

HEPATITIS B VIRUS REACTIVATION IN PATIENTS UNDERGOING CHEMOIMMUNOTHERAPY FOR HAEMATOLOGICAL MALIGNANCIES

VIOLETA MOLAGIC^{1#*}, CĂTĂLIN TILIȘCAN^{1,2#}, CRISTINA POPESCU^{1,2}, VICTORIA ARAMĂ^{1,2#}, MIHAELA RĂDULESCU^{1,2}, ANA MARIA VLĂDĂREANU^{2,3}, ȘTEFAN SORIN ARAMĂ^{1,2}

¹“Prof. Dr. Matei Balș” National Institute for Infectious Diseases, 1 Dr. Grozovici Street, Bucharest, Romania

²“Carol Davila” University of Medicine and Pharmacy Bucharest, 37 Dionisie Lupu Street, Bucharest, Romania

³Haematology Department, University Emergency Hospital Bucharest, 169 Splaiul Independenței Street, Bucharest, Romania

*corresponding author: violeta_molagic@yahoo.com

#Authors with equal contribution.

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Abstract

Hepatitis B virus (HBV) infection continues to be a major global health problem. HBV reactivation has been documented both in HBsAg positive patients and in individuals with occult hepatitis B infection (OBI) - negative for HBsAg but positive for anti-hepatitis B core IgG antibodies (IgG anti-HBc). HBV reactivation has a major clinical significance, especially in patients receiving chemoimmunotherapy for haematological malignancies (HM). We aimed to evaluate clinical and epidemiological patterns of HBV reactivation in the OBI group compared to the HBV reactivation in HBsAg positive patients (non-OBI group) during chemoimmunotherapy for HM. We enrolled 63 patients with HM and at least one serological marker of HBV infection, including 10 patients with OBI. Viral reactivation occurred overall in 29/63 patients (46.2%). In the OBI group, seven patients (70%) had reactivation with HBsAg reverse seroconversion after a mean of 5.5 chemotherapy cycles, compared to 22 patients (41.5%) in the non-OBI group ($p = 0.0097$). All patients with viral reactivation were treated with antiviral therapy (entecavir) with a favourable outcome. No deaths occurred due to hepatic failure and the risk of reactivation was not correlated with age, sex and type of HM in our patients.

Rezumat

Infecția cu virus hepatitic B (VHB) continuă să reprezinte o problemă majoră de sănătate publică la nivel mondial. Reactivarea infecției VHB a fost observată atât la pacienții AgHBs pozitivi, cât și la cei cu AgHBs negativ dar care prezentau anticorpi anti-HBc IgG (OBI). Reactivarea infecției VHB are o mare importanță clinică, mai ales la persoanele aflate sub chimio-imunoterapie pentru hemopatii maligne (HM). Studiul de față a avut ca obiectiv evaluarea caracteristicilor clinice și epidemiologice ale reactivării infecției VHB la pacienții cu OBI comparativ cu pacienții cu AgHBs pozitiv (non-OBI) în timpul chimio-imunoterapiei pentru HM. Lotul a cuprins 63 pacienți care aveau cel puțin un marker serologic pozitiv pentru VHB, dintre care 10 pacienți au avut OBI. În ansamblul lotului de studiu, reactivarea virală a fost observată în 29/63 cazuri (46,2%). În grupul OBI reactivarea virală VHB a fost identificată la 7 pacienți (70%) care au prezentat revers seroconversie AgHBs în medie după 5,5 cicluri chimioterapice, față de 22 cazuri (41,5%) în grupul non-OBI ($p = 0,0097$). Toți pacienții cu reactivare virală VHB au primit tratament antiviral cu entecavir și au avut o evoluție favorabilă. Nu s-a înregistrat nici un deces datorat insuficienței hepatice, iar riscul de reactivare nu s-a corelat cu vârsta, sexul sau tipul histologic al afecțiunii hematologice la pacienții aflați în studiu.

Keywords: hepatitis B virus, occult hepatitis B infection, haematological malignancies, chemoimmunotherapy, HBV reactivation

Introduction

Despite the introduction of universal hepatitis B virus (HBV) vaccination in the 1990s, it is estimated that worldwide 2 billion people have serological evidence of either present or past HBV infection [13]. Even on healthcare professionals, vaccine coverage is still moderate. Psarraou *et al.* reported a prevalence of vaccination coverage between 55 - 88% in a study carried out in Greece [11].

Occult hepatitis B infection (OBI) is defined as negative hepatitis B surface antigen (HBsAg), positive anti-hepatitis B core (anti-HBc) antibodies and HBV DNA

in the liver cells, with or without detectable HBV DNA in serum [4, 12].

