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Case report

Successful use of entecavir for a severe case of reactivation of hepatitis B virus following polychemotherapy containing rituximab☆

Mayra J. Sanchez[1], Maria Buti[1,*], Maria Homs[2], Andres Palacios[3], Francisco Rodriguez-Frias[2], Rafael Esteban[1]

[1]*Hospital Vall d'Hebron, Liver Unit, CIBERehd Institute Carlos III, Paseo de la Vall d'Hebron, 08035 Barcelona, Spain*
[2]*Hospital Vall d'Hebron, Biochemistry Department, CIBERehd Institute Carlos III, Barcelona, Spain*
[3]*Hospital Vall d'Hebron, Hematology Division, Barcelona, Spain*

***Backgrounds/Aims*: Hepatitis B virus (HBV) reactivation following treatment with rituximab has been reported in patients with either HBsAg-positive, or HBsAg-negative and anti-HBc positive infection. Patients with severe reactivation often have a fatal outcome despite treatment with lamivudine. The use of entecavir has not been reported in patients with severe HBV reactivation.**

***Methods*: We present a case of a HBsAg-negative patient diagnosed with chronic lymphocytic leukemia who received a chemotherapeutic regimen that included rituximab, who subsequently presented with severe HBV reactivation with ascites, jaundice and coagulopathy and was treated with entecavir. A review of the literature and underlying HBV associated mutations are discussed.**

***Results*: Entecavir produced a rapid and sustained suppression of HBV that was associated with rapid clinical improvement without any side effects.**

***Conclusion*: Entecavir is an efficacious and safe treatment for severe HBV reactivation.**

***Keywords*: Entecavir; HBV reactivation; Rituximab**

1. Introduction

Fatal hepatitis B virus (HBV) reactivation following treatment with chemotherapy has been reported to occur in two settings: in chronic carriers of hepatitis B surface antigen (HBsAg) and in patients with prior HBV infection who are HBsAg-negative and have antibodies against hepatitis B core antigen (anti-HBc) with or without antibodies to hepatitis B surface antigen (anti-HBs) [1,2]. HBV reactivation has important clinical and therapeutic implications; however, there is no clear consensus in the guidelines regarding HBV screening and management [3,4].

The consensus guidelines issued by the American Association for the Study of Liver Diseases (AASLD) recommend screening with HBsAg in patients at high risk for hepatitis B infection prior to the initiation of chemotherapy and prophylaxis for those who are HBsAg-positive. They do not recommend anti-HBc screening or prophylaxis for individuals who are HBsAg-negative or for those who are anti-HBc positive,

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* Corresponding author. Tel.: +34 93 2746140; fax: +34 93 2746068.
E-mail address: mbuti@vhebron.net (M. Buti).

Abbreviations: HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen.

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with or without anti-HBs positivity [3]. The Management of Hepatitis B guidelines issued by the European Association for the Study of the Liver (EASL) recommend screening of all the candidates for chemotherapy and immunosuppressive therapy with HBsAg and anti-HBc antibodies prior to initiation of treatment [4]. According to these guidelines, prophylaxis against HBV reactivation is only recommended for patients who are HBsAg-positive. For those who are anti-HBc positive with an undetectable serum HBV-DNA, they recommend following serum alanine transaminase (ALT) and HBV-DNA level and to start antiviral therapy if and when there is evidence of HBV reactivation. The same guidelines recommend prophylaxis and treatment with lamivudine, but mention that entecavir and tenofovir should be considered in patients with high levels of HBV-DNA, though the experience in this setting with these medications is very limited.

We describe a case of severe HBV reactivation with decompensated liver disease in an HBsAg-negative patient who received a chemotherapeutic regimen that included rituximab and fludarabine for treatment of chronic lymphocytic leukemia, and who was successfully treated with entecavir, achieving a rapid and sustained suppression of HBV replication.

