Reactivation of HBV Infection in Low Grade Lymphoma Patient

VICTORIA ARAMĂ[1,2], DANIELA MUNTEANU[1], IOANA OLARU[1], MIHAELA RĂDULESCU[1], RALUCA MIHĂILESCU[1], ANA-MARIA VLĂDĂREANU[2,3], MINODORA ONISÂI[2,3], ANAMARIA VINTILESCU[3], CAMELIA DOBREA[4], M. OLARIU[1], S.S. ARAMĂ[2]

[1]"Prof. Dr. Matei Balş" National Institute of Infectious Diseases, Bucharest
[2]"Carol Davila" University of Medicine and Pharmacy, Bucharest
[3]Emergency University Hospital Bucharest, Hematology Department
[4]"Victor Babeş" National Institute of Research and Development

Reactivation of hepatitis B virus is a complication of chronic or HBV infection in patients with malignancies, especially hematological disorders, under cytotoxic or immunosuppressive therapy. The immunosuppression favors HBV replication with the massive infection of hepatocytes. Once immunity is restored when chemotherapy therapy is discontinued, a rapid, immune-mediated destruction of the infected hepatocytes ensues, clinically manifested as hepatitis, liver failure or even death. We report a case of HBV reactivation in a patient with B cells non-Hodgkin lymphoma, with HBsAg negative and protective titre of anti-HBs, after 5 months of combined chemotherapy. Currently, there are no data to support routine pre-emptive anti-HBV therapy in patients with negative HBsAg and undetectable viremia before the initiation of chemotherapy.

The case presented in this paper is included in the group of patients that is studied in LIMFOVIR Grant (convention no 41012/2007). This research grant is funded by the National Center of Programs Management, program 4 – Partnerships in Priority Fields. The grant is coordinated by the National Institute of Infectious Diseases Prof. Dr. Matei Bals, Bucharest. The grant team include also the Emergency University Hospital Bucharest, Hematology Department, the "Carol Davila" University of Medicine and Pharmacy, Bucharest, the "Victor Babeş" National Institute of Research and Development, the Institute of Electrotechnical Research, Bucharest and the Polytechnic University, Bucharest. The manager of the grant is Associated Professor dr. Victoria Aramă.

Key words: hepatitis B virus, reactivation, lymphoma, chemotherapy.

The estimated number of people infected with hepatitis B virus (HBV), worldwide is 2 billion, and over 350 million are currently living with chronic infection. Annually, 600.000 deaths related to acute or chronic HBV infection are recorded [1]. In Romania, 6% of the population is chronically infected by the HBV, positioning it among the countries with intermediate endemicity [2].

Hepatitis B virus reactivation can be defined as the recurrence of active hepatic necroinflammation, in a person known to be an inactive hepatitis B surface antigen (HBsAg) carrier or with resolved HBV infection [3].

CASE REPORT

We present the case of a 64 years old female, diagnosed in 2004 with parotid gland B cells non-Hodgkin lymphoma. She underwent bilateral total parotidectomy and chemotherapy and she obtained remission. Unfortunately there is not much data from that period of time about the treatment and its duration and, also, the patient was not followed for the next years.

Between January and February 2009 the patient was admitted into the Infectious Disease Hospital for two months intermittent fever. Laboratory assessments showed only inflammatory syndrome. Chest and abdominal computed tomography (CT) revealed 2 cm mediastinal adenopathy and hypodense areas in both kidneys that indicated a possible hematological relapse. The symptoms improved with broad-spectrum antibiotic therapy (carbapenem + macrolide) and the investigations were stopped there because the patient demanded discharge from the hospital, against medical advise. After 3 months, the fever reappeared and she was readmitted into the Infectious Disease Hospital. The investigations showed persistent inflammatory syndrome without other changes. Because an infectious etiology could not be proven, the only possible explanation for the persistent fever was the hematological relapse and she was transferred to the Hematology Clinic. For the first

time, during the hospitalization in the Infectious Disease Department, the serological markers for the hepatitis B virus were performed. They showed an old spontaneous seroconverted infection: hepatitis B surface antigen (HBsAg) negative, hepatitis B e antigen (HBeAg) negative, anti-hepatitis B e antibodies (HBeAc) positive and anti-hepatitis B surface antibodies (HBsAc) positive, at a protective titre (> 1000 mUI/L).