The prevalence of OBI is estimated between 2% in Europe to over 50% in Asia [13-15]. OBI is associated with a significant potential for HBV reactivation in the context of chemotherapy or rituximab based therapy (chemoimmunotherapy) for haematological malignancies (HM), with a high risk of fulminant hepatitis and acute liver failure or progression of HM because of therapy discontinuation. HBV infection prevalence in Romania is still intermediate (4.2%), but the prevalence of OBI is 27.9% and remains significant [2].

We aimed to evaluate the clinical characteristics and outcomes of HBV reactivation in the OBI group *versus* HBsAg positive (non-OBI group) patients during chemoimmunotherapy for HM.

Materials and Methods

We performed a prospective observational study that enrolled patients diagnosed with the following HM: chronic lymphocytic leukaemia (CLL), Hodgkin lymphoma (HL), non-Hodgkin lymphoma (NHL), who associated at least one serological marker for HBV infection, including OBI. Study participants were enrolled between December 2007 and December 2010 and were monitored for HBV infection in the “Prof. Dr. Matei Balș” National Institute for Infectious Diseases (MBNIID), Bucharest, Romania until December 2018. The following inclusion criteria were used: patients with HM with at least one serological marker of HBV infection, aged over 18 years. Exclusion criteria were: HIV infection and mental disability to express their informed consent. The study was approved by the local ethics committee. Patients were informed about the purpose of the study and its role in the present scientific context. Written informed consent was obtained from all participants.

The diagnosis of HM was based on the bone marrow/ lymph node biopsy and flow-cytometry analysis according to the international guidelines and was made in the Haematology Department of the University Emergency Hospital of Bucharest (UEHB).

Baseline assessment included viral hepatitis markers and viral load level, FibroMax (using BioPredictive test), abdominal ultrasound, haematological and biochemical tests. HBV DNA levels were determined every 3 - 6 months and a haematological evaluation was performed before every cycle of chemoimmunotherapy. HBV DNA levels were performed for patients who tested positive for at least one serological marker of HBV infection. Serological tests for HBV infection were repeated at 3 and 6 months after the initiation of chemoimmunotherapy.

The following serological markers were used to identify patients with HBV infection: HBsAg, anti-HBs, HBeAg, anti-HBe, IgM anti-HBc, IgG anti-HBc, which were determined using the MEIA method (ARCHITECT i2000SR Immunoassay Analyzer, Abbott Laboratories, Libertyville, IL, USA). Real-time PCR quantification assays, with a lower detection limit of 6 IU/mL were used to determine the HBV DNA level (COBAS TaqMan 48 analyser - Roche Molecular Diagnostics, Pleasanton, CA, USA). Fibrosis and necroinflammation stages were evaluated using the FibroMax method (BioPredictive, Paris, France). Alanine amino transferase (ALT) levels were evaluated using Ortho Clinical Vitros 5,1FS chemistry analyser, New Jersey, USA.

Statistical analysis

Data were processed using IBM SPSS Statistics version 22 software (New York, USA). Normally-distributed variables were expressed as mean \pm standard deviation (SD) and non-Gaussian variables as median with interquartile range. In the univariate analysis, we used the *Chi-square test* for the association of dependable variables and possible risk factors. Statistical significance was defined as two-tailed $p < 0.05$.

HBV reactivation in HBsAg positive patients was defined as a ≥ 2 log increase in the HBV DNA level compared with baseline [17]. OBI reactivation was defined as reverse seroconversion from HBsAg negative to HBsAg positive and/or reappearance or rise of HBV DNA [10].

Results and Discussion

We enrolled 63 patients with HM and at least one serological marker of HBV infection. Their age ranged from 27 to 87 years (mean 57.35 ± 12.78 years), 42 were males (66.7%) and 21 were females (33.3%). The most common HM was NHL (38 cases) followed by CLL (20 cases), HL (3 cases) and multiple myeloma (2 patients). An aggressive histological type of HM was reported in 33 (52.4%) of patients (Figure 1).

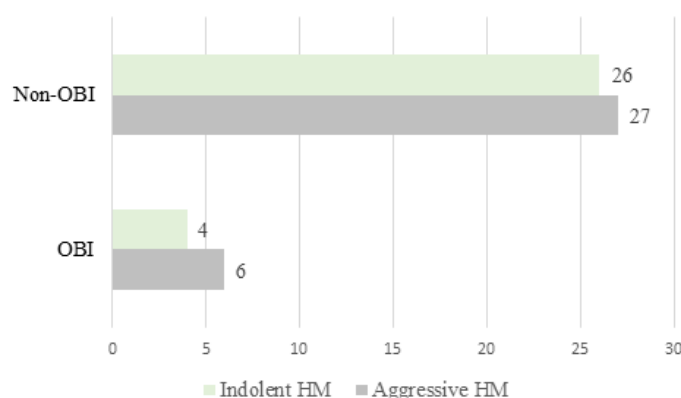


Figure 1.

