



### Chronic Hepatitis B Infection: New Approaches to Diagnosis and Management



## Volume 4 Issue 9

#### In this Issue...

How well defined are the various stages of HBV infection, and what assays can help identify them? How should patients with resolved infection be managed when undergoing immunosuppression or chemotherapy to minimize the risk of reactivation? What is the risk of transmission from blood donors with occult HBV infection? What progress has been made in gene editing techniques to eradicate HBV?

In this issue, Drs. Kenneth Sherman and Nadeem Anwar from the University of Cincinnati College of Medicine review recent publications that provide new insight into answering these questions.

#### Program Information

- [CME Information](#)
- [Accreditation](#)
- [Credit Designations](#)
- [Intended Audience](#)
- [Statement of Need](#)
- [Internet CME/CE Policy](#)
- [Faculty Disclosure](#)
- [Disclaimer Statement](#)

Length of Activity  
1.0 hour Physicians  
1.0 hour Nurses

Launch Date  
June 23, 2016

Expiration Date  
June 22, 2018

### LEARNING OBJECTIVES

After participating in this activity, the participant will demonstrate the ability to:

- Describe the stages of chronic hepatitis B infection.
- Discuss the role of newer assays and predictive algorithms in assessing the outcomes and management in HBV infection.
- Summarize factors predictive of HBV reactivation.

### GUEST AUTHORS OF THE MONTH

#### Commentary & Reviews



**Kenneth Sherman, MD, PhD**  
Professor of Medicine  
University of Cincinnati College of Medicine  
Cincinnati, Ohio

#### Guest Faculty Disclosure

**Dr. Sherman** has indicated that he has served as a consultant/advisor to MedImmune and has received grant/research support from MedImmune and Inovio.

**Dr. Anwar** has indicated that he has no financial interests or relationships with any commercial entity whose products or services are relevant to the content of this presentation.

#### COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN POST-TEST

NURSE POST-TEST



**Nadeem Anwar, MD**  
Associate Professor of Medicine  
University of Cincinnati College of  
Medicine  
Cincinnati, Ohio

***Unlabeled/Unapproved uses***

**Dr. Sherman and Dr. Anwar** have indicated that there will be references to Tenofovir Alafenamide (TAF), which is not currently approved for the treatment of hepatitis B in the United States.

## IN THIS ISSUE

**COMMENTARY**

**[Predicting Prognosis to Lamivudine Treatment for HBeAg-Negative Acute-On-Chronic Liver Failure](#)**

**[HBsAg Quantification Across Different Phases of Chronic HBV Infection](#)**

**[HBV Transmission by Donors with Occult Infection](#)**

**[HBV DNA Monitoring and the Risk of HBV Reactivation in B-Cell Lymphoma](#)**

**[HBV Eradication: CRISPR/Cas9 Nickase-Mediated Disruption of Hepatitis B Virus](#)**

***Program Directors***

***Mark S. Sulkowski, MD***

Professor of Medicine  
Medical Director, Viral  
Hepatitis Center  
Divisions of Infectious  
Diseases and  
Gastroenterology/Hepatology  
Johns Hopkins University  
School of Medicine  
Baltimore, Maryland

***Raymond T. Chung, MD***

Director of Hepatology  
and Liver Center  
Vice Chief, Gastroenterology  
Kevin and Polly Maroni  
Research Scholar  
Massachusetts General Hospital  
Harvard Medical School  
Boston, Massachusetts

***Julie McArthur, MS, CRNP***

Adult Nurse Practitioner  
Division of Infectious Disease  
Johns Hopkins University  
School of Medicine  
Baltimore, Maryland

Chronic hepatitis B virus infection is a major cause of liver-related morbidity and mortality worldwide. With more than 248 million infected persons, HBV is one of the most common infections on the planet, eclipsing diseases such as HIV and HCV infection.<sup>1</sup> The disease leads to development of cirrhosis and predisposes infected people to hepatocellular carcinoma. The natural history of the virus is quite complex and variable, and these features, along with the long timeline between infection and the appearance of symptoms, has made diagnostic classification quite challenging to health care providers. Knowledge of the stage of disease has significant implications for deciding individual treatments, screening and surveillance for HCC, and assessing risk for viral reactivation in those who have achieved either nonreplicative status or functional cure.<sup>2</sup>

When a patient presents with serologic and virologic markers of HBV infection, the clinician must either determine the stage of the disease from existing data or obtain tests that will help determine it. Depending on the stage, this may require a single visit or a series of visits to determine the true natural history of the process in each person. Following acute infection, the majority of patients will pass through a series of events that may lead to clearance of hepatitis B surface antigen from the serum.

