

Rapid loss of HBs antigen in patients with HBV reactivation and high level of transaminases during immunosuppressive therapy - case series

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Abstract

Reactivation of hepatitis B virus (HBV) infection has been described in patients with HBsAg negative and antiHBc positive (occult hepatitis B infection - OBI) receiving immunosuppressive therapy (IST). The lack of proper monitoring of patients with this HBV infection during IST can result in viral reactivations with high level of transaminases, jaundice and even acute liver failure. In these situations, it is mandatory to start antiviral therapy with nucleot(s) ide analogs (NA) which produce a strong viral suppression. We report a series of five cases of OBI patients with severe HBV reactivation during IST. One patient was diagnosed with hematologic malignancy (non-Hodgkin lymphoma), two with rheumatoid arthritis, one with psoriasis and one patient with renal transplant. All the patients were evaluated and treated for the reactivation of HBV in the Prof. Dr. Matei Bals National Institute of Infectious Diseases, a tertiary care hospital from Bucharest, Romania. At the time of HBV reactivation diagnosis, 3 patients were asymptomatic and two developed jaundice. All had acute ALT flares (more than 10 times the upper limit of normal range - ULN), very high HBV viral loads and anti-HBc serum IgM antibodies. All patients were immediately treated with ETV 0.5 mg /day and if it was possible, IST was stopped. In all cases was obtained quickly HBsAg loss under antiviral therapy.

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Background

Patients with HBV infection, especially those with OBI defined by serum negative surface antigen (HBsAg) and presence of hepatitis B core antibodies (anti-HBc), should be carefully

monitored during IST used for hematologic or rheumatologic diseases, because of the significant potential of reactivation. The risk of HBV reactivation depends on the immunosuppressive regimen used and the HBV serological and viro-

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logical profile. Anti-B-cell agents, such as Rituximab, have a high risk of HBV reactivation because they induce a broad and deep B-cell depletion (1).

The lack of proper monitoring of patients with OBI during IST can result in viral reactivations with high level of ALT, jaundice and even acute liver failure. In these situations, it is mandatory to start antiviral therapy with nucleot(s)ide analogs (NA) which produce a strong viral suppression.

All international guidelines (2,3) recommend prophylactic therapy regardless of HBsAg status to all patients receiving anti-CD20 therapy or undergoing stem cell transplantation. OBI patients may be at risk of reactivation and should undergo either monitoring (if they have high HBsAb titers) or prophylactic NA therapy with Entecavir (ETV), Tenofovir disoproxil fumarate (TDF) or Tenofovir disoproxil alafenamide (TAF); Lamivudine is no longer recommended as it has a low barrier to resistance.

The aim of our communication is to present a series of OBI patients with severe HBV reactivation during IST, treated with ETV, who experienced rapid HBsAg loss under antiviral therapy. All patients signed the informed consent before the inclusion, according to the latest version of World Medical Association Declaration of Helsinki.

Case description

We present five cases, three males and two females (mean age of 57.4 years), one patient with a hematologic malignancy, two with rheumatoid arthritis, one with psoriasis, and one patient with renal transplant. All the patients were evaluated and treated for the reactivation of HBV in the Prof. Dr. Matei Bals National Institute of Infectious Diseases, a tertiary care hospital in Bucharest, Romania.

Prior to IST, all the patients had negative serum HBsAg and positive IgG HBc antibodies. Two patients were negative for HBsAb, two had HBsAb titers above 10 UI/ml, and in one case no prior quantification of HBsAb was performed. In one patient, HBV DNA was measured prior to IST and the result was negative. Before initiating IST all patients had normal ALT.

Table I presents the most important clinical, biological, and virological characteristics of our patients.

At the time of HBV reactivation diagnosis, 3 patients were asymptomatic and two developed jaundice. All had acute ALT flares (> 10 times the upper limit of normal range - ULN), high HBV DNA and positive IgM HBc. Four patients were HBeAg positive. The epidemiological inquiry for other recent viral parenteral transmission was negative. All patients received ETV 0.5 mg /day and, if possible, IST was stopped.

The first patient was a 48-year-old female, with a severe form of psoriasis under therapy with methotrexate and cyclosporin for one year. At the start of IST, the patient was screened for HBV and was HBsAg and HBsAb negative but IgG HBc positive. Because of severe fatigue during IST (which was started one year before), the ALT was checked and a high level of ALT was discovered. The patient's HBV profile revealed: HBsAg and HBeAg positivity, IgM HBc antibodies, and HBV DNA= 16 million UI/ml. IST was stopped and ETV was started. The outcome was good with HBeAg loss after 4 months and HBsAg clearance after 6 months of therapy. The patient continued ETV one year after IST cessation without HBsAb seroconversion.