The histological type of HM

From the whole group of patients, 53 (84.1%) were HBsAg positive, of which 14 (26.4%) inactive carriers (with HBV DNA < 2,000 UI/mL) and 39 (73.6%) with higher HBV viral loads. At baseline, the median value of HBV DNA was 17,300 UI/mL. The remaining 10 patients (15.9%) were OBI patients from which four cases had serum antiHBs antibodies with titres > 10 mUI/mL.

Hepatomegaly and splenomegaly were present in 37 (58.6%) and respectively 21 (33.4%) cases of all 63 patients. A FibroMax test was performed in 30 (47.6%) of the 63 study participants. Of these patients, 18 (60%) had moderate/mild fibrosis (F1 and F2) and 23 (76.6%) had moderate necroinflammatory activity (A1).

Chemotherapy that included only CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone) regimen was used in 21 (33.3%) of the patients. Rituximab (anti CD20 monoclonal antibody) was added to CHOP in another 39 cases (61.9%); 3 patients did not require

chemotherapy at baseline. Of all patients, only 17 (26%) were already under antiviral treatment with entecavir (pre-emptive antiviral therapy).

Viral reactivation (VR) occurred overall in 29/63 (46.2%) of the patients (Figure 2). In the OBI group, 7 out of 10 patients (70%) had reactivation with HBsAg reverse seroconversion after a mean of 5.5 chemotherapy cycles, compared to 22 patients out of 53 (41.5%) in the non-OBI group (p = 0.0097). In the OBI group, the mean baseline ALT was 38 ± 5.39 UI/L and increased after reactivation to 126 ± 23.21 U/L. The patients with OBI and reactivation had a mean HBV-DNA of 25,140 ± 26.71 UI/mL. All patients with viral reactivation were treated with antiviral therapy (entecavir) with a favourable outcome. No deaths occurred due to hepatic failure. The risk of reactivation was not correlated with age, sex and type of HM (NHL, LLC, HL or MM) in OBI and non-OBI patients group.

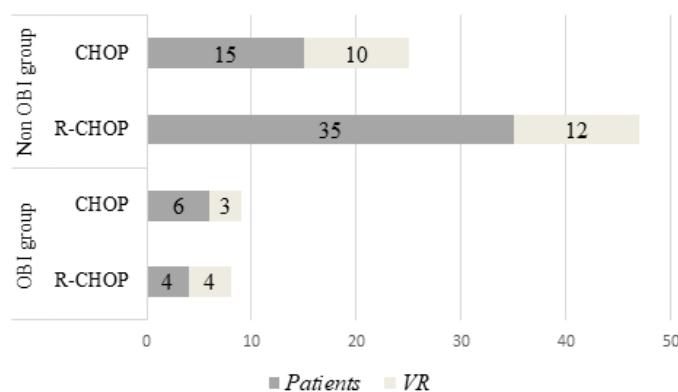


Figure 2. Viral reactivation (VR) after chemotherapy in OBI versus non-OBI group

The patients were long term followed-up by complex multidisciplinary monitoring in MBNIID and UEHB. We used serological and HBV-DNA monitoring during chemoimmunotherapy and until at least 2 years after the end of treatment for all study participants. During

the follow-up period, 15 patients died (23.8%): four cases in the OBI group and 11 cases in non-OBI group of patients, with all deaths due to the aggressive evolution of HM (Table I).

Table I Clinical characteristics and outcome of HBV reactivation in OBI versus non-OBI group

Variable	OBI group (n = 10)	Non OBI group (n = 53)	p
Male gender n (%)	6 (60)	36 (67.9)	0.441
Median age (years)	61.8	56.5	0.233
Non-Hodgkin lymphomas n (%)	8 (80)	30 (56.6)	0.523
Aggressive histologic type n (%)	6 (60)	27 (51)	0.572
Fibrosis (F1 - F2)	4 (40)	18 (34)	0.391
Necroinflammatory activity (A1)	3 (30)	20 (37.7)	0.920
ALT mean UI/L	126	186	0.292
HBV DNA mean UI/mL	25,140	155,080,972	-
Viral reactivation n (%)	7 (70)	22 (41.5)	0.0097
Mean no of chemotherapy cycles	5.5	5.6	-
Preemptive antiviral treatment n (%)	2 (20)	15 (28.3)	0.794
Death n (%)	4 (40)	11 (20.7)	0.231

HBV infection continues to be endemic in Romania. The number of reactivations in HBV-infected patients is likely to increase further, with the increasing prevalence of haematological malignancies and the more widespread use of chemoimmunotherapy. The risk of reactivation of HBV infection varies significantly according to different reports and is different for specific neoplastic disorders and the type of immunosuppressive drug. HBsAg positive cancer patients receiving chemo- or immunosuppressive therapy are up to 8 times more likely to have an episode of HBV reactivation compared to HBsAg negative/anti-HBc positive patients [10].