First, anti-core IgM antibodies appear and signal the beginning of an immune response. This is accompanied by elevated serum ALT levels as virus is cleared by an immunologically mediated death of hepatocytes. Hepatitis B anti-surface antibody, which is initially not detectable, becomes detectable when it is quantitatively greater than the HBV surface antigen that it is binding. The “window period” is defined as the segment of time (lasting days to weeks) that antigen:antibody binding are in equilibrium and neither is detectable by the typical serological assays used in commercial laboratories. In this period, only HBV anti-core IgM is detected. Eventually, excess HB anti-surface antibody appears. HBV DNA, which is initially high in serum, begins to drop as hepatocytes that replicate HBV are cleared, and new hepatocyte infections are prevented by the presence of high titers of circulating HBV anti-surface antibody. Finally, replication ends, surface antigen is absent, and the serum demonstrates both HBV anti-surface antibody and anti-core antibody, which change from IgM positive to IgG positive. This patient has functional cure, as inactive covalently closed DNA (cccDNA) *may* be present in some hepatocytes for years to come.<sup>3</sup> In other patients, complete viral nucleic acid clearance may occur. Over time, one or more serum antibodies (anti-surface and/or anti-core) may decline and become unmeasurable.

In neonates and young children, as well as in immunosuppressed hosts (eg HIV-infected), acute HBV infection may lead to a chronic disease state.<sup>4</sup> These patients have persistent HBsAg in serum and detectable HBV DNA. Anti-core IgG may be present, but anti-surface is not. In most patients, and particularly those infected at or near birth, an immune-tolerant phase characterized by high HBV DNA and low or normal serum ALT level is common. This may be related to the failure of the immune system to recognize the invading virus, probably due to the delay in thymic maturation early in life, which leads the host to incorrectly identify HBV as “self.” Since HBV injury is immunologically mediated, a failed immune response leads to productive infection without injury.<sup>5</sup> At some point, patients shift from this state to either a replicative and immunologically active state (high DNA, elevated ALT). The timing of this is variable and unpredictable. Furthermore, some patients will remain in this state for years, while others will move quickly, without intervention, to a nonreplicative chronic state (low DNA, low ALT). A few patients will move further to functional cure or even true cure, but this is both unpredictable and hard to prognosticate. This is why new tools, like quantitative hepatitis B surface antigen levels, could be useful in determining who needs treatment and who is responding to treatment that may lead to functional or true cure.



### COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN  
POST-TEST

NURSE  
POST-TEST

Patients who achieve functional cure are capable of returning to any prior stage of disease, depending upon the level of immunosuppression.<sup>6</sup> Recent data suggest that use of rituximab in cancer chemotherapy regimens is particularly likely to provoke this response.<sup>7</sup> Steroids, HIV-associated immunosuppression, and other disease states can also lead to sero-reversion and disease recrudescence.<sup>8</sup> For this reason, we need better markers of cccDNA that might identify those in functional cure vs those with true HBV viral cure, particularly if those assays can use serum rather than liver tissue.<sup>9-12</sup> Unfortunately, current technologies are not sufficient to allow routine testing of cccDNA.

After we have those markers, we will need new treatment interventions that would facilitate increased frequency of HBV cure. There is, therefore, particular interest in agents that can block different elements of the HBV life cycle.<sup>13</sup> In the end, however, the removal of cccDNA remains a blockade to final cure. New CRISPR/cas9 technologies hold the promise of cccDNA clearance, but the safe implementation of this and related technologies is still years away.

### References:

1. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. [Estimations of worldwide prevalence of chronic hepatitis B virus infection: a systematic review of data published between 1965 and 2013](#). *Lancet*. 2015;386:1546-1555.
2. Sarin SK, Kumar M, Lau GK, et al. [Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update](#). *Hepatol Int*. 2016;10:1-98.
3. Nassal M. [HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B](#). *Gut*. 2015;64:1972-1984.
4. McMahon BJ, Alward WL, Hall DB, et al. [Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state](#). *J Infect Dis*. 1985;151:599-603.
5. Chisari FV, Ferrari C. [Hepatitis B virus immunopathology](#). *Springer Semin Immunopathol*. 1995;17:261-281.
6. Francisci D, Falcinelli F, Schiaroli E, et al. [Reactivation of hepatitis B virus replication due to cytotoxic therapy: a five-year prospective study](#). *Tumori*. 2012;98:220-224.
7. Martin ST, Cardwell SM, Nailor MD, Gabardi S. [Hepatitis B reactivation and rituximab: a new boxed warning and considerations for solid organ transplantation](#). *Am J Transplant*. 2014;14:788-796.
8. Seetharam A, Perrillo R, Gish R. [Immunosuppression in Patients with Chronic Hepatitis B](#). *Curr Hepatol Rep*. 2014;13:235-244.
9. Takkenberg RB, Zaaijer HL, Menting S, et al. [Detection of hepatitis B virus covalently closed circular DNA in paraffin-embedded and cryo-preserved liver biopsies of chronic hepatitis B patients](#). *Eur J Gastroenterol Hepatol*. 2010;22:952-960.
10. Tajik Z, Keyvani H, Bokharaei-Salim F, et al. [Detection of hepatitis B virus covalently closed circular DNA in the plasma of Iranian hbeag-negative patients with Chronic hepatitis B](#). *Hepat Mon*. 2015;15:e30790.

