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Case Series

Hepatitis B Virus Reactivation with Discontinuation of Nucleoside Analogue in Patients Who Received Allogeneic Hematopoietic Stem Cell Transplantation

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Keywords

Hepatitis B virus reactivation · Allogeneic hematopoietic stem cell transplantation · Hepatitis B surface antigen seroclearance · Case report

Abstract

Reactivation of hepatitis B virus (HBV) is known to occur frequently after hematopoietic stem cell transplantation (HSCT). The reactivation can be prevented by nucleos(t)ide analogue (NA), but it is unclear how long NA should be continued. Here, we report 3 cases of HBV reactivation with discontinuation of NA following the discontinuation of immunosuppressive therapies after HSCT. Three male patients aged 34, 59, and 54 years received allogeneic HSCT (allo-HSCT) for chronic myeloid leukemia, mixed phenotype acute leukemia, and myelodysplastic syndrome, respectively. Before HSCT, 2 patients were positive for hepatitis B surface antigen (HBsAg) and 1 patient was negative for HBsAg and positive for antibodies to hepatitis B core

antigen. NA (lamivudine or entecavir) was started at the same time as HSCT and stopped after the discontinuation of immunosuppressive therapies. In all patients, the serum HBV DNA levels were increased after the discontinuation of NAs. Two of the three patients developed severe hepatitis with high levels of HBV DNA (7.5 and 7.4 log IU/mL, respectively). A patient without hepatitis was re-administered NA soon after the HBV DNA started to increase (3.3 log IU/mL). Interestingly, the 2 patients who developed hepatitis cleared HBsAg promptly after the recovery from hepatitis and they could stop NAs without the reversion of HBsAg. It was speculated that transplanted immune cells, which were naïve for HBV, react strongly with HBV antigens that were increased after the NA discontinuation. The discontinuation of NA after allo-HSCT is not recommended generally because strong hepatitis might be induced even after several years.

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Introduction

It has been estimated that almost one-third of people in the world experience infection with hepatitis B virus (HBV) and, among them, almost 292 million persons are considered to have hepatitis B surface antigen (HBsAg) in the sera [1]. Generally, HBsAg-positive persons are regarded to have HBV persistent infection, and HBsAg rarely disappears in the natural course of chronic infection or with antiviral therapies [2]. Once HBV infects hepatocytes, HBV utilizes the cellular functions and forms its life cycle [3]. During the cycle, covalently closed circular DNA (cccDNA), which is a template for viral proteins including HBsAg, is formed in the nucleus. Nucleos(t)ide analogues (NAs) are widely used for HBV chronic infection, but they do not efficiently reduce cccDNA. HBsAg-positive patients with high levels of serum HBV DNA and alanine aminotransferase (ALT) are at high risk of liver cirrhosis and hepatocellular carcinoma [4] and they are considered to be targets of antiviral therapies.

HBsAg disappearance is regarded as the optimal treatment endpoint, termed “functional cure” [4]. However, the loss of HBsAg does not mean the absence of cccDNA, and HBsAg-cleared patients with antibodies to hepatitis B surface antigen (HBsAb) and/or antibodies to hepatitis B core antigen (HBcAb) are at risk of HBV reactivation [5, 6]. Although cases with spontaneous HBV reactivation have been reported [7], the reactivation occurs mainly after anti-cancer chemotherapies or immunosuppressive therapies in HBsAg-positive or HBsAg-cleared patients [5]. Especially, patients who received allogeneic hematopoietic stem cell transplantation (allo-HSCT), including bone marrow transplantation (BMT), peripheral blood stem cell transplantation, and cord blood transplantation (CBT), are known to be at high risk of HBV reactivation [8]. The administration of NA is known to be effective for the prevention of the HBV reactivation [9]. However, the discontinuation of NA in such patients has rarely been reported and the prognosis is unclear. Also, it is unclear how long the NA should be continued. Here, we describe the clinical courses of 3 patients who discontinued NA after allo-HSCT. They experienced HBV reactivation and, interestingly, 2 patients who had ALT elevation cleared HBsAg after the recovery from hepatitis.