2. Case report

A 62 year-old male was diagnosed in May 2007 with chronic lymphocytic leukemia. He initially presented with Rai Stage 0, and was managed with observation until August 2008, when he developed cervical lymphadenopathy and was considered for chemotherapy. He had no history of liver disease and prior to initiating chemotherapy his liver enzymes were normal, HBsAg was negative and anti-HBc positive and anti-HBs were not determined. Therefore, no HBV prophylaxis was initiated. From September to November 2008 the patient was treated with four cycles of polychemotherapy (rituximab, fludarabine and cyclophosfamide), which resulted in complete tumoral remission. In December 2008, 4 weeks after the last cycle, his liver enzymes were abnormal with an AST 121 IU/L (normal <40.0 IU/L), ALT 257 IU/L (normal <44.0 IU/L) and normal alkaline phosphatase and total bilirubin. These elevations were thought to be related to chemotherapy and no intervention was undertaken. Two months later, the patient presented with fatigue, jaundice and ascites. On physical exam, the patient was alert and oriented, with significant jaundice, hepatosplenomegaly and ascitic, without asterixis. A paracentesis was performed. The ascites fluid was translucent with a protein of 0.7 g/dL, and showed no evidence of infection. Laboratory tests showed an increase in transaminases activity with an AST of 2180 IU/L, and an ALT of 3481 IU/L, total bilirubin and direct bilirubin were 10.29 mg/dL (normal <1.02 mg/dL), and 8.41 mg/dL (normal <0.57 mg/dL) respectively, creatinine was 1.49 mg/dL (normal <1.3 mg/dL) and INR was prolonged at 1.82. Hepatitis B serology showed HBsAg-positive, anti-HBc IgM negative, HBeAg negative, and anti-HBeAg positive. HBV-DNA level of 8.6×10^7 IU/mL, measured by PCR (COBAS TaqMan HBV test (Roche Diagnostics, Basel, Switzerland) with a limit of detection of 12 IU/mL. Other viral markers such as antibodies against hepatitis C virus (HCV) and hepatitis A virus IgM were negative.

On February 12, 2009, the patient was started on antiviral therapy with entecavir (Baraclude® Bristol-Myers Squibb) at a dose of 0.5 mg orally per day for HBV reactivation. Figs. 1 and 2 show the course of laboratory tests, including albumin serum levels, HBV-DNA levels, and HBsAg quantification during treatment with entecavir. Treatment was well tolerated and no side effects were observed. After 3 months of entecavir treatment, the patient was asymptomatic and his lab results showed HBsAg-positive, ALT 19 mg/dL, and undetectable HBV-DNA.

Complete molecular analysis of the HBV was performed prior to starting entecavir treatment, with amplification by nested PCR of the core gene, the surface gene and reverse transcriptation (RT) region of the polymerase gene as previously described [5]. The molecular results showed that the patient was infected with HBV genotype D and mutation G1896A within the pre-core region. Aminoacids from 99 to 160 of the surface gene, which include the main immunodominant epitope "a" region, were analyzed and the following changes: S122R, S127P, S134Y, S159G, and S160K were noted in relation to genotype D consensus surface region. In addition, mixed sequences were observed at positions L104LV and L109LI. At the polymerase gene, no mutations conferring drug resistance were detected. HBsAg was quantified using the Architect HBsAg assay (Abbott Laboratories, Abbott Park, IL; dynamic range, 0.05–250.0 IU/mL) after 1/100, 1/2 dilution and non-dilution of each sample (Fig. 1).

3. Discussion

HBV reactivation is more frequent in patients with chronic HBV infection. However, it can also occur in patients who have resolved the infection, as indicated by the presence of anti-HBc and/or anti-HBs, known as "*de novo* hepatitis B." Although "*de novo* hepatitis B" occurs only in 3.3–4% of patients after chemotherapy, it is an increasing concern to clinicians because of its higher morbidity and mortality rate [6,7]. HBV reactivation usually occurs in patients after solid organ transplant, allogenic and autologous hematopoietic stem-cell transplantation, and after immunosuppressive