11. Xu CH, Li ZS, Dai JY, Zhu HY, Yu JW, Lv SL. [\[Establishment and application of nested real-time quantitative polymerase chain reaction assay for detection of hepatitis B virus covalently closed circular DNA\]](#). *Zhonghua shi yan he lin chuang bing du xue za zhi = Zhonghua shiyan he linchuang bingduxue zazhi = Chinese journal of experimental and clinical virology* 2011;25:307-309.

12. Mu D, Yan L, Tang H, Liao Y. [A sensitive and accurate quantification method for the detection of hepatitis B virus covalently closed circular DNA by the application of a droplet digital polymerase chain reaction amplification system](#). *Biotechnology Letters*. 2015;37:2063-2073.

13. Zoulim F, Durantel D. [Antiviral therapies and prospects for a cure of chronic hepatitis B](#). *Cold Spring Harbor Perspect Med*. 2015;5.

[Back to top](#)

## Predicting Prognosis to Lamivudine Treatment for HBeAg-Negative Acute-On-Chronic Liver Failure

Lai J, Lin CS, Yang L, Chen SR, Zhang YQ, Ke WM. Pretreatment HBsAg level and an early decrease in MELD score predict prognosis to lamivudine treatment for HBeAg-negative acute-on-chronic liver failure. *Clin Res Hepatol Gastroenterol*. 2014 Jun;38(3):331-336.



[View Journal Abstract](#)



[View Full Article](#)

Hepatitis B continues to remain a disease with significant morbidity and mortality at a global level, being more prevalent in certain parts of Asia and Africa.<sup>1</sup> Worldwide, over 240 million people are infected with hepatitis B, and 780,000 die from it annually. Patients with this infection are at risk for cirrhosis (with all its sequelae), hepatocellular carcinoma, and death.<sup>2</sup> The diagnosis of hepatitis B includes testing for hepatitis B surface antigen (HBsAg) as well as other serologies. The quantification of HBsAg has recently been shown to have a correlation with the different stages of the hepatitis B infection.<sup>3</sup> Quantification of HBsAg in pregnancy has been shown to be able to predict the viral load in hepatitis B e antigen (HBeAg)-positive women.<sup>4</sup> HBsAg quantification has also been shown to predict the viral response in HBeAg- positive patients who are taken off entecavir according to the APASL-recommended stopping guidelines rules.<sup>5</sup>

In this 2014 study, the authors were aiming to see if there was a correlation between the changes in the HBsAg and the MELD score after initiation of lamivudine, and the overall prognosis in chronic hepatitis B patients with acute-on-chronic liver disease. The study is retrospective in design. Fifty-seven treatment naïve, HBsAg-positive and HBeAg-negative patients were included in the study and were started on lamivudine. Patients who were co-infected with other viruses, as well as treatment-experienced patients or those with decompensated liver disease or with other concurrent liver diseases or hepatocellular carcinoma, were excluded.

Participants were followed for three months or until death (if they died within the three month period). HBsAg was measured along with the standard MELD score at the time of enrollment as well as at the end of the follow-up period. In the patients who survived, for those on treatment, the MELD and total bilirubin decreased over the course of the follow up, compared to the patients who died, in whom these parameters increased ( $P < .05$ ). HBV

 RECOMMEND TO A COLLEAGUE

 NEWSLETTER ARCHIVE

COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN POST-TEST

NURSE POST-TEST

DNA decreased in both groups. HBsAg did not drop with treatment and was higher in the survival group, compared to the deceased group both at baseline and at the end of the follow-up period. The authors concluded that the patients with higher pretreatment HBsAg and early drop in MELD score with treatment had a lower three-month mortality.

The relationship of HBsAg quantification to the survival group vs the death group as described in this trial is interesting, as the survival group was noted to have higher HBsAg levels. Recently, Goyal et al have demonstrated an inverse relationship between the HBsAg level and degree of fibrosis in patients with HBeAg.<sup>6</sup> On the other hand, multiple studies have suggested that HBsAg decline is seen with hepatitis B treatment with interferon and can be used to assess response to treatment and even make decisions about stopping therapy at the 12-week mark.

More information is needed to verify the predictive value of this test in various phases of chronic hepatitis B and to better understand the dynamics of the HBsAg in response to treatment with interferon as well as oral NA agents. Going forward, studies should concentrate on evaluation of agents with greater efficacy in terms of viral clearance than lamivudine, including tenofovir, entecavir, and perhaps newer agents like tenofovir alafenamide (TAF). Overall, quantifying the HBsAg appears to be a promising marker and in the future may become a part of the standard of care in managing and following patients with chronic hepatitis B. At this time, however, FDA approved assays for HBsAg are not available in the US.