In the Hematology Clinic, the chest and abdominal CT was repeated and that time revealed generalized lymphadenopathy, under 2 cm diameter, multiple pulmonary nodules, hypodense areas into the liver and right kidney. Histopathology examination with supplementary immunohistochemistry techniques of biopsy samples from bone marrow and lung indicated the presence of reactive lymphoid infiltrates, which did not attest a relapse. After 2 more months, the patient presented for general status impairment, fatigue, weight loss, loss of appetite, night fever. An axilar lymphadenopathy biopsy revealed neoplastic lymphoid inflitrates. The final diagnosis was *B cell non-Hodgking lymphoma-MALT-type stage IV*.

In September 2009, the patient started combined chemotherapy, first CVP (cyclophosphamide, vincristine, prednisone) – one administration – and then R-CVP (rituximab, cyclophosphamide, vincristine, prednisone) – 4 times. In February 2010, prior to the sixth administration of R-CVP, the patient complained of significant fatigue for 2 weeks. The laboratory assessments revealed high ALT level (10 times over the normal range) and positive HBsAg. The chemotherapy was discontinued and the patient was referred to the infectious disease specialist. The investigations performed in the Infectious Disease Hospital showed a significant decrease in anti-HBs antibody titer (16.3 mUI/L against 1000 mUI/L), HBeAg positive, anti-HBe antibodies negative and HBV-DNA > 1,000,000,000 UI/ml. The diagnosis was: *Chronic hepatitis B reactivation* on immunosuppressed background secondary to non-Hodgkin lymphoma and chemotherapy. The therapy with entecavir 0.5 mg/daily was therefore initiated.

After 5 months of combined chemotherapy, anti-HBs antibody titre dropped dramatically to undetectable levels at a certain point and the presence of HBsAg indicated reactivation of HBV infection (Fig. 1).

Meanwhile, the ALT level reached 2912 UI/ml (56 times over the normal range), with a decreasing trend afterwards (Fig. 2). HBV-DNA under treatment showed an important reduction after only 4 weeks of therapy and until week 12 the viremia became almost undetectable (Fig. 3).

