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Patients with Coexistence of Circulating Hepatitis B Surface Antigen and Its Antibody May Have a Strong Predisposition to Virus Reactivation During Immunosuppressive Therapy: A Hypothesis

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Hepatitis B virus (HBV) reactivation is a well-recognized complication in patients who undergo immunosuppressive drug therapy. Although the recommendation of antiviral prophylaxis made by the American Gastroenterological Association in 2015 focuses on the risk stratification of different immunosuppressive drugs, risk factors for HBV reactivation are also worth identifying in clinical practice. Recent studies have shown that the uncommon serological pattern of coexistent circulating HBV surface antigen (HBsAg) and its antibody (anti-HBs) was associated with double mutations (A1762T/G1764A) in the basal core promoter (BCP) region of the HBV genome, which is critical for HBV replication. Here, we depicted rheumatoid arthritis (RA) patients with coexistent HBsAg and anti-HBs in our medical center, who developed HBV reactivation during immunosuppressive drug therapy. DNA sequencing analysis of the HBV genome revealed triple mutations (A1762T, G1764A, and T1753V) in the BCP region, which could further enhance the ability of HBV replication. Hence, a novel hypothesis is advanced for the first time that patients with coexistent HBsAg and anti-HBs may have a strong predisposition to HBV reactivation due to specific BCP mutations. This hypothesis would, if correct, justify the concurrent detection of HBsAg and anti-HBs in HBV screening in patients with rheumatic diseases and quickly recognize patients with high risk of HBV reactivation. Further controlled studies are needed to confirm this hypothesis.

MeSH Keywords: Hepatitis B Antibodies • Hepatitis B Antigens • Hepatitis B virus • Mutation

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Background

Hepatitis B virus (HBV) infection continues to be one of the most serious global disease burdens. Approximately 400 million people in the world have been infected with HBV, resulting in 786 000 deaths caused by HBV-related diseases in 2010 [1]. HBV surface antigen (HBsAg) is the first seromarker arising 2–10 weeks after exposure to HBV, and generally disappears within 4–6 months with the emergence of its antibody (anti-HBs) in patients with HBV resolved. The appearance of anti-HBs usually indicates resolution and immunity to HBV infection [2]. By contrast, if HBsAg persists in serum beyond 6 months, it indicates progression to chronic HBV infection (CHB). The serological profile of coexistence of circulating HBsAg and anti-HBs is uncommon in clinical practice, although an increasing number of reports have documented this phenomenon in some HBV-infected patients since 1976 by Arnold [3]. The prevalence of coexistent HBsAg and anti-HBs in patients with CHB investigated in different studies varies among countries, with 2–5% in China [4–7], 2–7% in Korea [8,9], 2–9% in France [10,11], and up to 26.1% in Japan [12] and 25% in Chicago (the United States) [13]. In addition, Shiels et al., at the Mayo Clinic, reported that the prevalence of coexistent HBsAg and anti-HBs was higher in patients with chronic active hepatitis (36/57, 63.2%) than that in patients with acute hepatitis (13/38, 34.2%) or asymptomatic carriers (24/133, 18.0%) [14]. The underlying mechanisms and clinical significance of coexistent HBsAg and anti-HBs remain largely obscure.

HBV reactivation is a well-recognized complication that may lead to high HBV-related morbidity and mortality in patients who undergo immunosuppressive drug therapy. A standardized definition of HBV reactivation has not yet been established. It is usually characterized by the reappearance of HBsAg in a patient with resolved HBV infection; the detection of previously undetectable HBV DNA or a >1 log10 (10-fold) IU/mL increase in the serum HBV DNA level; and HBV DNA levels rising above an arbitrary cutoff (for example, 20 000 IU) [15–17]. Templates for HBV mRNAs and replicative intermediate, also termed covalently closed circular DNA (cccDNA), persist in the nuclei of infected hepatocytes and play an important role in viral persistence and reactivation of the virus [18]. The prevalence of HBV reactivation was estimated as at least 50% in patients with leukemia or lymphoma and 20–40% in patients with breast cancer due to chemotherapy [16]. Recent data specific to rheumatic diseases have demonstrated HBV reactivation in 12% of HBsAg carriers and 1.7% of HBsAg-negative individuals who were positive for anti-HBc during TNF-α inhibitor therapy [15,19]. Our previous studies showed that discontinuation of antiviral prophylaxis and a past history of hepatitis and leflunomide (LEF) might increase the risk of HBV reactivation for rheumatoid arthritis (RA) patients with HBsAg carrier state during disease-modifying anti-rheumatic drugs (DMARDs) therapy [20]. HBV reactivation may cause severe outcomes, including hepatitis, liver cirrhosis, and acute liver failure, as well as a high rate of HBV-related liver mortality (5–30%) [17]. According to the recommendations, antiviral prophylaxis is suggested to be used before immunosuppressive therapy and continued for a minimum of 6–12 months after suspending immunosuppressant for CHB patients [21,22]. However, this is not easy to carry out in clinical practice, especially in China. There are many real difficulties, such as a huge number of patients with concurrent rheumatic diseases and CHB, high drug costs, and poor patient compliance [20,23]. Importantly, the recommendation of antiviral prophylaxis made by the American Gastroenterological Association in 2015 suggests antiviral prophylaxis for patients with CHB taking high-risk or moderate-risk immunosuppressants, but against routinely using antiviral prophylaxis in patients undergoing low-risk immunosuppressants (such as methotrexate and azathioprine) [16]. Besides the risk stratification of different immunosuppressive drugs, it is also important to identify other risk factors for HBV reactivation in patients undergoing immunosuppressive drug therapy.

