

## Molecular and Clinical Characteristics of Hepatitis B Surface Antigen

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### Technical advances in the measurement of hepatitis B surface (HBs) antigen

HBs protein is encoded by the pre-S and S genome, and the detection of HBs antigen (HBsAg) in sera is an initial step for the diagnosis of hepatitis B virus (HBV) infection. The “a” determinant region (amino acid residues 124–147) is the main epitope for inducing a protective immune response. Mutations in the “a” determinant region, known as escape mutants, affect antigenicity and hinder attempts to detect HBV in serum [1]. HBsAg mutation is usually induced by immune escape of post-exposure prophylaxis. In the 1970s, the introduction of the reversed passive hemagglutination (R-PHA) test revolutionized the detection of HBV [2]. Several enzyme immunoassays were then developed and showed increased sensitivity for the detection of HBV. However, it was still difficult to detect escape mutants using these methods [3]. Currently, chemiluminescent immunoassays (CLIA) and chemiluminescent enzyme immunoassays (CLEIA) are commonly used for HBV screening because they show higher sensitivities and specificities than the previous methods. In addition, CLIA and CLEIA allow us to quantify HBs-Ag, in terms of the HBs-Ag titer. The HBsAg titer in HB extracellular antigen (HBeAg)-positive chronic hepatitis B is positively correlated with serum HBV-DNA, intrahepatic double-stranded covalently closed circular DNA (cccDNA), and total HBV-DNA [4]. The HBsAg-HQ assay, the most recently introduced system, allows us to measure HBsAg at titers of > 0.005 IU/ml [5]. Advanced methods showing high sensitivity and low false-positive rates will become standard methods of measuring the HBsAg titer.

### Clinical significance of the HBsAg titer

The primary goal of chronic hepatitis B (CHB) treatment is to eradicate HBV or to at least suppress HBV replication. Despite recent advances in anti-viral agents for chronic HBV infection, complete eradication of the virus remains difficult. Thus, we hope to reduce viremia and improve the patient’s prognosis by improving hepatic function and preventing liver cancer. The natural history of CHB can be divided into five major phases: (i) high replicative, low inflammatory phase; (ii) immune clearance phase; (iii) HBeAg-negative chronic hepatitis phase; (iv) non-replicative phase; and (v) HBs-Ag loss/occult hepatitis phase.

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After HBs-Ag loss, patients showing anti-HBs antibody positive are recognized as clinically cured phase. HBV-DNA is a sensitive marker but its titer fluctuates widely, especially in the immune clearance phase, and it is difficult to make long-term predictions based on its titer. In general, the HBsAg level gradually decreases from 5 logIU/ml in the high replicative phase to 2 logIU/ml in non-replicative phase [6]. The cut-off level of 1,000 IU/ml can be used to differentiate the non-replicative phase from HBeAg-negative chronic hepatitis [7,8].

The eradication of HBsAg is the ultimate virological goal of treating CHB, but is still rare. In untreated patients, the HBsAg seroclearance rate is reported to be 0.9%–1.0% per year, and is increased by hepatic flares. The HBsAg titer at baseline is the only predictor of HBsAg seroclearance [9]. In European studies, the HBsAg seroclearance rate was approximately 3%–8% after 1 year of interferon treatment and occurred in 23% of patients during a median follow-up of 8.8 years [10–12]. By contrast, the accumulated HBsAg seroclearance rate in entecavir-treated patients was 0.2% and 3.5% at 1 and 5 years, respectively [13].

In a Taiwanese study, the frequency of liver cancer was significantly greater in patients with an HBsAg titer > 1,000 IU/ml than in patients with an HBsAg titer < 1,000 IU/ml [14]. In a Japanese study, the incidence of liver cancer was significantly greater in patients with an HBsAg titer > 2,000 IU/ml, even if they were treated with a nucleotide analog [15]. According to Chinese and Korean studies, the frequency of liver cancer recurrence was significantly greater in patients with an HBsAg titer of > 2,000 IU/ml and > 4,000 IU/ml, respectively [16,17].

### De novo hepatitis in HBsAg-negative patients

Although undetectable serum HBsAg is often perceived to indicate the cure of CHB, reactivation of disease, known as *de*

*novo* hepatitis, is relatively common following chemotherapy and immunosuppressive therapy in HBsAg-negative patients [18]. *De novo* hepatitis has also been reported in HBsAg-negative patients following liver transplantation from an anti-HBc-positive donor [19]. Hepatocytes infected with HBV produce cccDNA, which is stable in the nucleus. Systemic chemotherapy and immunosuppressive therapy sometimes re-initiates HBV replication, called reactivation, in HBsAg-positive patients and in HBsAg-negative patients positive for anti-HBc and/or anti-HBs. DNA replication typically commences 12 weeks after chemotherapy, and seroconversion to HBsAg positivity is detected about 12 weeks after the start of DNA replication

[18]. HBV reactivation was reported to occur in 6.25% of HBsAg-negative patients with diffuse large B-cell lymphoma who were treated with systemic chemotherapy, comprising rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone [20]. Several therapeutic regimens comprising nucleotide analogs with or without HBV immunoglobulin (HBIG) have been adopted in several liver transplant centers.