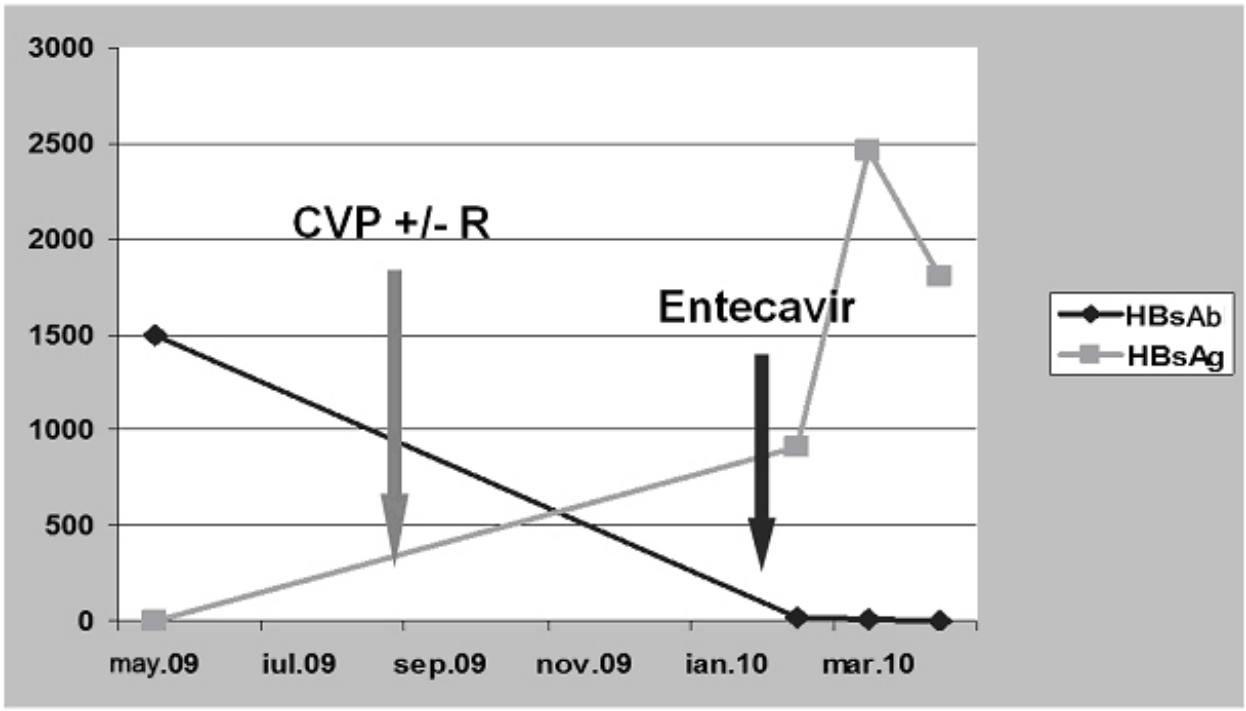

Fig.1. The variations of HBV serological markers after combined chemotherapy.