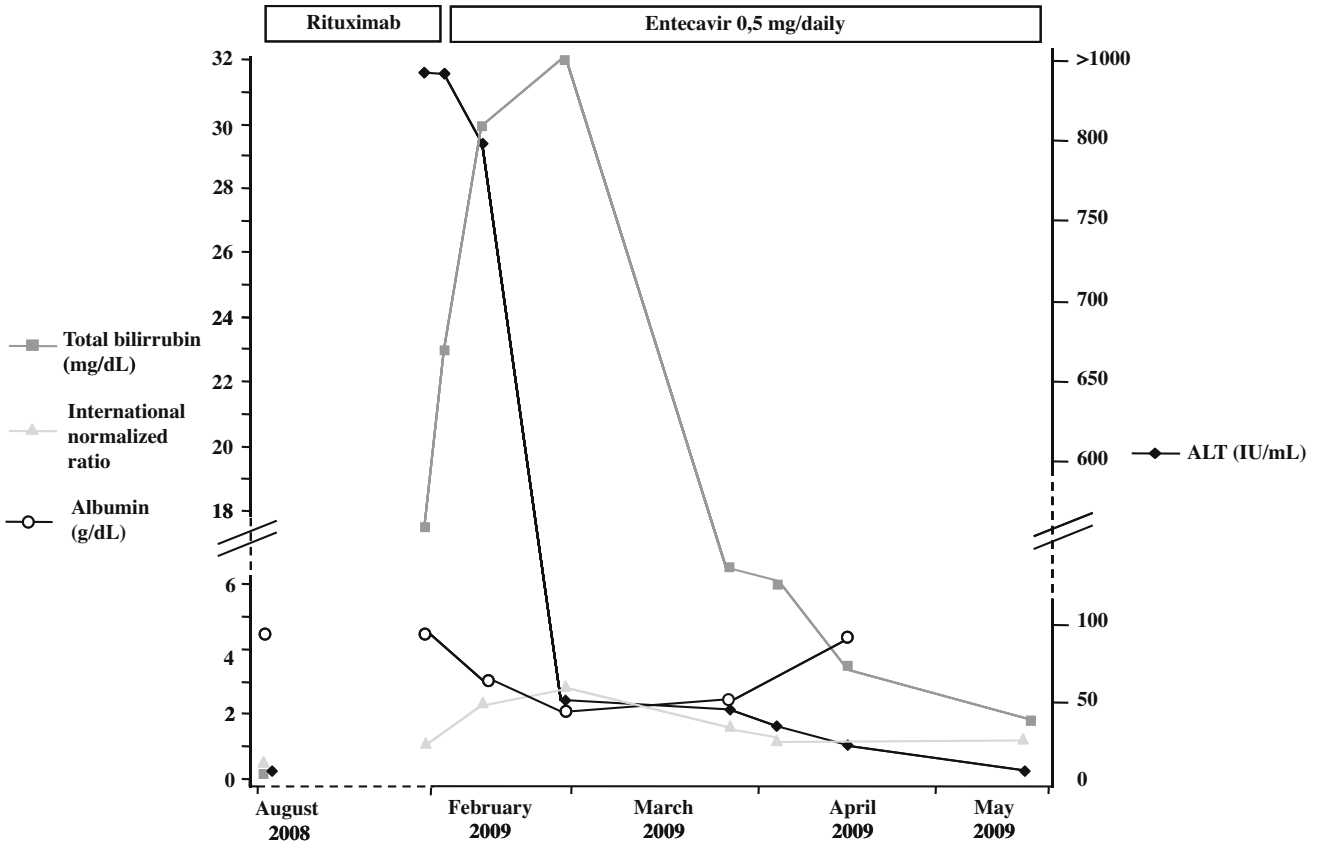

Fig. 1. Biochemical parameters before and during entecavir treatment.

therapy [8–11]. Reactivation of hepatitis B virus infection has also been observed with rituximab, a chimeric mouse human monoclonal antibody against CD20+ cells, used against certain types of malignancies. The predictive factors for HBV reactivation in patients undergoing polychemotherapy containing rituximab are male gender, absence of anti-HBs, high level of HBV-DNA [1,2]. The relationship between HBV reactivation and chemotherapy with rituximab was analyzed recently in a review including 46 patients with HBsAg-