We should be aware of HBV reactivation, even in HBsAg-negative patients treated with systemic chemotherapy and immunosuppressive therapy. Currently, serological tests of HBsAg, anti-HBc antibodies, and anti-HBs antibodies are recommended before and during treatment of HBV.

## References

- 1 Melegari M, Bruno S, Wands JR (1994) Properties of hepatitis B virus pre-S1 deletion mutants. *Virology* 199: 292-300
- 2 Wallace J, Barr A, Milne GR (1975) Which techniques should be used to screen blood donations for hepatitis B surface antigen? *British Medical Journal* 2: 412-414.
- 3 Coleman PF, Chen YC, Mushahwar IK, Coleman PF (1999) Immunoassay detection of hepatitis B surface antigen mutants. *J Med Virol* 59: 19-24.
- 4 Thompson AJ, Nguyen T, Iser D, Ayres A, Jackson K, et al. (2010) Serum hepatitis B surface antigen and hepatitis B e antigen titers: disease phase influences correlation with viral load and intrahepatic hepatitis B virus markers. *Hepatology* 51: 1933-1944.
- 5 Shinkai N, Matsuura K, Sugauchi F, Watanabe T, Murakami S, et al. (2013) Application of a newly developed high-sensitivity HBsAg chemiluminescent enzyme immunoassay for hepatitis B patients with HBsAg seroclearance. *J Clin Microbiol* 51: 3484-3491
- 6 Chan HL, Wong VW, Wong GL, Tse CH, Chan HY, et al. (2010) A longitudinal study on the natural history of serum hepatitis B surface antigen changes in chronic hepatitis B. *Hepatology* 52: 1232-1241.
- 7 Tseng TC, Liu CJ, Yang WT, Chen CL, Yang HC, et al. (2014) Hepatitis B surface antigen level complements viral load in predicting viral reactivation in spontaneous HBeAg seroconverters. *J Gastroenterol Hepatol* 29: 1242-1249.
- 8 Martinot-Peignoux M, Lapalus M, Laouenan C, Lada O, Netto-Cardoso AC, et al. (2013) Prediction of disease reactivation in asymptomatic hepatitis B e antigen-negative chronic hepatitis B patients using baseline serum measurements of HBsAg and HBV-DNA. *J Clin Virol* 58: 401-407.
- 9 Nagaoka S, Abiru S, Komori A, Sasaki R, Bekki S, et al. (2008) Hepatic flares promote rapid decline of serum hepatitis B surface antigen (HBsAg) in patients with HBsAg seroclearance: A long-term follow-up study. *Hepatology* 47: 547-554
- 10 Buster EH, Janssen HL (2006) Antiviral treatment for chronic hepatitis B virus infection--immune modulation or viral suppression? *Neth J Med* 64: 175-185
- 11 Lampertico P (2015) The royal wedding in chronic hepatitis B: the haves and the have-nots for the combination of pegylated interferon and nucleos(t)ide therapy. *Hepatology* 61: 1459-1461.
- 12 van Zonneveld M, Honkoop P, Hansen BE, Niesters GMH, et al. (2004) Long-term follow-up of alpha interferon treatment of patients with chronic hepatitis B. *Hepatology* 39: 804-810.
- 13 Hara T, Suzuki F, Kawamura Y, Sezaki H, Hosaha T, et al. (2014) Long-term entecavir therapy results in falls in serum hepatitis B surface antigen levels and seroclearance in nucleos(t)ide-naïve chronic hepatitis B patients. *J Viral Hepat* 21: 802-808.
- 14 Tseng TC, Liu CJ, Chen CL, Yang HC, Su TH, et al. (2013) Risk stratification of hepatocellular carcinoma in hepatitis B virus e antigen-negative carriers by combining viral biomarkers. *J Infect Dis* 208: 584-593.
- 15 Kawanaka M, Nishino K, Nakamura J, Oka T, Urata N, et al. (2014) Quantitative Levels of Hepatitis B Virus DNA and Surface Antigen and the Risk of Hepatocellular Carcinoma in Patients with Hepatitis B Receiving Long-Term Nucleos(t)ide Analogue Therapy. *Liver Cancer* 3: 41-52.
- 16 Zhou HB, Li QM, Zhong ZR, Hu JY, Jiang XL, et al. (2015) Level of hepatitis B surface antigen might serve as a new marker to predict hepatocellular carcinoma recurrence following curative resection in patients with low viral load. *Am J Cancer Res* 5: 756-771
- 17 Sohn W, Paik YH, Kim JM, Kwon CH, Joh JW, et al. (2014) HBV DNA and HBsAg Levels as Risk Predictors of Early and Late Recurrence after Curative Resection of HBV-related Hepatocellular Carcinoma. *Ann Surg Oncol* 21: 2429-2435
- 18 Hui CK, Cheung WW, Zhang HY, Au WY, Yueng N, et al. (2006) Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 131: 59-68.
- 19 Cholongitas E, Papatheodoridis GV, Burroughs AK (2010) Liver grafts from anti-hepatitis B core positive donors: a systematic review. *J Hepatol* 52: 272-279.
- 20 Yeo W, Chan TC, Leung NW (2009) Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* 27: 605-611.