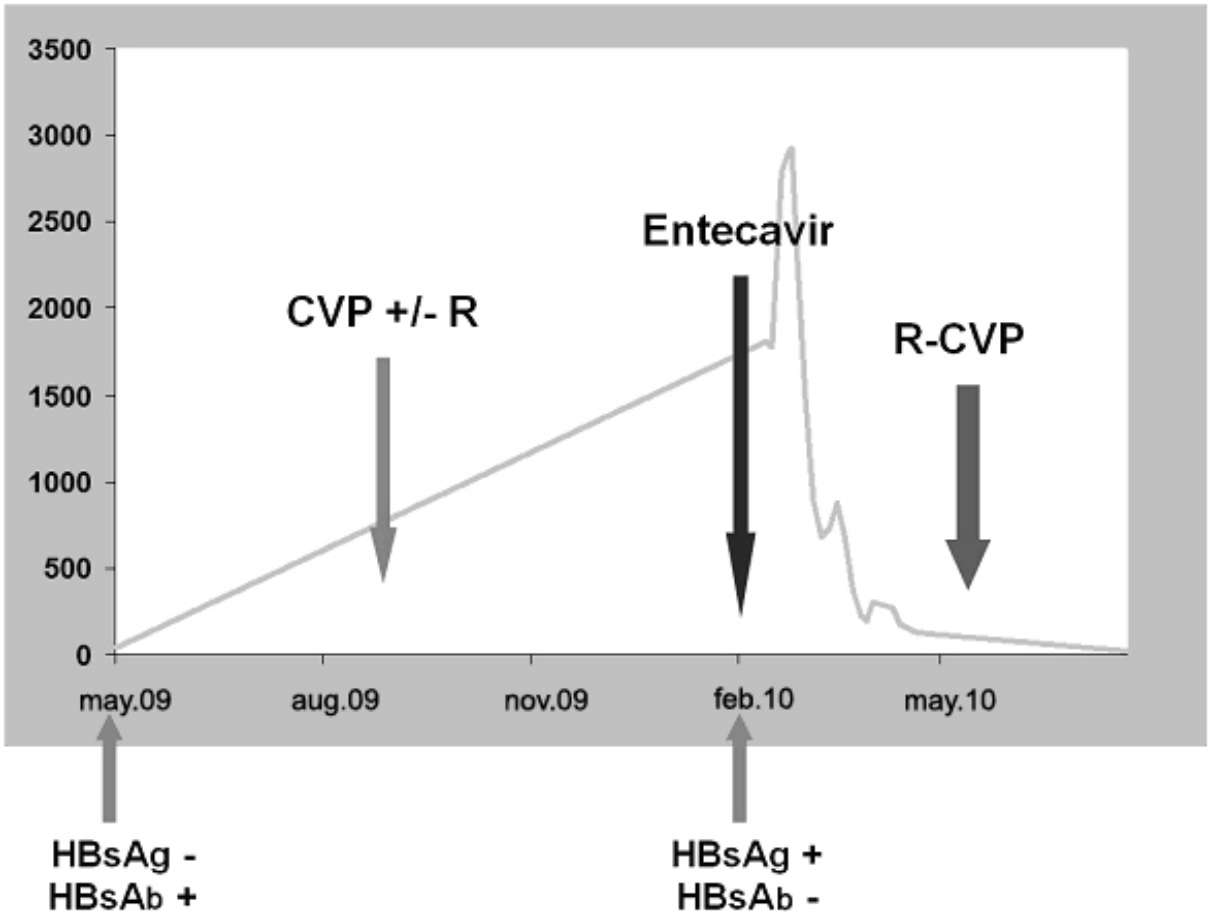

Fig. 2. The dynamics of ALT levels.

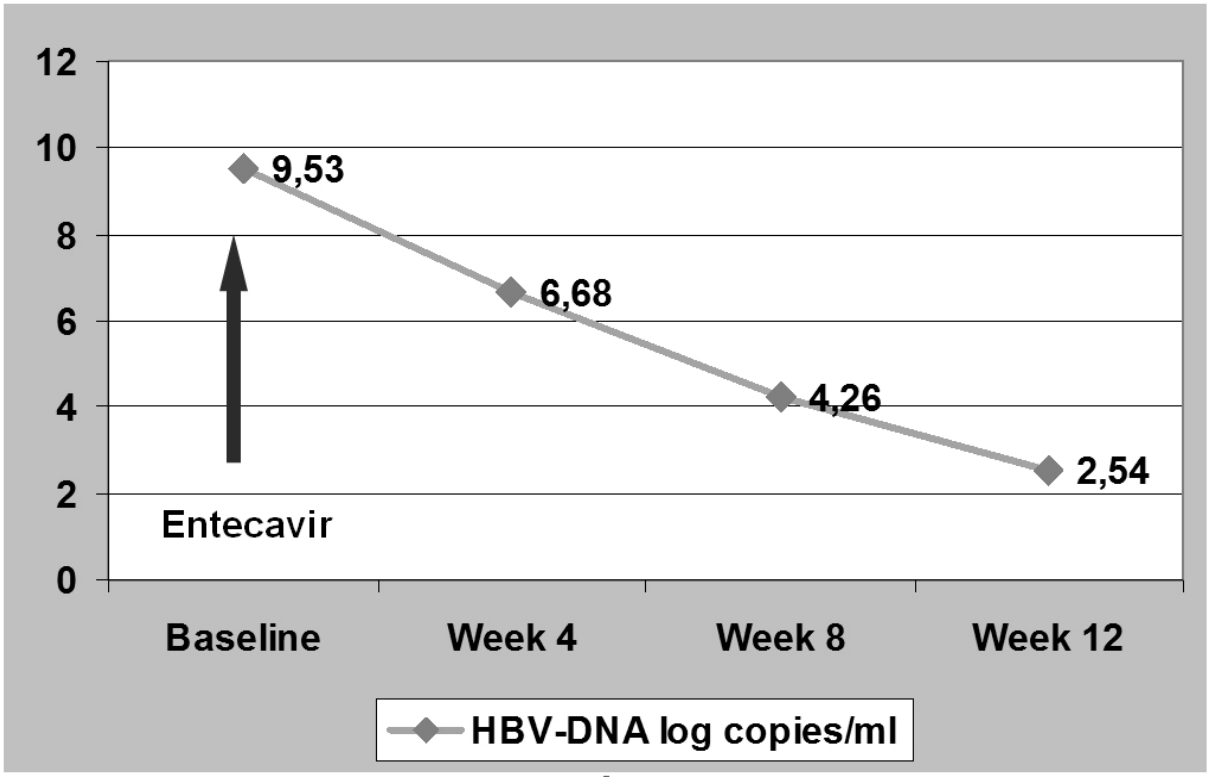

Fig. 3. The decrease of HBV-DNA after the initiation of Entecavir therapy.

