virions and defective particles. Thus serum HBsAg level not only reflects the cccDNA transcription or mRNA translation, but also host immune control over HBV infection. Our recent study showed that low serum HBsAg level (<100 IU/mL), alone or in combination with HBV-DNA levels, at 1 year after HBeAg seroconversion could predict HBsAg loss in patients with HBV genotype B or C infection. In addition, qHBsAg was better than serum HBV-DNA level for the prediction of spontaneous HBsAg loss in HBeAg-negative carriers with a low viral load (<2,000 IU/mL). Moreover, HBsAg level <10 IU/mL was the strongest predictor of HBsAg loss in patients with a low viral load.

Taken together, there indeed exists a correlation between qHBsAg quantification and the risk of HCC development in Asian HBV carriers.
sAg and liver disease progression. In the recent update of REVEAL-HBV study, qHBsAg was analyzed, and the results showed that both HBsAg and HBV-DNA levels are independent predictors of HCC development.30 Our hospital-based ERADICATE-B study (Elucidation of Risk Factors for Disease Control or Advancement in Taiwanese Hepatitis B Carriers) also showed similar findings. In brief, a total of 2,688 non-cirrhotic Taiwanese chronic hepatitis B patients followed for a mean of 14.7 years. HCC risk increased when patients had increased HBV DNA level.31 Although the incidence of HCC is significantly associated with baseline serum HBV-DNA levels, inactive or low risk HBV carriers (serum HBV-DNA levels <2,000 IU/ml) still have significantly higher hazard ratios for HCC compared with individuals without HBV infection. Therefore, it is important to identify factors predictive of HCC other than HBV-DNA level in these inactive or low risk HBV carriers. In the ERADICATE-B study, we evaluated 1,068 HBsAg-negative patients with low viral load (<2,000 IU/mL). Risk factors for HBeAg-negative hepatitis as well as HCC development included advanced age, male gender, elevated levels of ALT and high qHBsAg (≥1,000 IU/ml), but not levels of HBV DNA. Multivariate analysis revealed that qHBsAg ≥1,000 IU/mL was an independent risk factor for HCC development.31 Data from REVEAL-HBV study and ERADICATE-B study all showed that serum HBsAg and HBV DNA levels were complementary markers in predicting disease progression to cirrhosis and HCC.31,32

Risk calculator and algorithm for disease progression

Several host and viral factors predictive of HCC risk have been identified, and the REACH-B (Risk Estimation for Hepatocellular Carcinoma in Chronic Hepatitis B) study has developed and validated a predictive score for the risk of development of HCC in patients with chronic hepatitis B.33 This study included risk score development cohort with 3,584 non-cirrhotic CHB Taiwanese and a validation cohort with 1,050 patients from three independent hospitals of Hong Kong and Republic of Korea. The 17-point risk score is composed of 5 predictors of HCC, including sex, age, serum ALT level, HBeAg status, and serum HBV-DNA level. The risk score could precisely estimate the risk of HCC development at 3, 5, and 10 years of follow-up. Nevertheless, this risk scoring system of HCC may underestimate risk for patients with very low viral load at baseline. In ERADICATE-B study, the risk of HCC for carriers with HBV-DNA <2,000 IU/mL and HBsAg ≥1,000 IU/mL was much higher than those with HBV-DNA <2,000 IU/mL and HBsAg <1,000 IU/mL.31 This finding indicated that high HBsAg level itself may reflect inadequate host immune control against HBV infection, leading to an increased HCC risk over time in patients with low viral load. Therefore, serum HBsAg level should be integrated into the HCC risk calculator for future management of patients with chronic HBV infection, particularly in those with low and intermediate viral loads. Furthermore, the relationships between HCC risk and dynamic changes of serum HBV-DNA, HBsAg, and ALT levels were determined in the ERADICATE-B study. Compared to patients with persistently low levels of HBV-DNA, HBsAg, or ALT, those with persistently high levels of these 3 factors were at a higher risk of HCC.32 Therefore, it is essential to incorporate qualitative and quantitative hepatitis B viral factors in risk calculation model to make it more comprehensive and to be clinically useful in various forms of chronic liver disease, including inactive carrier state, chronic hepatitis and cirrhosis (Fig.).