#### References:

1. Chen CJ, Wang LY, Yu MW. [Epidemiology of hepatitis B virus infection in the Asia-Pacific region](#). *J. Gastroenterol. Hepatol.* 2000;15 (Supl.):E3–E6.
2. WHO fact sheet (<http://www.who.int/mediacentre/factsheets/fs204/en/>)
3. Tseng TC, Kao JH. [Clinical utility of quantitative HBsAg in natural history and nucleos\(t\)ide analogue treatment of chronic hepatitis B: new trick of old dog](#). *J. Gastroenterology* 2013;48:13–21.
4. Fujiko M, Chalid MT, Turyadi, et al. [Chronic hepatitis B in pregnant women: is hepatitis B surface antigen quantification useful for viral load prediction?](#) *Int J Infect Dis.* 2015 Dec;41:83-89.
5. Qiu YW, Huang LH, Yang WL, et al. [Hepatitis B surface antigen quantification at hepatitis B e antigen seroconversion predicts virological relapse after the cessation of entecavir treatment in hepatitis B e antigen-positive patients](#). *Int J Infect Dis.* 2015 Oct 30;43:43-48.
6. Goyal SK, Jain AK, Dixit VK, et al. [HBsAg Level as Predictor of Liver Fibrosis in HBeAg Positive Patients With Chronic Hepatitis B Virus Infection](#). *Clin Exp Hepatol.* 2015 Sep;5(3):213-20.

[Back to top](#)

# HBsAg Quantification Across Different Phases of Chronic HBV Infection

Chung KH, Kim W, Kim BG, et al. Hepatitis B Surface Antigen Quantification across Different Phases of Chronic Hepatitis B Virus Infection Using an Immunoradiometric Assay. *Gut Liver*. 2015 Sep;9(5):657–664

 [View Journal Abstract](#)

 [View Full Article](#)

The disease course of hepatitis B is divided into phases based on the immune response to the infection.<sup>1</sup> These include the immune-tolerant phase, immune-clearance phase, immune-reactive phase, low-replicative phase, and the reactivation phase.<sup>2</sup> Other phase characterizations are also noted in the literature. Patients may move from phase to phase, return to a prior phase, or skip or never enter a particular phase. Thus, individual patient categorization is difficult, especially without longitudinal evaluation of markers. Several studies have looked at the HBsAg quantification as a marker for disease activity as well as for response to treatment.<sup>3-6</sup>

HBsAg is synthesized in the hepatocytes infected with hepatitis B, and its serum concentration correlates to the amount of intrahepatic covalently closed circular DNA (ccc-DNA). This article looks at the relation of HBsAg serum load and the HBV DNA during the various phases of chronic HBV infection. The use of HBsAg as a marker of treatment response is also examined.

The authors quantified serum HBsAg levels of 785 patients at the Seoul Metropolitan Government Seoul National University Boramae Medical Center over a period of seven months. Of these, 534 test results were included in the analysis. Definitions of the four phases of CHB were adopted from EASL guidelines: immunotolerant (IT), immunoreactive (IR), low replicative (LR) and HBeAg-negative chronic HBV (ENH). Definitions of response to treatment were determined based on the HBeAg status as well as the degree of decrease in HBsAg.

All subjects were Asian, and 64.3% were male. Samples were divided into eight groups, based on their treatment status (IT, IR, LR, ENH) for the treated vs untreated patients, and their titers were compared. The HBsAg titer was higher in patients in IT compared to those in IR phase ( $P = .043$ ). However, no difference was noted in patients in LR compared to ENH phases. Virologic response was defined as undetectable HBV DNA, while partial response was defined as decrease in HBV DNA of more than  $1 \log_{10}$  IU/ml. In the treatment groups, HBsAg titers in HBeAg+ patients with virologic response (E+VR) were much lower than in those with partial response (E+pVR;  $P = .027$ ). For HBeAb negative patients with virologic response (E-VR), titers of HBsAg were significantly lower than those for HBeAg negative patients with partial response (E-pVR;  $P = .009$ ). Comparing different phases, HBsAg titers were higher in the IT and IR phases compared to low replicative (LR) and ENH groups ( $P < .001$ ). Between the eAg+ and eAg- groups, HbsAg titers were higher in the former ( $P < .001$ ).

A correlation was noted between the HbsAg and the HBV DNA in the IT and IR phases ( $P < .001$ ), but not in the LR or the ENH phases. No correlation was noted between the two in all types of the treatment response groups as described above.

HbsAg decreased gradually with each 10-year patient age stratum ( $P < .001$ ) and was five times higher in noncirrhotic patients than in those with cirrhosis ( $P < .001$ ) when divided into patients older or younger than 60 years. Similar trends were seen when comparing patients without HCC (higher titers) compared to those with HCC, though the trend was only

 RECOMMEND TO A COLLEAGUE

 NEWSLETTER ARCHIVE

COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN POST-TEST

NURSE POST-TEST

statistically significant in patients older than 60 years.