The combined chemotherapy was resumed in May 2010, after 3 months of antiviral therapy and the levels of aminotrasferases remained normal, but with a persisting cholestatic syndrome (2–3 times the normal levels). Computer-tomography reevaluation performed in May 2010 revealed multiple profound lymphadenopathies, hepatosplenomegaly with liver nodular involvement, pulmonary condensations. R-CVP protocols were administered, which were followed by severe leucopenia, with a difficult response to treatment. The patient gradually developed pancytopenia – predominantly neutropenia and thrombocytopenia, and superficial lymphadenopathies. The biopsy of one of the lymph nodes revealed a different histopathological aspect, respectively a transformation of the disease to Diffuse large B-Cell Non-Hodgkin lymphoma. Unfortunately, continued chemotherapy was not followed by any response, and the patient died in November 2010.

DISCUSSION

The reactivation of HBV infection can occur spontaneously, but more often, it is seen in immunocompromised individuals like those receiving chemotherapy for hematological malignancies or solid tumors. It can also occur in subjects with non-malignant conditions who require immunosuppressive therapy and in patients with rheumatic and inflamematory intestinal diseases receiving biological therapy [4].

Risk factors associated with HBV reactivation are: male sex, younger age, the presence of HBsAg, the presence of HBeAg, detectable HBV viremia, the use of corticoids and antracyclines, the diagnosis of lymphoma or breast cancer, the use of an intensive immunosuppressive regimen. The length of chemotherapy, the pre-chemotherapy HBV viral load, the intrahepatic level of covalently closed circular DNA (cccDNA), the alanine transaminase (ALT) plasma levels, and the infection with B and C genotypes are also thought to influence HBV reactivation [4–6]. It was also noted that patients with lymphoma who have a positive HBsAg have a higher risk of HBV reactivation than patients with other types of cancer. The more intense immunosuppression caused by the chemotherapeutic drugs employed in this situation, as well as the inherent immunosuppressive effect of the lymphoma are thought to contribute to the higher reactivation risk in these particular patients [7].

Reactivation of HBV replication with an increase in plasma viral load and the level of AST and ALT was noted in 20 – 50% of patients receiving chemotherapy or immunosuppressive therapy [3]. In 30–60% of patients with lymphoma and positive HBsAg, who are not given specific antiviral prophylaxis significant liver dysfunction, associated with an increase in liver function tests can occur; and over a half of these patients can become jaundiced [8]. Hepatitis B viral reactivation was also observed in subjects with solid tumors with a reactivation frequency of 25 to 40%, with the exception of patients with hepatocellular carcinoma who had a reactivation rate of 60%. In the recipients of a stem cell transplant, the reactivation rate was 50%. The level of this rate can depend on the intensity of the chemotherapy regimen used, and the coexistence of graft versus host disease [5]. The mortality rate observed in patients with HBV reactivation ranged from 5 to 40% [8][18].

Time to HBV reactivation can vary. Most frequently, it takes place at the end of the immunosuppression period, but it can also occur during chemotherapy. The median time to reactivation from the moment of chemotherapy initiation is of approximately 4 months, but it was also observed as late as 3 years [8][18][21].

Most patients with HBV reactivation have positive HBsAg, although cases of reactivation in patients with absent HBsAg and positive anti-HBc antibodies and/or positive anti-HBs antibodies have been described. These patients, with negative HBsAg, but with detectable plasma or intrahepatic hepatitis B viral load are regarding as having occult HBV infection [4]. It is thought that HBV reactivation in subjects who cleared HBsAg occurs because of the persistence of HBV in hepatocytes in the form of cccDNA [9]. The hepatitis B virus can also be found in other sites, HBV-DNA was also identified in mononuclear cells isolated from the peripheral blood of transplant patients, under immunosuppressive therapy who had positive anti-HBc antibodies and HBsAg/anti-HBs antibodies, and undetectable HBV viral load [10].