Case Presentation

Case 1

A 34-year-old Japanese male received unrelated CBT for chronic myeloid leukemia in a local hospital. Three months before the CBT, he was positive for HBsAg and lamivudine (LAM) was administered to prevent HBV reactivation. After the discontinuation of immunosuppressive drugs (tacrolimus) for graft-versus-host disease prophylaxis, LAM was stopped 3 years after the CBT. Two months after the LAM discontinuation, he was found to have liver dysfunction of ALT 329 U/L and entecavir (ETV) was started. However, 3 days later, his liver function tests revealed a marked elevation of ALT (10,296 U/L), total bilirubin (8.7 mg/dL), and decrease in the prothrombin time (20%) and he was transferred to our hospital. Regarding HBV markers, HBsAg was positive (>250 IU/mL), hepatitis B e antigen (HBeAg) was negative, antibodies to hepatitis B e antigen (HBeAb) were positive, and HBV DNA was 7.5 log IU/mL. Of note, immunoglobulin M class antibodies to hepatitis B core antigen (IgM-HBc), which increase in patients with HBV acute infection but generally not in those with chronic infection, were strongly positive (25.2 index). No hepatic encephalopathy was observed, and he recovered with conservative therapies and was discharged on day 28. His serum HBsAg disappeared and HBsAb turned positive 1 year and 10 months after the start of ETV, and the ETV was then stopped. Subsequently, his serum HBsAg remained negative for 3 years and 8 months, and he was referred to a local hospital. His clinical course is shown in Figure 1. Direct sequencing for the HBV S region using a stored serum sample revealed the subgenotype of HBV as B2/Ba.

Case 2

A 59-year-old Japanese male received BMT (from an HLA-matched unrelated donor) for mixed phenotype acute leukemia in the Department of Hematology of our hospital. At that time, he was negative for HBsAg and HBV DNA but was positive for both HBsAb and HBcAb. His primary physician decided to administer ETV at the time of BMT, and it was discontinued 1 year later following the discontinuation of tacrolimus. Nine months after the discontinuation of ETV, his serum HBV DNA turned positive and gradually increased. One year and 4 months after the ETV discontinuation, HBV DNA increased to 7.4 log IU/mL and liver dysfunction appeared (ALT 633 U/L), and he was referred to the Department of Gastroenterology. At that time, his HBsAg level was high (>250 IU/mL), HBeAg was positive, and HBeAb was negative. The HBV genotype determined by enzyme immunoassay was B. Similarly to case 1, IgM-HBc was strongly positive (29.7 index). He was treated with ETV again, HBsAg then disappeared, and HBsAb became positive 2 years and 8 months after the restart of ETV. Then, ETV was discontinued but HBsAg remained negative for 9 years. His clinical course is shown in Figure 2.

Case 3

A 54-year-old male received BMT from an HLA-matched unrelated donor for myelodysplastic syndrome in the Department of Hematology of our hospital. He had been pointed out for positivity of HBsAg when he was 35 years old. Five months before BMT, because his serum

HBV DNA was positive (3.2 log IU/mL), an antiviral treatment with ETV was started in a previous hospital. Then, the HBV DNA remained at an undetectable level in our hospital during the ETV treatment. He was referred to the Department of Gastroenterology because of an ALT elevation without an increase of HBV DNA 1 year and 6 months after BMT. He soon recovered from the ALT flare and, after that, such an ALT elevation was not observed again. Eight years after BMT, he was still positive for HBsAg but, because of the stable status of liver function and HBV markers, his physician decided to discontinue ETV. Then, HBV DNA increased to 3.3 log IU/mL 4 months after the discontinuation of ETV, and ETV was restarted before an ALT elevation. After that, HBV DNA decreased soon and HBsAg remained positive at a similar level as before the transient HBV DNA increase. Enzyme immunoassay showed that the HBV genotype was B. His clinical course is shown in Figure 3.