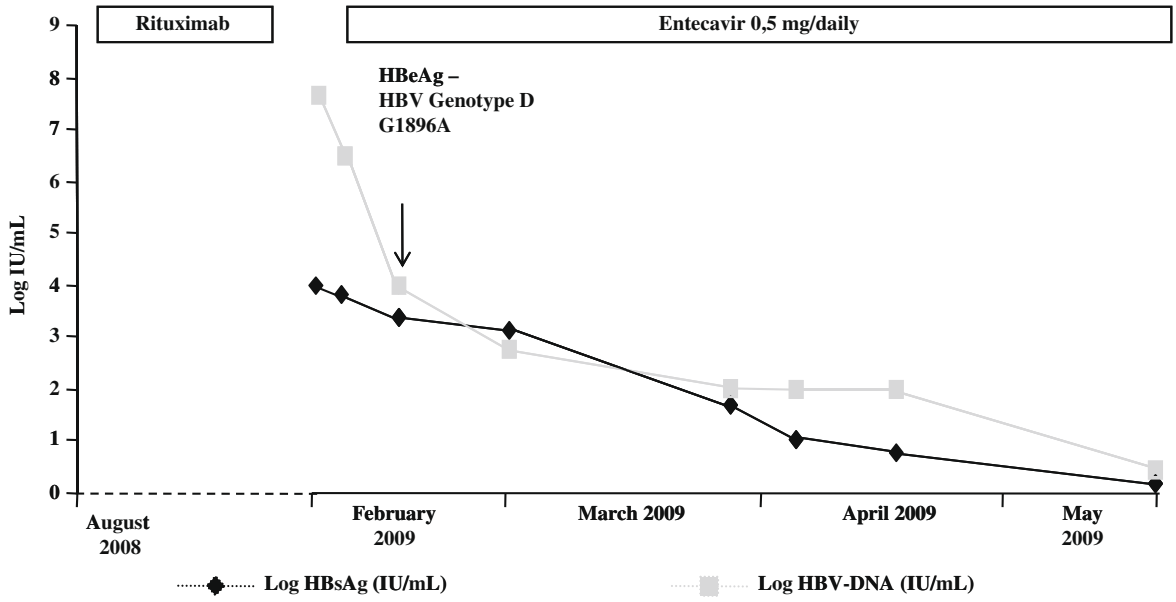

Fig. 2. Virologic parameters before and during entecavir treatment.

negative and anti-HBc positive. HBV reactivation, defined as detectable HBV-DNA and elevated ALT levels during and up to 6 months after completion of chemotherapy was detected in 5 (25%) of 21 patients treated with rituximab and in none of those treated without rituximab [12]. In addition, due to the increasing reports the United States Food and Drug Administration emitted a black box warning regarding the risk of hepatic dysfunction when using this medication [13].

To our knowledge, this is the first case of severe HBV reactivation leading to hepatic decompensation successfully treated with entecavir. In this case, entecavir achieved a rapid and complete viral suppression despite very high levels of HBV-DNA and the delay in its administration due to late diagnosis. The treatment also led to reversal of decompensation without any adverse effects. Upon review of the literature, only one case of HBV reactivation treated with entecavir as first-line therapy has been published [14] and, in contrast with our patient, this patient was asymptomatic with only an increase in ALT activity and without jaundice and liver decompensation. Table 1 summarizes a literature review of 15 cases of HBV reactivation following a chemotherapeutic regimen including rituximab who were treated with oral antivirals. Like our case, the majority were anti-HBc positive, had elevated ALT peaks after a median of 4–5 cycles of treatment and 10 died of liver related complications despite lamivudine treatment.

In our patient, entecavir was chosen as initial treatment, taking into consideration the high mortality rate during lamivudine therapy and the presence of high levels of HBV-DNA associated with a negative HBeAg, factors that suggested that a long, even indefinite, treatment, could be necessary. Entecavir offers advantages over lamivudine, as has been demonstrated in several studies [15]. Entecavir achieves a faster and stronger HBV-DNA suppression than lamivudine and has a high resistance barrier, which is important in a long treatment course. In cases of HBV reactivation the presence of HBV precore mutations, G1986A, such as the one present in our patient, has been associated with the development of severe hepatitis, including fulminant hepatitis [16]. This virulence could be explained by the loss of the immunomodulatory effect associated with the HBeAg, which cannot be produced by the presence of this mutation and suggests that in patients with HBV reactivations carrying the pre-core mutations, more potent antiviral drugs like entecavir and tenofovir should be used to prevent the development of severe liver complications.

Moreover, the presence of several aminoacid changes in the "a" determinant of HBV surface region known to impair HBsAg antigenicity, as detected in our case, are more frequent in cases of HBV reactivation, which may suggest some mismatch effect between HBsAg and anti-HBs antibodies, assuming that antibodies were present prior to HBV reactivation [17].