The chemotherapeutic combinations that are most frequently associated with reactivation are the regimens employed in hematological malignancies (acute leukemia, lymphoproliferative and myeloproliferative disorders, plasma cells dyscrasias). The patients suffer an intense marrow suppression with an important decrease in white cell counts [6][11][21]. The immunosuppression favors HBV

replication with the massive infection of hepatocytes, once immunity is restored when chemotherapy or the immunosuppressive therapy are discontinued, a rapid, immune-mediated destruction of the infected hepatocytes ensues, clinically manifested as hepatitis, liver failure or even death [6].

As previously mentioned, certain chemotherapeutic regimens have a higher risk of HBV reactivation. For example, the corticoid-containing regimens are associated with a higher risk of reactivation because the viral DNA has a glucocorticoid-responsive fragment, which can facilitate HBV replication and genetic expression [12][13], it has been demonstrated that antracyclines stimulate *in vitro* the secretion of HBV-DNA by the hepatocytes in a dose-dependent manner. Both drugs are frequently used in cancer patients [14].

Monoclonal antibodies, like rituximab (anti-CD20 chimeric monoclonal antibodies) and alemtuzumab (anti-CD52 humanized monoclonal antibodies) have also been associated with an increased risk of HBV reactivation. Therapy with these types of antibodies induces an important and persistent B and T-cell depletion. Infliximab (anti-TNFα antibody) can also increase the risk of reactivation [6] [17][18].

Hepatitis B virus reactivation can be associated with a wide array of clinical manifestations ranging from a slight, asymptomatic increase of ALT and AST levels, or only serological proof of viral reactivation, to fulminant liver failure and death [4].

Depending on the clinical and laboratory features, the following conditions can be defined: hepatitis as an increase in transaminases exceeding 3-fold the upper limit of normal (ULN) by 2 consecutive measurements, at least 5 days apart, and in the absence of other causes; icteric hepatitis – hepatitis associated with jaundice and a serum bilirubin exceeding 1.8 mg/dl; HBV reactivation – an increase in HBV-DNA viral load exceeding by 1 log the pre-flare level or detectable HBV viral load in a patient with previously undetectable viremia; liver failure: the presence of hepatic encephalopathy and coagulopathy (prothrombin time $\geq$ 10 sec) [6].

To ascertain that the HBV flare is the cause of hepatic dysfunction, other causes of hepatitis should be excluded (e.g. hepatitis A, hepatitis C, HDV superinfection, cytomegalovirus hepatitis, Epstein-Barr virus hepatitis). Drug-induced hepatitis should also be ruled out [8][18][21].

Hepatitis B virus reactivation, in the absence of previous prophylaxis can lead to major negative consequences. On one hand chemotherapy must be discontinued, which will negatively impact on the prognosis of the underlying malignancy, and on the other hand the mortality of the HBV flare can be substantial, despite of early initiation of antiviral treatment, because the immune-mediated hepatocyte destruction had already occurred [8].

This is the reason why European and American guidelines recommend that patients requiring immunosuppressive therapy should be tested before the start of chemotherapy for the presence of HBsAg, anti-HBs antibodies, HBeAg, anti-HBe antibodies, and for anti-HBc antibodies. Active immunization of naïve patients is recommended [3][19].

In patients with positive HBsAg, the HBV viral load should be assessed, and they should receive pre-emptive therapy with nucleoside/ nucleotide analogues during the whole course of immunosuppressive therapy (regardless of the viral load) and for the 6–12 months following the discontinuation of therapy [3][8][19][21]. Most of the experience in these patients is with lamivudine, which will suffice for the patients with low viral loads and a small risk of resistance. In patients with high viral loads, pre-emptive therapy with more efficient drugs which have a higher genetic barrier is recommended (e.g. entecavir or tenofovir) [3] [19]. Adefovir, tenofovir, and entecavir can be used as an alternative to lamivudine, especially in patients who require a course of therapy exceeding 12 months – these patients have a higher risk of resistance to lamivudine [3].