Discussion

In this report, we show 3 patients who received allo-HSCT who discontinued NAs that were administered for the prevention of HBV reactivation. The characteristics of these patients are summarized in Table 1. In all 3 patients, the serum level of HBV DNA increased after the NA discontinuation, although it was after the discontinuation of immunosuppressive drugs for graft-versus-host disease prophylaxis. Two patients experienced hepatitis and then the serum HBsAg became negative and NA could be stopped without further reactivation. We recommend to not stop NA after allo-HSCT because it can lead to severe hepatitis, although HBsAg may be cleared frequently. Such HBsAg clearance is consistent with a previous report showing a high rate of HBsAg clearance in patients with reactivation of HBV from resolved infection [10]. In that study, HBsAg-negative/HBcAb-positive patients who underwent rituximab-containing chemotherapy or HSCT for hematological malignancies were included and antiviral treatments were performed only for HBV-reactivated patients.

Regarding the mechanisms of HBV reactivation in patients who received allo-HSCT even after the discontinuation of immunosuppressive agents, loss of the acquired immune system that controls active HBV proliferation is considered [11]. Then, HBV replication occurs from cccDNA in the hepatocytes and, after the spread of HBV-infected hepatocytes, the immune cells derived from the transplanted stem cells react vigorously with the HBV-infected cells. The responses might be similar to those in patients with acute self-limiting HBV infection. In such patients, robust, polyclonal, and multi-specific CD4 and CD8 T-cell responses and neutralizing antibody responses were observed [12]. Actually, the levels of IgM-HBc were high in cases 1 and 2, similar to acute infection with HBV. In contrast, adaptive immune responses in chronically HBV-infected patients are known to be depleted qualitatively and/or quantitatively [12], which is characterized by the impairment of effector cytotoxic activity and cytokine production. Also, the expression of inhibitory receptors, such as programmed cell death-1 and cytotoxic T lymphocyte-associated antigen-4, is increased on the surface of exhausted T cells [13]. Moreover, patients with HBV acute infection showed high levels of cytokines/chemokines including interleukin-21 in their serum, and acutely infected patients who developed persistent infection or chronically infected patients who experienced acute exacerbation did

not show such increases [14]. Therefore, a functional restoration of immune responses, including cytokines/chemokines, in patients who received allo-HSCT might be important for HBsAg clearance. It would be interesting to analyze the cytokines/chemokines in HBV-reactivated patients after allo-HSCT in a future study. Another important point in the present cases is that such immune reactions were not induced under the NA administration. Increased antigen levels might be required to induce effective responses in the post-HSCT state.

Interestingly, all 3 patients in this study were infected with HBV of genotype B. In Japan, genotype C HBV is found most frequently in chronically infected patients [15]. In comparison, genotype B HBV is found more frequently in northeast Japan where our hospital is located [16]. Genotype B HBV is considered to cause less progression of liver diseases in chronically infected patients [17], but it might cause severe hepatitis, such as acute liver failure, more frequently than genotype C [18]. Because the pathogenesis of HBV reactivation is similar to acute infection in patients who received allo-HSCT as mentioned above, genotype B HBV may have a risk of reactivation.

As factors for HBV reactivation, the association with HBV mutations, including immune escape mutations, has been reported [7, 19, 20]. In HBeAg-negative patients in the inactive state, G1896A mutation in the precore region of HBV genome and/or A1762T/G1764A mutations in the core promoter region were frequently found [21, 22]. Also, such mutations were found in fulminant hepatitis patients and high replication capacity in cell culture models has been reported [18, 23]. In an immune state that is naïve for HBV and lacks acquired immunity after allo-HSCT, HBV with these mutations might replicate rapidly after the discontinuation of NA, as in cases 1 and 3. Because case 2 was positive for HBeAg, HBV in this case might not have the precore mutation. Probably, this is one of the reasons why there was a delay in the HBV DNA elevation after the discontinuation of ETV. However, as a limitation of this study, we could not obtain serum samples that contained enough HBV DNA for sequencing analysis including the core promoter and precore regions.

Some previous reports showed that HBsAg-positive patients who received BMT from HBsAb-positive donors cleared HBsAg more frequently than those who received BMT from HBsAb-negative donors [24]. The mechanisms of HBsAg clearance in the previous reports might be different from that in the present report. In a previous report in which NA was not administered before/after BMT, ALT flare occurred during or soon after immunosuppressive therapy, and then HBsAg disappeared. Even under such an immunosuppressive state, acquired immunity against HBsAg derived from HBsAb-positive donors might work more efficiently because of the high levels of viral antigens.