The second patient was a 66-year-old female, with rheumatoid arthritis and who was enrolled in a clinical trial with a biological agent (Baricitinib). The HBV profile was at the start of IST:

HBsAg negativity, IgG HBc antibodies positive, HBsAb positive (titer > 1000ui/ml). After 6 months of IST, the patient developed jaundice, a high level of ALT with HBsAg, IgM HBc and HBeAg positive with HBV DNA > 170 million UI/ml. The investigational product was stopped and ETV was started with HBeAg loss after 4 months and HBsAg clearance after 6 months. After 2 months of ETV, adalimumab was started and the patient continued ETV, with persistence of HBsAg negativity, but no HBsAb were subsequently detected.

The third patient was a 35-year-old male who had kidney transplantation 2 years before HBV reactivation. He had been receiving tacrolimus since transplantation. The HBV profile was: negative HBsAg and HBsAb, but had positive IgG HBc antibodies. Because at that time the local guidelines for patients with solid organ transplantation were not recommending prophylaxis for HBV reactivation in this situation, the patient did not receive any medication for HBV. After two years, he developed jaundice with a high level of ALT and was referred to our clinic. The profile of HBV was: HBsAg, IgM HBc antibodies and HBeAg positive with viral load 1.3 million UI/ml. ETV was started with HBe seroconversion and HBsAg clearance after 6 months and with HBs seroconversion after 12 months of antiviral treatment. The patient continues to receive ETV and is monitored in our clinic every 6 months.

The fourth patient was a 69-year-old male with B-cell non-Hodgkin lymphoma treated with Rituximab-containing regimens (R-CHOP). The HBV profile was: HBsAg, HBsAb and HBeAg negative, but IgG HBc positive. He received R-CHOP for 6 months without any antiviral prophylaxis. The HBV reactivation occurred during the 7th cycle of IST, with ALT > 10ULN, reverse seroconversion of HBsAg, HBeAg, IgM HBc antibodies and HBV DNA > 38 million UI/ml. The patient received ETV with ALT nor-

malization after two months and had HBs seroconversion with HBsAb titer of 200 mUI/ml and undetectable viral load after 6 months of therapy.

The fifth case was a 69-year-old male diagnosed with rheumatoid arthritis, for 6 years on anti-TNF-alpha therapy (Infliximab, followed by Adalimumab). The anti-TNF-alpha therapy was followed by Rituximab from December 2009 until March 2015. Prior to the start of Rituximab he was negative for HBsAg, but positive for IgG HBc and HBsAb (35 mUI/ml). After 12 cycles of Rituximab his ALT level was high and he was admitted to our hospital. The viral pattern showed HBsAg, IgM HBc and HBeAg positive, HBsAb negative and HBV DNA > 14 million UI/ml.

He received Entecavir 0.5 mg/day with normalization of ALT level and HBe seroconversion after four months. After six months, the viral load became undetectable and he achieved HBs seroconversion with positive HBsAb (263mUI/L).

Discussions

HBV reactivation may occur either spontaneously, after discontinuation of antiviral therapy or in patients with conditions requiring IST (4,5). The evolution depends on the complex interplay between the host's immune response and viral replication. HBV reactivation was reported during IST both in patients with HBsAg positive chronic HBV infection and with OBI. We present **5 cases** with OBI who had HBV reactivation, all with acute hepatitis flares (defined as an abrupt ALT elevation > 5 x ULN) (6), serum presence of IgM HBc antibodies, and reverse seroconversion from HBsAg negative to HBsAg positive due to different causes of iatrogenic immunosuppression. All patients achieved HBsAg loss and had a favourable clinical outcome after stopping IST and received ETV treatment, which signified the return to the initial HBV status, prior to the initiation of IST. None of the pa-