A recent study comparing the efficacy of pre-emptive therapy with entecavir vs lamivudine in patients with lymphoma suggests that the former may have a superior effect in preventing hepatitis B reactivation and the disruption of chemotherapy; this effect was best seen in patients with advanced stage lymphoma [20]. The study population, however, was small, and further studies are needed to establish which patient groups might derive the most benefit from tailored pre-emptive therapy.

Patients with negative HBsAg, positive anti-HBc antibodies and/or anti-HBs antibodies, and undetectable HBV viral load, who require immunosuppressive therapy or chemotherapy should be closely monitored by assessing ALT and HBV-DNA levels. Treatment with analogues should promptly be initiated when HBV reactivation is confirmed (detectable HBV viral load), before the elevation of ALT and AST levels. Currently, data to support routine pre-emptive anti-HBV therapy in patients with negative HBsAg and undetectable viremia are lacking [3][19].

Acknowlegments: We wish to thank The Roumanian National Center of Program Management, who funded the research grant LIMFOVIR (convention no 41012/2007), in the Program 4 – Partnerships in Priority Fields. The case presented in this paper is included in the group of patients that is studied in this research grant. The grant is coordinated by the "Prof. Dr. Matei Balş" National Institute of Infectious Diseases, Bucharest. The grant team includes also the Emergency University Hospital Bucharest, Hematology Department, the "Carol Davila" University of Medicine and Pharmacy, Bucharest, the "Victor Babeş" National Institute of Research and Development, the Institute of Electrotechnical Research, Bucharest and the Polytechnic University, Bucharest. The manager of the grant is dr. Victoria Aramă.

Reactivarea hepatitei cu virus B reprezintă o complicaţie a infecţiei cronice cu VHB, la pacienţii cu afecţiuni maligne, în special hematologice, aflaţi sub terapie imunosupresivă sau citotoxică. Imunosupresia favorizează replicarea VHB, ceea ce conduce la o infectare masivă a hepatocitelor. În momentul în care se sistează chimioterapia şi imunitatea se reface, are loc distrugerea rapidă a hepatocitelor infectate prin mecanism mediat umoral, avînd drept rezultat hepatita manifestă clinic, insuficienţa hepatică sau chiar decesul. Prezentăm un caz de reactivare a infecţiei VHB, la 5 luni de chimioterapie combinată, la o pacientă cu limfom non-hodgkinian cu celulă B, cu AgHBs negativ şi titru protector de AcHBs. La ora actuală, la pacienţii cu AgHBs negativ, nu există date care să susţină terapia pre-emptivă anti-VHB de rutină, anterior iniţierii chimioterapiei.

Cazul prezentat face parte din grupul de pacienţi incluşi în Grantul de cercetare LIMFOVIR (nr. contract 41012–2007). Acest proiect este finanţat de Centrul Naţional de Management de Programe, în cadrul programului 4 – Parteneriat pe Domenii Prioritare. Grantul este coordonat de Institutul Naţional de Boli Infecţioase Prof. Dr. Matei Balş, Bucureşti. Din echipă fac parte: Spitalul Universitar de Urgenţă Bucureşti – Clinica de Hematologie, Universitatea de Medicină şi Farmacie Carol Davila, Bucureşti, Institutul Naţional de Cercetare şi Dezvoltare Victor Babeş, Institutul de Cercetare, Proiectare Electrotehnică, Bucureşti şi Universitatea Politehnică Bucureşti. Directorul de proiect este Conferenţiar Doctor Victoria Aramă.

Corresponding author: Victoria Aramă
"Prof. dr. Matei Balş" National Institute of Infectious Diseases, Bucharest
E-mail: ralsan @gmail.com