Rituximab affects both the cellular and the humoral arms of the immune system. In fact, it has been shown that besides depleting the circulating population of B cells, it increases both activated and regulatory T cells [18]. After the disappearance of B cells from the circulation, and elimination of the previously formed antibodies, disequilibrium in the immunological response in patients with a history of exposure to hepatitis B may occur and this would allow for viral replication and reactivation of HBV infection. In the majority of cases, Rituximab is given concomitantly with other drugs which also have immunomodulatory effects producing an enhanced immunosuppression. In this case, fludarabine was used concomitantly and there have been reports of HBV reactivation with this drug [19,20]. Fludarabine produces a pronounced decreased in the T cells populations, which for CD4+ cells is still present up to 13 months after the end of therapy [21].

It is at this state where the conditions are favorable for HBV proliferation, evidenced by increased serum HBV-DNA but without clinical or biochemical evidence of hepatitis (akin to the state of immune tolerance in newborns). The restoration of immune function following withdrawal of chemotherapy would lead to rapid cytolysis of infected hepatocytes and hence hepatitis and liver damage. This could be an explanation for the lag seen between the chemotherapy and hepatitis manifestations [22,23].

From the currently available literature, it is apparent that reactivation of hepatitis B virus infection following chemotherapy treatment ("*de novo*" hepatitis B) can be severe and associated with high fatality. We pointed out before that the AASLD only recommended screening of these patients with HBsAg. By following this recommendation, we would be missing patients with HBsAg-negative and anti-HBc positive infection who are also at risk of reactivation. We believe that patients being considered for chemotherapy should be screened for hepatitis B infection with HBsAg, anti-HBs and anti-HBc, and if needed, subsequent HBV-DNA determination.

In those patients with negative HBV-DNA levels in serum, periodic determination of HBV-DNA levels and liver enzymes during treatment should be carried out and antiviral treatment should be instituted if the HBV-DNA level is detected, as prompt treatment initiation seems to have an impact on the clinical course. For patients with positive HBV-DNA levels, prophylaxis should be implemented before the start of the chemotherapeutic regimen with lamivudine or entecavir and continue at least for 12 months after cessation of chemotherapy. In the case of severe reactivation, we recommend treatment with entecavir as a first option due to its greater efficacy in decreasing the HBV levels.

Table 1
Baseline characteristics and clinical outcome of patients treated with oral antiviral for HBV reactivation following a chemotherapy regimen containing rituximab reported in the literature.

Author	Age (Yrs)	Sex	Baseline disease	HBV serology[a] pre-chemotherapy	Chemotherapy treatment	Peak ALT (U/L)	Peak TB (mg/dL)	Time of reactivation	Type of antivirals	Clinical outcome
Westhoff et al. (2003) [24]	73	M	DLC	anti HBs+ anti-HBc+	R	NR	NR	After 5 cycles	Lamivudine	Liver-related death
Sarrechia et al. (2005) [25]	53	M	CLL	anti-HBs+ anti-HBc+	R	2110	17	After 3 months	Lamivudine	Liver-related death
Law et al. (2005) [26]	67	M	NHL	anti-HBs+ anti-HBc+	R-CHOP	2204	NR	After 8 cycles	Lamivudine	Liver-related death
Niscola et al. (2005) [27]	51	M	CLL	anti-HBs + anti-HBc+	R	NR	NR	After 7 months	Lamivudine	Liver-related death
Sera et al. (2006) [28]	59	M	NHL	anti-HBs+ anti-HBc+	R+ VP16+ P + Dex	359	26.4	2 months after stopping R	Lamivudine	Liver-related death
Ozgonenel et al. (2006) [29]	21	M	DCL	NR	R-CHOP	NR	NR	After 3 cycles	Lamivudine	Liver-related death
Yamagata et al. (2007) [30]	54	M	DCL	anti-HBc+	R-CHOP	531		After 7 cycles	Lamivudine	Liver-related death
Dillon et al. (2008) [22]	21	F	DCL	NR	R-CHOP	10 × UL	NR	After 4 cycles	Lamivudine	Liver-related death
Garcia-Rodriguez et al. (2008) [23]	68	F	DCL	NR	R-CHOP	97	14	1 year after 6 cycles	Lamivudine	Liver-related death
Garcia-Rodriguez et al. (2008) [23]	53	F	FL	NR	R-CHOP	177	1.2	9 months after 3 cycles	Lamivudine, then Entecavir	Alive
Colson et al. (2008) [14]	48	M	DCL	anti-HBs+ anti-HBc+	R-CHOP	1800	WNL	After 4 cycles	Entecavir	Alive
Yeo et al. (2009) [1]	77	M	DCL	anti-HBc+	R-CHOP	2110	4.2	After 6 cycles	Lamivudine	Liver-related death
Yeo et al. (2009) [1]	58	M	DCL	anti-HBc+	R-CHOP	362	1.1	After 5 cycles	Lamivudine	Alive
Yeo et al. (2009) [1]	46	M	DCL	anti-HBc+	R-CHOP	809	0.5	After 6 cycles	Lamivudine	Malignancy-related death
Yeo et al. (2009) [1]	63	M	DCL	anti-HBc+	R-CHOP	649	0.32	After 6 cycles	Lamivudine	Alive