M/35 year	Kidney transplantation/ Tacrolimus	HBsAg negative active IgG HBc positive	HBsAg positive QHBsAg= 4460 HBsAg positive HBsAb negative ALT= 961ui/ML BT = 3.3mg/dl HBV-DNA= 1.3 million UI/ml START ETV	HBsAg positive IgM HBc positive HBsAg positive HBsAb positive ALT= 354UI/ml BT =1.9mg/dl HBV-DNA= 1.3 million UI/ml START ETV	HBsAg positive Q HBsAg=120 HBsAg positive HBsAb negative ALT=32UI/ml BT=0.9mg/dl	HBsAg negative HBsAb negative HBsAb=100UI/l ALT=23UI/ml BT=0.6mg/dl HBV-DNA negative	Continue ETV After one year HBsAb positive= 190 UI/L
M/69 year	B-cell non-Hodgkin lymphoma (NHL) / Rituximab	HBsAg negative active IgG HBc positive HBsAb negative active	HBsAg positive IgM HBc positive HBsAb positive HBV-DNA= 38,700,000 UI/ml ALT=457UI/ml BT= 1.9mg/dl Start ETV Stop IST	HBsAg positive IgG HBc positive HBsAg positive HBV-DNA= 230UI/ml BT=1 mg/dl HBV-DNA=2,300UI/ml Start ETV Stop IST	HBsAg positive ALT=30 UI/ml	HBsAg negative HBsAb positive=200 UI/l HBV-DNA=negative	Continue ETV Hematological remission
M/69 year	Rheumatoid arthritis / 6 years with Adalimumab/ Infliximab Rituximab (6,5 years) and metotrexat 12 years	HBsAg negative active IgG HBc positive HBsAb positive positive mUI/ml	HBsAg positive QHBsAg=230 HBsAg positive IgM HBc positive HBsAb negative HBV-DNA= 14,135,879UI/ml ALT= 658UI/ml BT=1.8mg/dl Stop IST Start ETV	HBsAg positive HBsAg positive HBV-DNA= 267,908UI/ml ALT= 2183UI/ml BT= 1.3mg/dl	HBsAg positive HBsAg positive HBV-DNA= 188 UI/ml ALT=206UI/ml BT=1.5mg/dl	HBsAg negative HBsAb positive=263UI/L HBsAb positive HBV-DNA negative ALT= 20UI/ml BT= 1.5mg/dl	Continue ETV Continue Rituximab Still HBsAb positive =260 UI/L

HBV=hepatitis B virus; IST=immunosuppressive therapy; ETV=entecavir; CHT=chemotherapy; HBsAg= Hepatitis B surface antigen; QHBsAg= quantitative hepatitis B surface antigen; HBsAb=antibody against hepatitis B surface antigen; HBsAg=Hepatitis B e antigen; HBsAb= antibody against hepatitis B e antigen; IgM HBsAb= Hepatitis B core antibodies IgM; IgG HBsAb= Hepatitis B core antibodies IgG; ALT: Alanine aminotransferase; BT=total bilirubin; UI/L= upper limits of normal

tients developed liver failure and no death was recorded.

We hypothesize that the restoration of immune antiviral responses after IST cessation led to achieving again viral control and HBsAg loss for all cases and presence of HBsAb in three patients. Interestingly, one patient who initially had serum HBsAb failed to develop them after one year since the moment of IST cessation and initiation of ETV treatment, while two other patients, negative for HBsAb before IST achieved seroconversion to HBsAb following reactivation. One patient had prior serum HBsAb and managed to reacquire HBsAb, with a higher titer, after HBV reactivation and treatment. In one case no prior HBsAb testing was performed, which represents a limitation of the present study. All of our patients achieved HBeAg clearance and had normal ALT after 4 months of ETV therapy. HBsAg clearance occurred within 6 months of therapy and HBV viral load became also undetectable in less than 6 months after the initiation of antiviral treatment.

The prevalence estimates for OBI vary widely between 1% to 87% according to different studies in various geographical regions of the world and many clinical and pathogenic characteristics of OBI are still incompletely defined (7-9). In spite of strong immune responses, that limit HBV replication, in OBI patients HBV DNA is preserved in the nuclei of hepatic cells as covalently-closed-circular DNA (cccDNA).

OBI is increasingly recognized as having a major clinical relevance, especially in patients during IST, when loss of immune surveillance may lead to significant HBV replication, with rapid viral protein expression in hepatocytes, causing their direct destruction (10).

Another major mechanism of hepatic destruction, which may significantly increase the risk of hepatic decompensation and failure is the immune restoration syndrome, following cessation

of IST. Rapid reconstitution may lead to exaggerated immune responses against hepatocytes that express hepatitis B viral proteins, with potential major necrosis of liver cells (11).