Patients with HM seem to have the highest risk of experiencing HBV reactivation [1, 10], with percentages varying between 18 - 73% for HBsAg positive patients. Among OBI patients the reported prevalence of HBV reactivation varies between 18 - 25% [7, 20]. Biological therapy combined with other therapies has proven effective both in patients with rheumatoid polyarthritis and in patients with HM [16].

The use of B-cell depleting agents (rituximab) carries a high risk of reactivation (30 - 60%) in HBsAg positive-patients, compared to approximately 10% in patients with OBI. Contrary to other studies, we report a significantly higher rate of reactivation in the OBI group, compared to the non-OBI group. Approximately two-thirds of our OBI patients experienced reactivation with reverse seroconversion to HBsAg, compared to less than half in the non-OBI group. This result may be due to the careful monitoring of the patients as well as the fact that the group of OBI patients was smaller compared to the non-OBI group. Also, this unexpected result emphasizes the fact that OBI has a major clinical significance in patients receiving chemoimmunotherapy. The host's immune system plays a major role in strongly suppressing HBV replication. However, immunosuppression consecutive to chemoimmunotherapy can disrupt the balance that may exist for a long time between the host and the virus which persists as covalently-closed-circular DNA (cccDNA) in the nuclei of the hepatocytes. Rituximab is a monoclonal antibody, which targets CD20 receptors on the surface of B-cells and has both direct effects that induce CD20+ cell toxicity (*via* complement mediation and antibody-dependent cell-mediated cytotoxicity) and indirect effects such as apoptosis and increase of chemotherapy sensitization of cancer cells [18]. The addition of rituximab to CHOP chemotherapy is a highly effective strategy in the treatment of HM, especially in B-cell lymphoproliferative disorders, but also carries a significant risk of HBV reactivation that can be life-threatening in both non-OBI and OBI patients. Consequently, international guidelines [6, 9, 17] recommend prophylaxis of HBV reactivation using nucleotide analogues, which can be extended 12 months after the end date of immunosuppressive

intervention. In our study, less than one-third of our patients received prophylaxis of HBV reactivation, without a significant difference between the OBI and non-OBI group. In Romania the rates of HBV complete screening before immunosuppressive therapy are unknown. Until recently, anti-HBc antibody testing was not used by onco-haematologists in our country to screen patients to determine previous exposure to hepatitis B virus. In the light of our results, we strongly advocate for the use of IgG anti-HBc, besides HBsAg and anti-HBs testing in the initial evaluation for HBV infection, for patients who are about to start chemotherapy or chemoimmunotherapy. We believe that currently occult HBV infections are underdiagnosed in Romania and a significant number of cancer patients do not have proper monitoring and early detection of HBV reactivation under chemoimmunotherapy.

The use of prophylaxis antiviral therapy for both HBsAg-positive patients and OBI patients group is justified by the increased risk of HBV reactivation, which can also worsen the evolution of the haematological disorder. A recent medical protocol [3] approved in Romania regarding the prophylaxis of HBV reactivation in candidates for immunosuppressive therapy recommends the use of a third-generation high genetic barrier to resistance nucleot(s)ide analogue with high antiviral efficiency: tenofovir disoproxil fumarate, tenofovir alafenamide or entecavir. In our study, all patients who developed HBV reactivation received antiviral treatment with entecavir 0.5 mg/day with a favourable outcome. Lamivudine, which has been used for a long time in the treatment of chronic B hepatitis, is no longer recommended by current guidelines due to the increased risk of developing resistance, compared to the third generation drugs [6, 9].

Different authors reported that HBV reactivation was most commonly associated with male sex and younger age groups in patients under immunosuppressive therapy [8, 19]. We did not find other significant differences in respect to epidemiology and clinical characteristics between the two groups: in both cohorts, viral reactivation occurred predominantly in male patients, with a mean age above 50 years. More than half of patients in both groups had been diagnosed with an aggressive HM. HBV reactivation was observed after more than 5 chemoimmunotherapy cycles, in concordance with the results of other studies. A comprehensive literature review reported that HBV reactivation was observed after a median number of 6 chemoimmunotherapy cycles [5]. We did not find statistically significant differences between the two groups regarding fibrosis, necroinflammation and liver enzymes.

Conclusions

To our knowledge, this is the first paper in Romania that evaluated OBI reactivation, compared to overt

HBV infection, in patients with HM in the context of rituximab-based chemoimmunotherapy. We report a high incidence of OBI reactivation in these patients and we suggest that IgG anti-HBc screening should be mandatory before chemoimmunotherapy for HM. Prophylaxis with entecavir (third-generation nucleoside analogues) should be recommended to reduce the risk of reactivation in all cases that have serological evidence of HBV infection.

Conflict of interest

The authors declare no conflict of interest.

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