REFERENCES

1. *Hepatitis B*, WHO fact sheet 204, accessed March 2010.
2. GRIGORESCU M., *Tratat de hepatologie*, 2004; p. 375.
3. LOK A.S., MCMAHON B.J., AASLD, *Practice Guidelines. Chronic Hepatitis B*: Update 2009. Hepatology, 2007;**45**:507–539, updated in 2009.
4. CHARBEL H., LEWIS J.H., *Hepatitis B reactivation in the setting of chemotherapy and immunosupression.* Chapter in Chronic viral hepatits. Diagnosis and therapeutics, Edited by Shetty K. and Wu G.Y.; Humana Press, 2009; p. 307–25.
5. YEU W., JOHNSON P.J., *Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy*. Hepatology. 2006 Feb; **43**(2):209–20.
6. LAU G.K., *Hepatitis B reactivation after chemotherapy: two decades of clinical research*. Hepatol Int. 2008 Jun; **2**(2):152–62.
7. LIANG R., LOK A.S., LAI C.L. *et al*., *Hepatitis B infection in patients with lymphomas.* Hematol Oncol. 1990; **8**:261–270.
8. LIANG R., *How I treat and monitor viral hepatitis B infection in patients receiving intensive immunosuppressive therapies or undergoing hematopoietic stem cell transplantation.* Blood. 2009 Apr 2; **113**(14):3147–53.
9. LOCARNINI S., *Molecular virology of hepatitis B virus.* Semin Liver Dis 2004; **24**(Suppl. 1): 3–10.
10. MASON A.L., XU L., GUO L., KUHNS M., PERRILLO R.P., *Molecular basis for persistent hepatitis B infection in the liver after clearance of serum hepatitis B surface antigen.* Hepatology 1998; **27**:1736–42.
11. LAU G.K., YIU H.Y., FONG D.T., CHENG H.C., AU W.Y., LAI L.Y. *et al*., *"Early" is superior to "deferred" pre-emptive lamivudine therapy for hepatitis B patients undergoing chemotherapy.* Gastroenterology. 2003; **125**:1742–9.

12. TUR-KASPA R., BURK R.D., SHAUL Y., SHAFRITZ D.A., *Hepatitis B virus DNA contains a glucocorticoid-responsive element*. Proc Natl Acad Sci U S A 1986; **83**:1627–1631.
13. LIAW Y.F.,*Hepatitis viruses under immunosuppressive agents.* J Gastroenterol Hepatol 1998; **13**:14–20.
14. HSU C.H., HSU H.C., CHEN H.L., GAO M., YEH P.Y., CHEN P.J. *et al*., *Doxorubicin activates hepatitis B virus (HBV) replication in HBV-harboring hepatoblastoma cells. A possible novel mechanism of HBV reactivation in HBV carriers receiving systemic chemotherapy.* Anticancer Res 2004; **24**: 3035–3040.
15. WESTHOFF T.H., JOCHIMSEN F., SCHMITTEL A. *et al*., *Fatal hepatitis B virus eactivation by an escape mutant following rituximab therapy.* Blood, 2003; **102**, 1930.
16. IANNITTO E., MINARDI V., CALVARUSO G. *et al*., *Hepatitis B virus reactivation and alemtuzumab therapy*. European Journal of Haematology, 2005; **74**, 254–258.
17. CALABRESE L.H., ZEIN N.N., VASSILOPOULOS D., *Hepatitis B virus reactivation with immunosuppressive therapy in rheumatic diseases:assessment and preventive strategies.* Annals of Rheumatic Diseases, 2006; **65**, 983–989.
18. LALAZAR G., RUND D., SHOUVAL D., *Screening, prevention and treatment of viral hepatitis B reactivation in patients with haematological malignancies.* Br J Haematol. 2007 Mar; **136**(5):699–712.
19. European Association for the Study of the Liver: *EASL Clinical Practice Guidelines: Management of chronic hepatitis B*. Journal of Hepatology, 2009; **50**:227–242.
20. LI H.R., HUANG J.J., GUO H.Q. *et al*., *Comparison of entecavir and lamivudine in preventing hepatitis B reactivation in lymphoma patients during chemotherapy*. J Viral Hepat, Nov 2010; epub. ahead of print.
21. VLĂDĂREANU A.M., ARAMĂ V., MOLAGIC V., Capitolul 2. *Infecţia cu VHB şi limfoproliferările maligne*, p. 66–70. În monografia Limfoamele în corelaţie cu virusurile limfotrope. Sub redacţia Ana Maria Vlădăreanu. Editura Medicală Amalteea, 2007.

Received December 2, 2010