Transient elastography-based risk estimation

The severity of hepatic fibrosis is known to be associated with HCC risk; however, this parameter has not been incorporated in the risk model for HCC development because of the invasive nature of liver biopsy. Recently, liver stiffness measurement by using transient elastography has been introduced as a noninvasive method for assessing the severity of hepatic fibrosis.35 A previous study showed that the baseline liver stiffness values could be an independent predictor of HCC in patients with chronic HBV infection, where the 3-year cumulative incidence of HCC was significantly higher in patients with a higher liver stiffness value.34 A recent Korean study included 1,250 CHB patients with baseline liver stiffness values to construct a predictive model for HCC occurrence based on a Cox proportional hazards model.36 By using
multivariate analysis, age, male gender, and liver stiffness values were independent predictors of HCC, whereas HBV-DNA level $\geq 20,000$ IU/L showed borderline statistical significance. A predictive model for HCC was developed using these 4 variables, with a correlation coefficient of 0.905 between predicted risk and observed risk of HCC occurrence. The suggested formula for 3-year probability of HCC occurrence is $1 - PA(A=\exp (0.05306\times\text{age}+1.106\times\text{male gender}+0.04858\times\text{liver stiffness value}+0.50969\times\text{HBV DNA} \geq 20,000\ \text{IU/L})$. Thus, this novel model may accurately estimate the risk of HCC occurrence in Asian patients with chronic HBV infection.

**CONCLUSIONS**

Several host and HBV factors such as age, gender, ALT level, hepatic fibrosis stage, serum HBsAg level, viral load, genotype and mutants are shown to affect disease progression and HCC development in chronic hepatitis B patients. On the basis of population and hospital-based cohort studies, risk stratification for disease progression and HCC in patients with chronic HBV infection has been established step by step. In the future, multivariate risk assessment profiles for disease progression and HCC should be integrated with current HBV treatment guidelines to enable practicing physicians to have better management of HBV carriers with different HCC risks.

**Acknowledgements**

The study was supported by grants from the National Taiwan University Hospital, the Ministry of Health and Welfare, and the Ministry of Science and Technology, Executive Yuan, Taiwan.

**Conflicts of Interest**

The author has no conflicts to disclose.