Abbreviations: CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; CLL, chronic lymphocytic leukemia; Dex, dexamethasone; DCL, diffuse large cell lymphoma; FL, follicular lymphoma; NHL, non-hodgkin' lymphoma; NR, no reported; P, prednisone; R, rituximab; ULN, upper limit of normal; VP16, etoposide; WNL, within normal limits; ×, times.

[a] All the patients have HBsAg if serology is reported.

In summary, this case demonstrates the usefulness and safety of entecavir in the treatment and reversal of severe HBV reactivation and provides evidence for the use of this drug in HBV reactivation associated with a high viral load, as suggested by European guidelines [4]. It also demonstrates that, in addition to HBsAg testing, anti-HBc and anti-HBs determinations have to be included in the screening process prior to the use of rituximab or other potent monoclonal antibodies, in order to prevent the development of HBV reactivation.

Note added in proof

Two months after this manuscript was submitted the patient remains asymptomatic, and his labs showed HBsAg negative, normal ALT levels and undetectable HBV DNA.

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References

[1] Yeo W, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, et al. Hepatitis B reactivation in Lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. J Clin Oncol 2009;27:605–611.

[2] Zhong S, Yeo W, Schroder C, Chan PK, Wong WL, Ho WM, et al. High hepatitis B virus (HBV) DNA viral load is an important risk factor for HBV reactivation in breast cancer patients undergoing cytotoxic chemotherapy. J Viral Hepat 2004;11:55–59.

[3] Lock ASF, McMahom BJ. AASLD practice guidelines: chronic hepatitis B. Hepatology 2007;45:507–539.

[4] European Association for the Study of Liver Disease. EASL clinical practice guidelines. J Hepatol 2009;50:227–242.

[5] Jardi R, Rodriguez F, Buti M, Costa X, Valdes A, Allende H, et al. Mutations in the basic core promoter region of hepatitis B virus. Relationship with precore variants and HBV genotypes in a Spanish population of HBV carriers. J Hepatol 2004;40: 507–514.

[6] Hui CK, Cheung WWW, Zhang HY, Au WY, Yueng YH, Leung AYH, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. Gastroenterology 2006;131:59–68.

[7] Umemura T, Tanaka E, Kiyosawa K, Kumada Hthe Japan de novo Hepatitis B research group. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. Clin Infect Dis 2008;47:e52–e56.

[8] Savas N, Colak T, Yilmaz U, Emiroglu R, Haberal M. Hepatitis B reactivation after renal transplantation: report of two cases. Transplant Int 2007;20:303–304.

[9] Pérez-Grande R, Gutiérrez-Zufiaurre N, Muñoz-Criado S, Blázquez de Castro AM, Vázquez López L, González San Martín F, et al. Hepatitis B reactivation in a hepatitis B surface antigen-negative patient after allogeneic bone marrow transplant: successful treatment with lamivudine and seroconversion. Diagn Microbiol Infect Dis 2009;64:80–82.

[10] Uhm JE, Kim K, Lim TK, Park BB, Park S, Hong YS, et al. Changes in serologic marker of hepatitis B following autologous hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2007;13:463–468.