The Hypothesis

The risk of HBV reactivation depends on the balance between the ability of HBV replication and the immune response of the host. Coexistence of circulating HBsAg and anti-HBs was reported to be associated with A1762T/G1764A double mutations in the basal core promoter (BCP) region in HBV genome, which might enhance the ability of HBV replication [6]. Therefore, we hypothesize that patients with coexistent HBsAg and anti-HBs may have a strong predisposition to HBV reactivation during immunosuppressive drug therapy due to specific BCP mutations.

Evaluation of the Hypothesis

HBV reactivation and coexistence of circulating HBsAg and anti-HBs in clinical practice

Between January 2012 and June 2016, a total of 97 RA patients with CHB were recruited from the Department of Rheumatology of Sun Yat-sen Memorial Hospital. Among them, 2 (2.0%) were positive for both HBsAg and anti-HBs (patient 1 and patient 2). Their serum HBV DNA were undetectable at baseline, but increased to 500 IU/mL at week 60 (patient 1) and 2680 IU/mL at week 48 (patient 2), which indicated HBV reactivation. The immunosuppressive drugs they used and fluctuation of HBsAg, anti-HBs, HBV DNA, and aminotransferases levels during the follow-up is demonstrated in Figure 1. DNA sequencing analysis of the whole HBV genome of these 2 patients revealed triple mutations at positions nt1753, nt1762, and nt1764 in the

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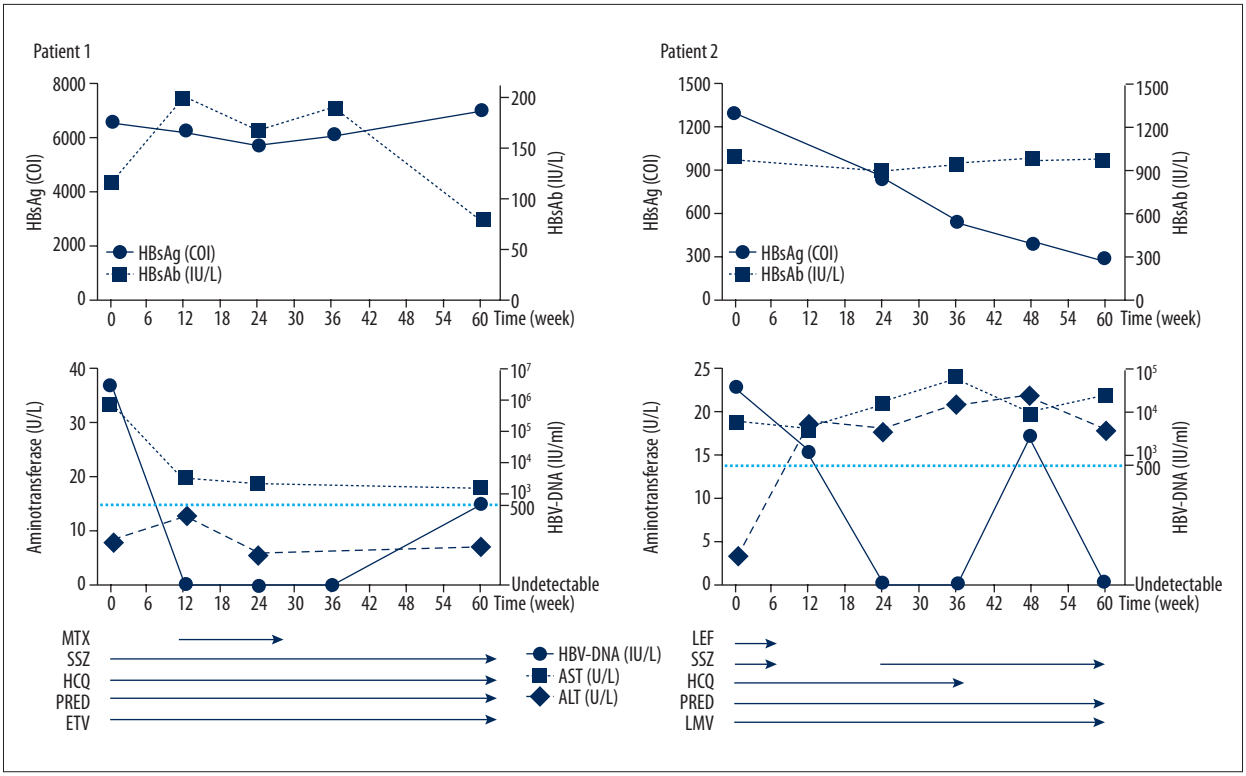