**REFERENCES**

1. Kao JH, Chen DS. Global control of hepatitis B virus infection. Lancet Infect Dis 2002;2:395-403.
2. Kao JH, Chen PJ, Chen DS. Recent advances in the research of hepatitis B virus-related hepatocellular carcinoma: epidemiologic and molecular biological aspects. Adv Cancer Res 2010;108:21-72.
3. Kao JH. Hepatitis B virus genotypes and hepatocellular carcinoma in Taiwan. Intervirology 2003;46:400-407.
4. Lin CL, Kao JH. Hepatitis B Viral factors and clinical outcomes of chronic hepatitis B. J Biomed Sci 2008;15:137-145.
5. Lin CL, Kao JH. Hepatitis B viral factors and treatment responses in chronic hepatitis B. J Formos Med Assoc 2013;112:302-311.
6. Chen CL, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65-73.
7. Tseng TC, Kao JH. Clinical utility of quantitative HBsAg in natural history and nucleos(t)ide analogue treatment of chronic hepatitis B: a new trick of old dog. J Gastroenterol 2013;48:13-21.
8. Lin CL, Kao JH. Risk stratification for hepatitis B virus-related hepatocellular carcinoma. J Gastroenterol Hepatol 2013;28:10-17.
9. Liu CL, Kao JH. Global perspective on the natural history of chronic hepatitis B: role of hepatitis B virus genotypes A to J. Semin Liver Dis 2013;33:97-102.
10. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B virus genotypes and spontaneous hepatitis B e antigen seroconversion in Taiwanese hepatitis B carriers. J Med Virol 2004;72:363-369.
11. Kao JH, Chen PJ, Lai MY, Chen DS. Genotypes and clinical phenotypes of hepatitis B virus in patients with chronic hepatitis B virus infection. J Clin Microbiol 2002;40:1207-1209.
12. Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. Gastroenterology 2000;118:554-559.
13. Yang HI, Yeh SH, Chen PJ, Iloeje UH, Jen CL, Su J, et al. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. J Natl Cancer Inst 2008;100:1134-1143.
14. Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 2003;124:327-334.
15. Yuen MF, Tanaka Y, Mizokami M, Yuen JC, Wong DK, Yuan HJ, et al. Role of hepatitis B virus genotypes Ba and C, core promoter and precore mutations on hepatocellular carcinoma: a case control study. Carcinogenesis 2004;25:1593-1598.
16. Liu CL, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B viral load and basal core promoter mutation in hepatocellular carcinoma in hepatitis B carriers. J Infect Dis 2006;193:1258-1265.
17. Liu CL, Chen BF, Chen PJ, Lai MY, Huang WL, Kao JH, et al. Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case-control study. J Infect Dis 2006;194:594-599.
18. Liu S, Zhang H, Gu C, Yin J, He Y, Xie J, et al. Associations between hepatitis B virus mutations and the risk of hepatocellular carcinoma: a meta-analysis. J Natl Cancer Inst 2009;101:1066-1082.
19. Hsieh YH, Su IJ, Wang HC, Chang WW, Lei HY, Lai MD, et al. Pre-S mutant surface antigens in chronic hepatitis B virus infection induce
oxidative stress and DNA damage. Carcinogenesis 2004;25:2023-2032.
20. Chen CH, Hung CH, Lee CM, Hu TH, Wang JH, Wang JC, et al. Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. Gastroenterology 2007;133:1466-1474.
21. Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. Gastroenterology 2006;130:1153-1168.
22. Kao JH. Diagnosis of Hepatitis B virus infection through serological and virological markers. Expert Rev Gastroenterol Hepatol 2008;2:553-562.
23. Liaw YF, Kao JH, Piratvisuth T, Chan HLY, Chien RN, Liu CJ, et al. Asian-Pacific consensus statement on the management of chronic hepatitis B: a 2012 update. Hepatol Int 2012;6:531-561.
24. Chen CJ, Yang HI. Natural history of chronic hepatitis B REVEALed. J Gastroenterol Hepatol 2011;26:628-638.
25. Tseng TC, Liu CJ, Chen CL, Wang CC, Su TH, Kuo SF, et al. Serum hepatitis B virus-DNA levels correlate with long-term adverse outcomes in spontaneous hepatitis B e antigen seroconverters. J Infect Dis 2012;205:54-63.
26. Chan HL, Thompson A, Martinot-Peignoux M, Piratvisuth T, Cornberg M, Brunetto MR, et al. Hepatitis B surface antigen quantification: why and how to use it in 2011 - a core group report. J Hepatol 2011;55:1121-1131.
27. Su TH, Hsu CS, Chen CL, Liu CH, Huang YW, Tseng TC, et al. Serum hepatitis B surface antigen concentration correlates with HBV DNA level in patients with chronic hepatitis B. Antivir Ther 2010;15:1133-1139.
28. Tseng TC, Liu CJ, Su TH, Wang CC, Chen CL, Chen PJ, et al. Serum hepatitis B surface antigen levels predict surface antigen loss in hepatitis B e antigen seroconverters. Gastroenterology 2011;141:517-525.
29. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Determinants of spontaneous surface antigen loss in hepatitis B e antigen-negative patients with a low viral load. Hepatology 2012;55:68-76.
30. Lee MH, Yang HI, Liu J, Batria-Utermann R, Jen CL, Iloeje UH, et al. Prediction models of long-term cirrhosis and hepatocellular carcinoma risk in chronic hepatitis B patients: risk scores integrating host and virus profiles. Hepatology 2013;58:546-554.
31. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. High levels of hepatitis B surface antigen increase risk of hepatocellular carcinoma in patients with low HBV load. Gastroenterology 2012;142:1140-1149.
32. Tseng TC, Liu CJ, Yang HC, Su TH, Wang CC, Chen CL, et al. Serum hepatitis B surface antigen levels help predict disease progression in patients with low HBV loads. Hepatology 2013;57:441-450.
33. Yang HI, Yuen MF, Chan HL, Han KH, Chen PJ, Kim DY, et al. Risk estimation for hepatocellular carcinoma in chronic hepatitis B (REACH-B): development and validation of a predictive score. Lancet Oncol 2011;12:568-574.
34. Jung KS, Kim SU, Ahn SH, Park YN, Kim Do Y, Park JY, et al. Risk assessment of hepatitis B virus-related hepatocellular carcinoma development using liver stiffness measurement (FibroScan). Hepatology 2011;53:885-894.
35. Wong GL, Wong VW, Choi PC, Chan AW, Chan HL. Development of a non-invasive algorithm with transient elastography (Fibroscan) and serum test formula for advanced liver fibrosis in chronic hepatitis B. Aliment Pharmacol Ther 2010;31:1095-1103.
36. Kim Do Y, Song KJ, Kim SU, Yoo EJ, Park JY, Ahn SH, et al. Transient elastography-based risk estimation of hepatitis B virus-associated occurrence of hepatocellular carcinoma: development and validation of a predictive model. Onco Targets Ther 2013;6:1463-1469.