[11] Shibolet O, Ilan Y, Gillis S, Hubert A, Shouval D, Safadi R. Lamivudine therapy for prevention of immunosuppressive-induced hepatitis B virus reactivation in hepatitis B surface antigen carriers. Blood 2002;100:391–396.

[12] Hambali A, Khaled Y. Incidence of hepatitis B reactivation following rituximab therapy. Am J Hematol 2009;84:195.

[13] http://www.fda.gov/MedWatch/SAFETY/2004/safety04.htm#Rituxan.

[14] Colson P, Borentain P, Coso D, Chabannon C, Tamalet C, Gerolami R. Entecavir as a first line treatment for HBV reactivation following polychemotherapy for lymphoma. Br J Hematol 2008;143:148–150.

[15] Lai CL, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, et al. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. N Engl J Med 2006;354:1011–1020.

[16] Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutation in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 1991;324: 1699–1704.

[17] Rodriguez-Frias F, Buti M, Jardi R, Vargas V, Quer J, Cotrina M, et al. Genetic alterations in the S gene of hepatitis B virus in patients with acute hepatitis B, chronic hepatitis B and hepatitis B liver cirrosis before and after liver transplantation. Liver 1999;19:177–182.

[18] Vallerskog T, Gunnarsson I, Widhe M, Risselada A, Klareskog L, van Vollenhoven R, et al. Treatment with rituximab affects both the cellular and the humural arm of the immune system in patients with SLE. Clin Immunol 2007;122:62–74.

[19] Alexopoulou A, Dourakis SP, Pandelidaki H, Archimandritis AJ, Karayiannis P. Detection of a hepatitis B surface antigen variant emerging in a patient with chronic lymphocytic leukaemia treated with fludarabine. J Med Virol 2006;78:1043–1046.

[20] Picardi M, Pane F, Quintarelli C, De Renzo A, Del Giudice A, De Divitiis B, et al. Hepatitis B virus reactivation after fludarabine-based regimens for indolent non-Hodgkin's lymphomas: high prevalence of acquired viral genomic mutations. Haematologica 2003;88:1296–1303.

[21] Wijermans PW, Gerrits WB, Haak HL. Severe immunodeficiency in patients treated with fludarabine monophosphate. Eur J Haematol 1993;50:292–296.

[22] Dillon R, Hirschfield GM, Allison ME, Rege KP. Fatal reactivation of hepatitis B after chemotherapy for lymphoma. BMJ 2008;337:756–758.

[23] Garcia-Rodriguez MJ, Canales MA, Hernandez-Maraver D, Hernandez-Navarro F. Late reactivation of resolved hepatitis B virus infection: an increasing complication post rituximab-base regimens treatment? Am J Hematol 2008;83:673–675.

[24] Westhoff TH, Jochimsen F, Schmittel A, et al. Fatal hepatitis B virus reactivation by an escape mutant following rituximab therapy. Blood 2003;102:1930.

[25] Sarrechia C, Cappelli A, Aiello P. HBV reactivation with fatal fulminating hepatitis during rituximab treatment in a subject negative for HBsAg and positive for HBsAb and HBcAb. J Infect Chemother 2005;11:189–191.

[26] Law JK, Ho JK, Hoskins PJ, et al. Fatal hepatitis B virus reactivation post-chemotherapy in a hepatitis B core antibody-positive patient: Potential implications for future prophylaxis recommendation. Leuk Lymphoma 2005;46:1085–1089.

[27] Niscola P, Del Principe MI, Maurillo L, et al. Fulminant B hepatitis in a surface antigen-negative patient with B-cell chronic lymphocytic leukaemia after rituximab therapy. Leukemia 2005;19:1840–1841.

[28] Sera T, Hiasa Y, Michitaka K, et al. Anti-HBspositive liver failure due to hepatitis B virus reactivation induced by rituximab. Intern Med 2006;45:721–724.

[29] Ozgonenel B, Moonka D, Savasan S. Fulminant hepatitis B following rituximab therapy in a patient with Evans syndrome and large B-cell lymphoma. Am J Hematol 2006;81:302.

[30] Yamagata M, Murohisa T, Tsuchida K, et al. Fulminant B hepatitis in a surface antigen and hepatitis B DNA-negative patient with diffuse large B-cell lymphoma after CHOP chemotherapy plus rituximab. Leuk Lymphoma 2007;48:431–433.