Considering these pathogenic mechanisms, the management of HBV reactivation must include both cessation of IST (which permits the restoration of antiviral immune responses) (12) and direct antiviral treatment (which limits the initial uncontrolled viral replication, the expression of hepatitis B-associated viral proteins and decreases the cytopathic effect associated with rapid immune restoration). Immune recovery after stopping IST has the potential of achieving better viral control, as observed in our case series in two patients that achieved HBsAg seroconversion after OBI reactivation.

HBV reactivation associated with IST may appear in the first months of therapy or can be delayed; it may even be observed a long time after cessation of immunosuppression intervention. Muraishi et al. (13) reported OBI reactivation in a 68-year-old man with lymphoma, which occurred 27 months after the end of rituximab-associated chemotherapy. Rituximab is associated with prolonged immunosuppression, due to major B-lymphocyte depletion effects (14), followed by a delayed immune restoration phase, which may explain why in Rituximab-based regimens HBV reactivation may be observed more than 12 months after the end of IST (15).

In our case series, all patients had HBV reactivation after more than 6 months of IST, including two patients with Rituximab-containing regimens.

A few other studies reported that an ALT level of more than 200 UI/l was associated with HBsAg clearance (16,17). Ueda et al. in Japan and Sanchez et al. in Spain (2009) reported HBsAg loss after 2 and 6 months of Entecavir treatment in patients who received chemotherapy for B-cell lymphoma (18,19). Brost et al. (20) reported in

Germany four cases with hematological malignancies and HBV reactivation. All were associated with rapid ALT normalisation and undetectable viral load between 3 and 13 months of antiviral treatment. In a retrospective study of 392 HBV patients followed for more than 5 years, Shinya N et al. observed that hepatic flares promote rapid and greater reduction of HBsAg levels in patients with HBsAg seroclearance (21). International guidelines recommend HBV screening for all patients receiving IST. This should include evaluation of HBsAg, IgG HBc, and HBsAb. HBV reactivation prophylaxis must be recommended to patients with positive HBsAg and also to those who only have positive IgG HBc. Current guidelines recommend the use of Entecavir or Tenofovir for prophylaxis of HBV reactivation (2,3).

We emphasize that in contrast to HBV chronic hepatitis treated with NA, severe OBI reactivation has a particular outcome, with a higher rate of HBsAg clearance. According to our experience, the rate of HBsAg loss in patients with chronic HBV hepatitis undergoing NA therapy is under 5% (22,23). All of our OBI patients with severe reactivation cleared AgHBs. Further studies in this direction could be important.

There are studies who have shown that atypical serological profiles and high HBV viral load may be an indicator of S gene mutations causing OBI, HBV reactivations and progress to severe forms of liver damage which is why it is important to detect gene mutations by molecular analysis and monitor them (24).

In summary, screening for HBV (HBsAg, HBsAb, HBcAb) should be performed in all patients who need IST, followed by prophylaxis, when required, and careful monitoring, as reactivation in OBI patients may occur even late after the start of immunosuppression regimen. OBI reactivation may occur during various IST regimens and ETV treatment and cessation of IST

both contribute to rapid viral control and may induce HBsAg seroconversion.

Abbreviations

HBV=hepatitis B virus

IST=immunosuppressive therapy

ETV=entecavir

CHT=chemotherapy

HBsAg= Hepatitis B surface antigen

QHBsAg= quantitative hepatitis B surface antigen

HBsAb=antibody against hepatitis B surface antigen

HBeAg=Hepatitis B e antigen

HBeAb= antibody against hepatitis B e antigen

IgM HBcAb= Hepatitis B core antibodies IgM

IgG HBcAb= Hepatitis B core antibodies IgG

ALT: Alanine aminotransferase

BT=total bilirubin

ULN= upper limits of normal

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Authors' Contributions

VM (Conception and design; Acquisition of data; Analysis and Interpretation of data; Writing Manuscript; Final approval), CP (Conception and design; Acquisition of data; Analysis and Interpretation of data; Writing Manuscript; Final approval); CT (Conception and design; Acquisition of data; Analysis and Interpretation of data; Writing Manuscript; Final approval), VA (Conception and design; Acquisition of data; Analysis and Interpretation of data; Writing Manuscript; Final approval), SSA (Conception and design; Acquisition of data; Analysis and Interpretation of data; Writing Manuscript; Final approval). All the authors had equally contributed in elaborating the paper.

Conflict of Interest

None to declare.

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