There is evidence to suggest that the concentration of HBsAg decreases with increasing fibrosis and varies from genotype to genotype.<sup>7</sup> The relationship of the HBsAg to various phases, as well as the drop in HBsAg and the HBV DNA titers as predictors of response, have also been outlined in previous studies.<sup>8</sup> Although there is a clear correlation to phases of disease, clear cutoffs were not assigned and receiver-operator curves of sensitivity/specificity were not provided. However, this work leading to more precisely characterizing disease stages has significant implications for decisions about treatment candidacy and in treatment-intervention clinical trials. In the future, the HBsAg quantification may become a routine part of monitoring disease activity as well as the response to treatment.

## References:

1. Lok AS, Lai CL. [A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children](#). *Hepatology*. 1988;8:1130-1133.
2. Sarin SK, Kumar M, Lau GK, et al. [Asian-Pacific clinical practice guidelines on the management of hepatitis B: a 2015 update](#). *Hepatol Int*. 2016 Jan;10(1):1–98.doi: 10.1007/s12072-015-9675-4.
3. Boyd A, Maylin S, Moh R, et al. [Hepatitis B surface antigen quantification as a predictor of seroclearance during treatment in HIV-hepatitis B virus coinfecting patients from Sub-Saharan Africa](#). *J Gastroenterol Hepatol*. 2016 Mar;31(3):634-644.
4. Kuo YH, Chang KC, Wang JH, et al. [Changing serum levels of quantitative hepatitis B surface antigen and hepatitis B virus DNA in hepatitis B virus surface antigen carriers: a follow-up study of an elderly cohort](#). *Kaohsiung J Med Sci*. 2015;31:102–107
5. Suh SJ, Bae SI, Kim JH, Kang K, Yeon JE, Byun KS. [Clinical implications of the titer of serum hepatitis B surface antigen during the natural history of hepatitis B virus infection](#). *J Med Viro.*, 2014;86:117–123.
6. Park H, Lee JM, Seo JH, et al. [Predictive value of HbsAg quantification for determining the clinical course of genotype C HBeAg-negative carriers](#). *Liver Int*. 012;32:796–802.
7. Goyal SK, Jain AK, Dixit VK, et al. [HBsAg Level as Predictor of Liver Fibrosis in HBeAg Positive Patients With Chronic Hepatitis B Virus Infection](#). *J Clin Exp Hepatol*. 2015 Sep;5(3):213-20. doi: 10.1016/j.jceh.2015.04.008. Epub 2015 May 27.
8. Martinot-Peignoux M, Lapalus M, Asselah T, Marcellin P. [HBsAg quantification: useful for monitoring natural history and treatment outcome](#)

[Back to top](#)

## HBV Transmission by Donors with Occult Infection

Lieshout-Krikke RW, van Kraaij MG, Danovic F, Zaaijer HL. Rare transmission of hepatitis B virus by Dutch donors with occult infection. *Transfusion*. 2016 Mar;56(3):691-698.



[View Journal Abstract](#)



[View Full Article](#)

In cases of hepatitis B acquired in adulthood through sexual transmission or IV drug use, a large majority of patients are able to overcome the infection. With time the HBsAg is lost, patients develop anti-HBs antibody, and HBV DNA becomes undetectable. However, the phenomenon of persistent HBV DNA has been described in several reports.<sup>1-3</sup> This happens in the setting of undetectable HBsAg and is termed occult hepatitis B infection (OBI). OBI has been associated with a risk of transmission of disease, chronic infection, cirrhosis, and hepatocellular carcinoma.<sup>4,5</sup> This 2016 paper looks at the rate of HBV transmission using blood products from patients with occult hepatitis B.

The authors reviewed the blood donations in Holland from 2008 (when NAT testing was introduced for screening) to 2011 to identify patients with occult hepatitis B and all the transfusion products that resulted from these blood donations. Some 2.3 million samples were tested for HBV DNA. Twenty-two donors were found to have the HBV DNA as the only marker of hepatitis B. Four of these were in the seroconversion stage, two had had some viral suppression from vaccination, and 16 had true occult HBV infection. One donor's OBI was diagnosed posthumously and was included, as he had donated 50 times. The hospitals that received the blood product donations from these donors were asked to identify and test the recipients of these products for hepatitis B infection using NAT, surface Ag, and anti-HBc serology.

Overall, 442 patients received products from OBI repeat donors. Eighty of them (18%) were tested, 193 (44%) had died, and 169 (38%) were not tested. Among those recipients tested, four of 80 were HBsAg positive (but one recipient had previously diagnosed chronic hepatitis B). Another two of 80 were positive for anti-HBc but negative for HBsAg. Three of the positive results came from recipients who received products from a single donor. One recipient came from an HBV-endemic area, so it was difficult to relate HBV status to the transfusion. This overall rate of transmission was deemed to be 5% (four of 80). Similar studies in Japan revealed a rate of 3% and in Australia, 0.85%.