In conclusion, in this series of cases, NA discontinuation after allo-HSCT induced HBV reactivation, and HBsAg was cleared in patients with ALT flare. Reconstruction of immune cells and strong responses against increased viral antigens could lead to HBsAg clearance, which is a goal of anti-HBV therapy. However, we consider that NA discontinuation is not recommended after allo-HSCT in recipients with HBsAg because it induces severe hepatitis. To clear HBsAg safely, further studies are required for the development of novel therapies.

Statement of Ethics

Written informed consent for publication of this case report and any accompanying images was obtained from the patients.

Conflict of Interest Statement

All authors declare that they have no conflicts of interest related to this study.

Funding Sources

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author Contributions

M. Tsuruoka and J. Inoue wrote the manuscript; Y. Onishi, M. Ninomiya, E. Kakazu, T. Iwata, A. Sano, and K. Sato diagnosed and treated the patients; Y. Onishi, H. Harigae, and A. Masamune helped to draft the manuscript and revised it critically.

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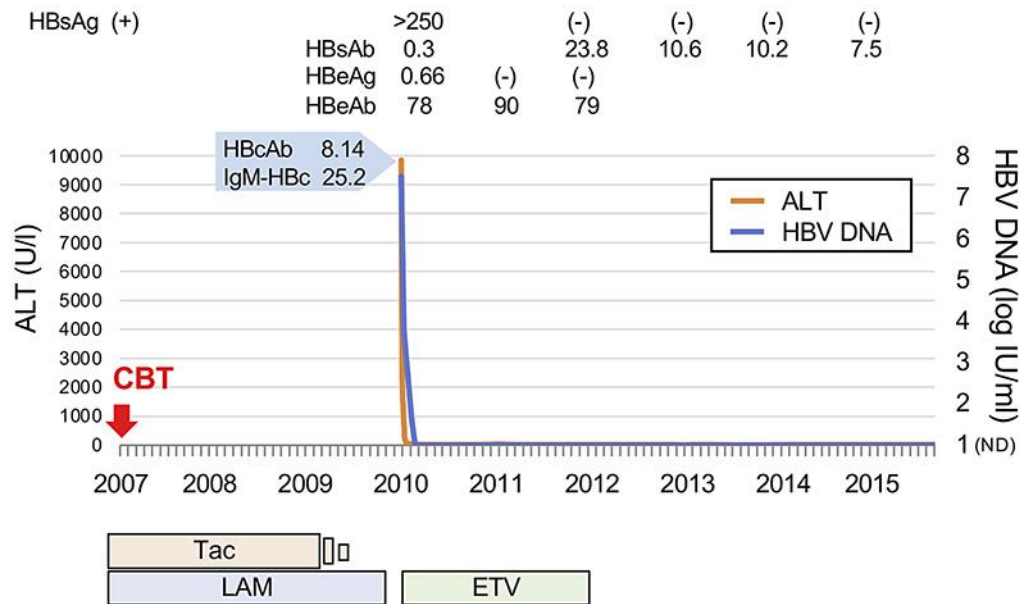


Fig. 1. Clinical course of case 1. ALT, alanine aminotransferase; CBT, cord blood transplantation; ETV, entecavir; HBcAb, antibodies to hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBeAb, antibodies to hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBsAb, antibodies to hepatitis B surface antigen; HBV, hepatitis B virus; HSCT, hematopoietic stem cell transplantation; IgM-HBc, immunoglobulin M class antibodies to hepatitis B core antigen; LAM, lamivudine; ND, not detected; Tac, tacrolimus; T-Bil, total bilirubin.