Figure 1. Change in HBsAg, anti-HBs, HBV DNA and aminotransferases levels of the 2 RA patients with coexistent HBsAg and anti-HBs and the immunosuppressive drugs they used during follow-up. PRED – prednisone; MTX – methotrexate; SSZ – sulfasalazine; HCQ – hydroxychloroquine; LEF – leflunomide; ETV – entecavir; ADV – adefovir.

BCP region (Figure 2). In this scenario, we suspected that patients with coexistent HBsAg and anti-HBs had a strong predisposition to HBV reactivation.

The possible mechanisms and clinical significance of coexistent HBsAg and anti-HBs

HBV is classified into 10 genotypes (A–J) that may vary primarily due to geographical distribution. It contains 4 partially overlapping open reading frames (ORFs), including the pre-S/S region, core gene, P region, and X region, which encode viral envelope proteins, the nucleocapsid (HBcAg), viral polymerase/reverse transcriptase, and proteins with transcriptional trans-activating function, respectively [24,25].

Previous studies demonstrated that coexistent HBsAg and anti-HBs was associated with mutations in several key areas of the HBV genome. It has been conferred that HBV escape mutants in the S region, especially within the major hydrophilic region (MHR), were responsible for coexistent HBsAg and anti-HBs in HBV infection [4,10,11]. In addition, a significantly higher prevalence of deletion mutations in the pre-S region was reported in patients with both HBsAg and anti-HBs compared to those with HBsAg alone [8,26]. Moreover, a recent study showed that the prevalence of BCP double mutations (A1762T/G1764A) in patients with coexistent circulating HBsAg and anti-HBs was significantly higher than that in patients with positive HBsAg but negative anti-HBs [6]. Interestingly, DNA sequencing analysis of the whole HBV genome of these 2 patients revealed mutations at positions nt1753, nt1762, and nt1764 in the BCP region, while no mutations conferring immunological escape in the S gene or deletion mutations in the pre-S region were observed.

Although the clinical significance of coexistent HBsAg and anti-HBs remains controversial, many studies have revealed that it might be associated with chronic active hepatitis [14], advanced fibrosis [11], and advanced liver diseases [8]. A total of 1042 non-HCC patients were recruited and followed up for a median 4.3 years, and it was found that coexistent HBsAg and anti-HBs independently increased the risk of hepatocellular carcinoma (HCC) development in chronic HBV infection [9].

Triple mutations (A1762T, G1764A, and T1753V) and enhanced ability of HBV replication

The BCP region is important for viral replication as a pregenomic RNA forms a hairpin loop structure that binds the polymerase [27]. The protein-RNA interaction is reported to be the first step of encapsidation of the pregenomic RNA

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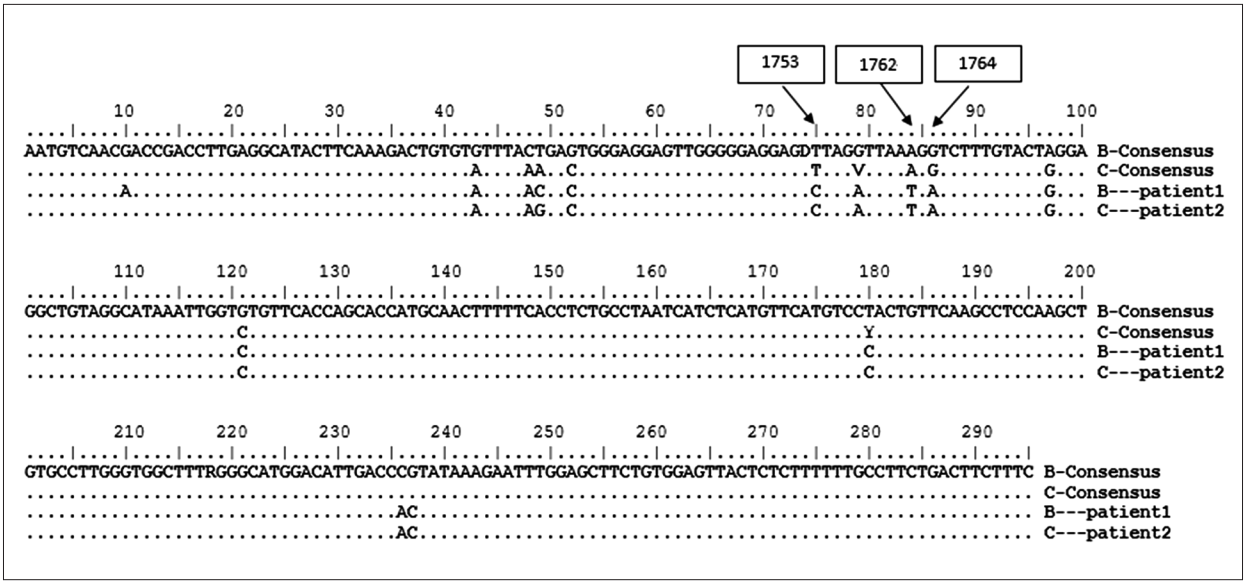