In the current study, the rate could have been higher if all the recipients had been tested. In areas that are endemic for HBV, the number of OBI donors is higher.<sup>6</sup> A larger study with more comprehensive tracking and testing of the recipients would be important in identifying the true incidence of disease transmission of hepatitis B from OBI donors. HBV NAT testing of donated blood is required to transfusion in the US (Federal Register, October 2012). However, this practice is not standard worldwide. Consideration must be given to making NAT testing a standard practice all over the world

### References:

1. Assar S, Arababadi MK, Ahmadabadi BN, Salehi M, Kennedy D. [Occult hepatitis B virus \(HBV\) infection: a global challenge for medicine](#). *Clin Lab*. 2012;58:1225-1230.
2. Mu SC, Lin YM, Jow GM, Chen BF. [Occult hepatitis B virus infection in hepatitis B vaccinated children in Taiwan](#). *J Hepatol*. 2009;50:264-272.

RECOMMEND TO A COLLEAGUE

NEWSLETTER ARCHIVE

### COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN POST-TEST

NURSE POST-TEST

3. Huang X, Hollinger FB. [Occult hepatitis B virus infection and hepatocellular carcinoma: a systematic review](#). *J Viral Hepat*. 2014;21:153–162.
4. Raimondo G, Allain JP, Brunetto MR, et al. [Statements from the Taormina expert meeting on occult hepatitis B virus infection](#). *J Hepatol*. 2008;49:652–657.
5. Pollicino T, Squadrito G, Cerenzia G, et al. [Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection](#). *Gastroenterology*. 2004;126:102–110.
6. Olotu AA, Oyelese AO, Salawu L, Audu RA, Okwuraiwe AP, Aboderin AO. [Occult Hepatitis B Virus Infection in Nigerian Blood Donors and Hepatitis B Virus Transmission Risks](#). *PLoS One*. 2015 Jul 6;10(7):e0131912.

[Back to top](#)

## HBV DNA Monitoring and the Risk of HBV Reactivation in B-Cell Lymphoma

Kusumoto S, Tanaka Y, Suzuki R, et al. Monitoring of Hepatitis B Virus (HBV) DNA and Risk of HBV Reactivation in B-Cell Lymphoma: A Prospective Observational Study. *Clin Infect Dis*. 2015 Sep 1;61(5):719-729.



[View Journal Abstract](#)



[View Full Article](#)

Reactivation of hepatitis B is seen sometimes with the use of immunosuppressive or chemotherapeutic agents. Treating physicians are becoming more aware of this possibility in patients with HBsAg positivity and increasingly will initiate antiviral therapy in anticipation of the oncoming chemotherapy. Rituximab-based therapy in patients with HBsAg positivity is associated with a high rate of HBV reactivation, resulting in liver failure and even death.<sup>1</sup> Risk factors for this complication have been identified.<sup>2,3</sup> However, patients who may have serological evidence of resolved hepatitis B infection can also be at risk for disease reactivation. The authors of this manuscript prospectively assessed the rate of HBV infection reactivation in patients with serologies suggestive of resolved HBV infection (positive HBcAb with or without anti-HBs Ab positivity).

A total of 269 patients with non-Hodgkin's lymphoma (NHL) who were going to receive rituximab-steroid chemotherapy were enrolled in the study. Subjects had to have negative HbsAg and positive anti-HBcAb and/or anti-HBsAb. During the six cycles of chemotherapy, their HBV DNA titers were measured every four weeks. The duration of follow up was 1.5 to 2.5 years. The endpoint was to verify the reactivation of HBV infection in the setting of chemotherapy.

Two hundred thirty-three patients finished the monitoring for 1.5 years. No HBV prophylaxis was used. Twenty-one patients developed HBV reactivation, 19 of them in the first year of follow up. Of these, 17 were male, with a median age of 69. Only five of those 21 patients had a detectable (but not quantifiable) HBV DNA at baseline. In patients with low detectable HBV DNA, initiation of antiviral therapy prevented hepatitis, and their viral load dropped below detectable levels within one to two months of starting antiviral therapy. Seven of 21 patients discontinued antiviral therapy but continued to follow with DNA measurements.

Reactivation appeared to be more associated with older age ( $P = .008$ ), male gender ( $P = .02$ ), diffuse large B-cell lymphoma ( $P = .04$ ), lower HBsAb titers ( $P < .001$ ), and a detectable (below quantification) HBV DNA at baseline ( $P < .001$ ). Anti-HBsAb titers  $< 100$  mIU/ml were associated with HBV reactivation, compared to titers greater than 100 mIU/ml. The authors



COMPLETE THE  
POST-TEST

Click on link to  
download instructions  
for the post-test and  
evaluation



concluded that frequent measurement of HBV DNA could be used to monitor for reactivation of hepatitis B.

The relation between the anti-HBs Ab titer to risk of reactivation has also been confirmed in a recent study by Yilmaz et al.<sup>4</sup> However, that study also showed that anti-HBs Ab titers drop during chemotherapy. It might be useful, therefore, to check the HBs Ab titers prior to initiating the chemotherapy and if needed, considering a booster immunization. Patients with low titers should be monitored, and if there is any evidence of reactivation, anti-viral therapy must be initiated.