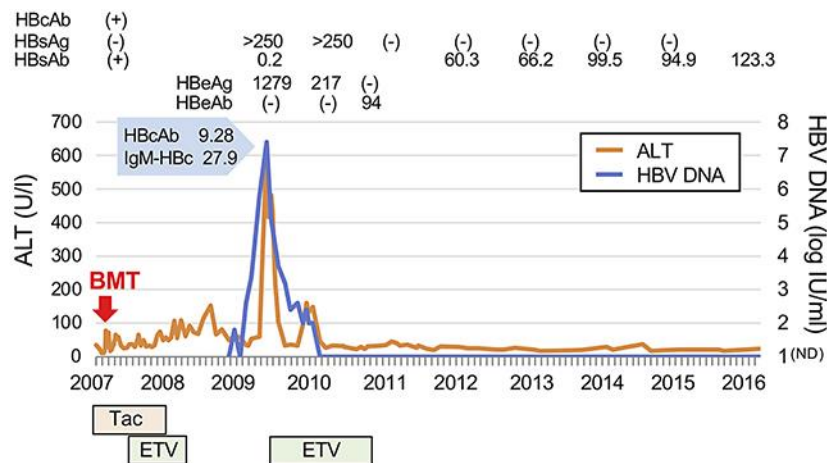


Fig. 2. Clinical course of case 2. BMT, bone marrow transplantation. See Figure legend 1 for other abbreviations.

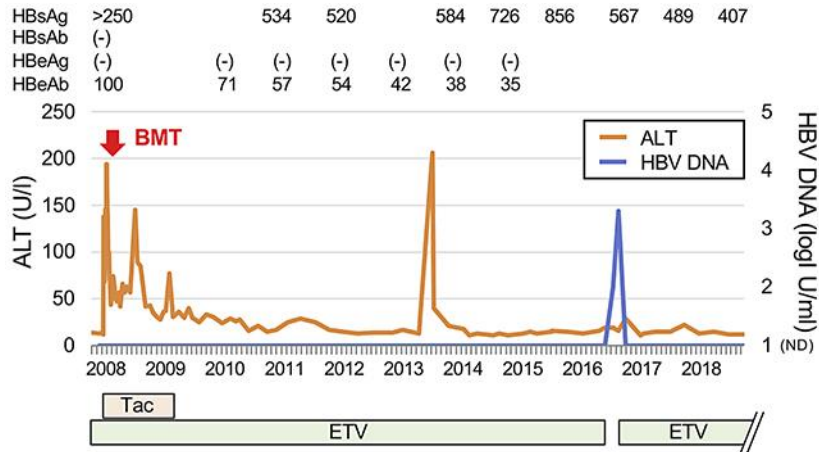


Fig. 3. Clinical course of case 3. BMT, bone marrow transplantation. See Figure legend 1 for other abbreviations.

Table 1. Clinical characteristics of the 3 cases in this study

	Case 1	Case 2	Case 3
Sex	Male	Male	Male
Age at the time of HSCT/HBV reactivation, years	34/37	59/61	54/62
Hematological disease	Chronic myeloid leukemia	Mixed phenotype acute leukemia	Myelodysplastic syndrome
Type of HSCT	CBT	BMT	BMT
GVHD prophylaxis	Tacrolimus + short-term MTX	Tacrolimus + short-term MTX	Tacrolimus + short-term MTX
HBV genotype (subgenotype)	B (B2/Ba)	B	B
NA (duration after HSCT)	LAM (3 years)	ETV (1 year)	ETV (8 years)
Duration from NA stop to ALT elevation	2 months	1 year and 4 months	Not applicable
Peak of ALT (U/L)/T-Bil (mg/dL)	10,296/17.4	633/0.8	29/1.1
HBV markers at the time of HSCT	HBsAg (+)	HBsAg/Ab (-/+) HBcAb (+)	HBsAg/Ab (+/-) HBeAg/Ab (-/+)
HBV markers at the time of HBV reactivation	HBsAg/Ab (+/-) HBeAg/Ab (-/+)	HBsAg/Ab (+/-) HBeAg/Ab (+/-)	HBsAg (+) HBeAg/Ab (-/-)
HBV markers after reactivation	HBsAg (-) HBeAg/Ab (-/+)	HBsAg (-) HBeAg/Ab (-/+)	HBsAg (+) HBeAg/Ab (-/-)

HSCT, hematopoietic stem cell transplantation; HBV, hepatitis B virus; CBT, cord blood transplantation; BMT, bone marrow transplantation; GVHD, graft-versus-host disease; MTX, methotrexate; NA, nucleos(t)ide analogue; LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T-Bil, total bilirubin; HBsAg, hepatitis B surface antigen; HBsAb, antibodies to hepatitis B surface antigen; HBcAb, antibodies to hepatitis B core antigen; HBeAg, hepatitis B e antigen; HBeAb, antibodies to hepatitis B e antigen.