Figure 2. Nucleotide substitutions in the BCP gene and neighboring regions (nt1679-1973) in the 2 RA patients with coexistent HBsAg and anti-HBs. Arrows denote the mutation positions nt1753, nt1762, and nt1764. The HBV consensus sequences (B-consensus and C-consensus) are obtained according to the corresponding genotypes available on the NCBI website. A short line indicates that there is no mutation in the locus.

into the nascent core particles in the HBV replication cycle [24,28]. Several lines of *in vitro* evidence have indicated that the simultaneous presence of A1762T and G1764A mutations in the BCP region of HBV can enhance the viral replication, which possibly occurs through up-regulated synthesis of the core protein and increased transcription of the pregenomic RNA [29–31]. Site-directed mutagenesis (A1762T, G1764A, and T1753V) of the wild-type clone exhibited appreciably stronger replication capacity of the virus than double mutations (A1762T/G1764A) [32,33]. Hepatitis was one of the most common consequences in patients with HBV reactivation. Accordingly, BCP double mutations was identified as an independent risk factor that correlated with active hepatitis. A significantly lower prevalence of BCP double mutations was observed in the inactive HBsAg carriers (anti-HBe-positive carriers with persistently normal ALT levels over 10 years) compared to that in the controls with HBeAg-negative and anti-HBe-positive chronic hepatitis B [34]. Thus, patients with double mutations (A1762T/G1764A) or triple mutations (A1762T/G1764A/T1753V) in the BCP region could be more likely to develop HBV reactivation due to a higher HBV replicative ability.

On the other hand, previous studies have revealed that the BCP double mutations A1762T/G1764A were associated with an increased risk of HCC [35-37]. Baptista et al. reported a significantly higher prevalence of 1762T/1764A mutations in the BCP region of HBV isolated from Africans with HCC (66%) compared to that in asymptomatic HBsAg carriers (11%) [37]. Recently, a meta-analysis showed a statistically significant summary odds ratios (OR) of HCC obtained for A1762T/G1764A (3.79, 95% CI=2.71 to 5.29) and T1753V (2.35, 95% CI=1.63 to 3.40) in the BCP region [38].

Taken together, it could be speculated that coexistence of circulating HBsAg and anti-HBs in CHB patients, possibly due to mutations (T1753V, A1762T, and G1764A) in the BCP region, may be associated with an augmented HBV replicative capacity and HBV reactivation, which could even contribute to a poor prognosis, including HCC.

Therefore, patients with coexistent HBsAg and anti-HBs may have a strong predisposition to HBV reactivation during immunosuppressive therapy. Serum HBV DNA and transaminase should be monitored carefully and antiviral prophylaxis should be initiated for these patients. Additionally, it would also be of great significance in the process of HBV screening before immunosuppressive therapy in patients with rheumatic diseases. The result of a high anti-HBs level might offer a false sense of security if only anti-HBs was tested in patients with coexistent HBsAg and anti-HBs. Thus, both HBsAg and anti-HBs should be examined in HBV screening in patients with rheumatic diseases. To the best of our knowledge, the present article is the first to propose the hypothesis that patients with coexistent HBsAg and anti-HBs are at high risk of HBV reactivation. Additional research in controlled studies should be performed to confirm this hypothesis.

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Conclusions

Patients with coexistent HBsAg and anti-HBs may have a strong predisposition to HBV reactivation due to specific mutations in the BCP region during immunosuppressive drug therapy. This hypothesis would justify the concurrent detection of HBsAg and anti-HBs in HBV screening in patients with rheumatic diseases in the setting of immunosuppressive drug therapy. Serum HBV DNA and transaminase in these patients should be monitored carefully and antiviral prophylaxis should be recommended regardless of the immunosuppressive drugs used.

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Conflict of interest

None.

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