This study has several takeaway messages. First: there appears to be a role for monthly monitoring of HBV DNA during therapy. Second: the titer of anti-HBs does seem to display a dose dependent effect related to risk of reactivation. This suggests (but does not prove) that booster dosing of HBV vaccine could be a useful component of management when repeated cycles of rituximab-based therapy are used. Finally: the risk of reactivation was found to be related to type of lymphoma. Whether this is due to greater immunosuppression or some other factor remains to be explored.

#### References:

1. Shih LN, Sheu JC, Wang JT, et al. [Serum hepatitis B virus DNA in healthy HBsAg-negative Chinese adults evaluated by polymerase chain reaction](#). *J Med Virol*. 1990 Dec; 32(4):257-260.
2. Chen KL, Chen J, Rao HL, et al. [Hepatitis B virus reactivation and hepatitis in diffuse large B-cell lymphoma patients with resolved hepatitis B receiving rituximab-containing chemotherapy: risk factors and survival](#). *Chin J Cancer*. 2015 May 28;34(5):225-234.
3. Hayashi K, Ishigami M2, Ishizu Y, et al. [Clinical characteristics and molecular analysis of hepatitis B virus reactivation in hepatitis B surface antigen-negative patients during or after immunosuppressive or cytotoxic chemotherapy](#). *J Gastroenterol*. 2016 Mar 4.
4. Yilmaz B, Erdem D, Teker F, et al. [The effect of anticancer therapy on anti-hepatitis B antibody titers in patients with haematological malignancies and solid tumours](#). *J Int Med Res*. 2016 Apr 5. pii: 0300060516638992.

[Back to top](#)

# HBV Eradication: CRISPR/Cas9 Nickase-Mediated Disruption of Hepatitis B Virus

Karimova M, Beschoner N, Dammermann W, et al. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. *Scientific Report* 5, Article number: 13734 (2015)



[View Journal Abstract](#)



[View Full Article](#)

Due to the large global disease burden of hepatitis B, and significant progress in vaccine-based disease prevention, no major breakthrough has been made for the eradication of established infections. The current goal of therapy is to decrease viremia and thereby minimize hepatic necro-inflammation and the progression of fibrosis. Covalently closed circular DNA (cccDNA) is not eradicated by current approved therapies in the majority of treated patients.<sup>1</sup> Hence the treatment is aimed at viral suppression, which occasionally leads to functional cure.<sup>2</sup> Treatment with antiviral agents decreases the risk for hepatocellular carcinoma<sup>3</sup> as well as the risk for hepatic decompensation. Several novel approaches to achieve eradication of the disease are currently being studied, including prevention of entry of HBV into hepatocytes,<sup>4</sup> inhibition of the HBV virion secretion,<sup>5</sup> and cleavage of the cccDNA. The latter mechanism holds the promise of true viral cure.

With their series of in vitro experiments, the authors studied the genome editing nuclease known as CRISPR/Cas9 RNA guided system to see if the cccDNA present in the nuclei of the HBV-infected hepatocytes could be targeted and cleaved to induce an error prone repair, which could help eradicate HBV infection. Two regions on S and X genes of the cccDNA that are well preserved across all the genotypes of HBV were selected as the target for the Cas9 nickase (Cas9n). A double strand break was achieved with the help of single guided RNAs (sgRNAs), which then resulted in a nonhomologous repair of the DNA break and indel formation (error prone DNA). Cells from two human hepatocyte cell lines were studied. Cells transfected with HBV alone (without the Cas9n) served as controls. Cas9n activity was quantified and was higher in cells transfected with HBV as well as the Cas9n along with single guided RNA (Cas9n/sgRNA), compared to that in cells infected with HBV only (7.8%-8% vs 0.5%-1.5%). This result was reproducible in both hepatic cell lines that were studied.

Cas9n/sgRNA induced mutations were studied using an assay with T7endonuclease I. Smaller DNA products that are indicative of these mutations were only present in HBV-infected cells treated with Cas9n/sgRNA. The indel formation was verified with sequence analysis and was noted in cells treated with the Cas9n/sgRNA. HbsAg ELISA was also done and showed a progressive increase in the untreated cells, while no HBV particle release was noted in the treated cells.

This data suggests that delivery of the Cas9n system to the infected hepatocytes in vitro can result in inactivation the cccDNA.

This elegant study shows that the use of the Cas9n system is a potential therapy that may become a part of the treatment of hepatitis B in the future and could promise eradication of the virus from the hepatocytes. Words of caution are in order, however. The delivery methods to ensure that the functional enzymes get to HBV-infected hepatocytes in a human host are challenging. Within hepatocytes, the enzymes must be transported to the nucleus and the targeting must be exact. For example, near-homologous introns or exons could be cleaved, resulting in significant functional damage to the host. The treatment must be effective in 100% of hepatocytes, or a nidus of cccDNA (which can lead to replicative

 RECOMMEND TO A COLLEAGUE

 NEWSLETTER ARCHIVE

COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN POST-TEST

NURSE POST-TEST

infection) will remain present for a period of time. For this reason, it is quite likely that the future treatment of hepatitis B will be multipronged and will include suppressing the replication of the virus at one or more key sites and preventing entry of the virus into uninfected hepatocytes, as well as cleavage of the cccDNA in the infected hepatocytes. There may be a concomitant role for immune-based therapies (eg, therapeutic vaccines with immunogenic complexes)<sup>6,7</sup> before complete HBV cure is achieved.

## References:

1. Wong DK, Seto WK, Fung J, et al. [Reduction of hepatitis B surface antigen and covalently closed circular DNA by nucleos\(t\)ide analogues of different potency](#). *Clin Gastroenterol Hepatol*. 2013;11:1004-1010.e1.
2. Block TM, Gish R, Guo H, et al. [Chronic hepatitis B: what should be the goal for new therapies?](#) *Antiviral Res*. 2013;98 (2013):27–34.
3. Sung JJ, Tsoi KK, Wong VW, Li KC, Chan HL. [Meta-analysis: treatment of hepatitis B infection reduces risk of hepatocellular carcinoma](#). *Aliment Pharmacol. Ther*. 2008;28:1067–1077.
4. Kaneko M, Watashi K, Kamisuki S, et al. [A Novel Tricyclic Polyketide, Vanitaracin A, Specifically Inhibits the Entry of Hepatitis B and D Viruses by Targeting Sodium Taurocholate Cotransporting Polypeptide](#). *J Virol*. 2015 Dec;89(23):11945-11953.
5. Yan W, Zhang C, Li B, et al. [A Series of Oleanolic Acid Derivatives as Anti-Hepatitis B Virus Agents: Design, Synthesis, and in Vitro and in Vivo Biological Evaluation](#). *Molecules*. 2016 Mar 24;21(4) pii:E402.
6. Xu DZ, Zhao K, Guo LM, et al. [A randomized controlled phase IIb trial of antigen-antibody immunogenic complex therapeutic vaccine in chronic hepatitis B patients](#). *PLoS One*. 2008 Jul 2;3(7):e2565.
7. Yao X, Zheng B, Zhou J, et al. [Therapeutic effect of hepatitis B surface antigen–antibody complex is associated with cytolytic and non-cytolytic immune responses in hepatitis B patients](#). *Vaccine*. 2007;10;1771–1779.

[Back to top](#)

## KEY TAKEAWAYS

- Newer HBV assays being investigated may help monitor disease activity as well as response to treatment.
- These assays can help elucidate better various stages of chronic hepatitis B infection.
- There is a small but real risk of transmission of HBV infections from blood donors with occult HBV infection.
- Factors affecting the reactivation of hepatitis B in the setting of immunosuppression with chemotherapy have been identified and can be used to help identify patients who may need suppressive antiviral therapy.
- New treatment modalities, including gene editing therapies, are being actively



pursued and may become a part of the treatment regimen for eradicating HBV infection in the future.

[Back to top](#)

## IMPORTANT CME/CE INFORMATION

### ACCREDITATION STATEMENTS

#### Physicians

This activity has been planned and implemented in accordance with the accreditation requirements and policies of the Accreditation Council for Continuing Medical Education (ACCME) through the joint providership of the Johns Hopkins University School of Medicine and The Institute for Johns Hopkins Nursing. The Johns Hopkins University School of Medicine is accredited by the ACCME to provide continuing medical education for physicians.

#### Nurses

The Institute for Johns Hopkins Nursing is accredited as a provider of continuing nursing education by the American Nurses Credentialing Center's Commission on Accreditation.

### CREDIT DESIGNATION STATEMENT

#### Physicians

eNewsletter: The Johns Hopkins University School of Medicine designates this enduring material for a maximum of 1.0 *AMA PRA Category 1 Credit(s)*<sup>™</sup>. Physicians should claim only the credit commensurate with the extent of their participation in the activity.

#### Nurses

eNewsletter: This 1 contact hour Educational Activity is provided by the Institute for Johns Hopkins Nursing. Each Newsletter carries a maximum of 1 contact hour, or a total of 8 contact hours for the 8 newsletters in this program.

All rights reserved - The Johns Hopkins University School of Medicine. Copyright 2016.

This activity was developed in collaboration with DKBmed.

### [INTERNET CME/CE POLICY](#)

### [DISCLAIMER STATEMENT](#)

### [STATEMENT OF RESPONSIBILITY](#)

### [STATEMENT OF NEED](#)

### [CONFIDENTIALITY DISCLAIMER FOR CME](#)

### [ACTIVITY PARTICIPANTS](#)

### [INTENDED AUDIENCE](#)

### [HARDWARE & SOFTWARE REQUIREMENTS](#)

### COMPLETE THE POST-TEST

Click on link to download instructions for the post-test and evaluation

PHYSICIAN  
POST-TEST

NURSE  
POST-TEST