

Prospective evaluation of seropositive occult hepatitis B viral infection in lymphoma patients receiving chemotherapy

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Objective To serially evaluate the viral kinetics of occult hepatitis B virus infection in lymphoma patients and perform a correlation with clinical outcomes.

Design Case series with 1-year follow-up.

Setting Regional hospital, Hong Kong.

Patients Consecutive patients who were newly diagnosed to have lymphoma in the hospital between 1 April 2007 and 31 March 2008 were tested for hepatitis B (HB) surface (s) antigen (Ag), anti-HBs antibody (Ab) and anti-HB core (c) Ab. Seropositive occult hepatitis B patients as defined by being negative for HBsAg but positive anti-HBsAb and/or anti-HBcAb without a hepatitis B vaccination history were recruited. Serum HBsAg, anti-HBsAb, anti-HBcAb, hepatitis B virus deoxyribonucleic acid (DNA) level, and liver biochemistry were checked at baseline and every 4 weeks during and after chemotherapy until 12 months after the completion of chemotherapy or death. Entecavir was started if patients developed biochemical flare-up of hepatitis B associated with virological rebound. The prevalence and course of hepatitis B virus-related hepatitis, as well as any temporal relationship to viral kinetics and clinical hepatitis, were assessed.

Results Of 47 patients tested, 10 (21%) with lymphoma were seropositive occult hepatitis carriers. Their median baseline hepatitis B virus DNA level was 89 IU/mL (range, <34–807 IU/mL). Virological rebound (as defined by a 10-fold increase in serum hepatitis B virus DNA level from pre-chemotherapy level persisted for 4 weeks) occurred in one of the 10 patients, followed by biochemical reactivation. Whereupon entecavir treatment was started and no liver failure ensued. Regarding the other seropositive occult patients, their serum hepatitis B virus DNA levels fluctuated, but there was no associated biochemical reactivation.

Conclusion Detectable baseline serum hepatitis B virus DNA is not uncommon in patients with occult hepatitis B who receive chemotherapy. Transient elevation in serum hepatitis B virus DNA levels does not predict biochemical reactivation, but antiviral treatment might be considered if virological rebound persists.

Key words

Chemotherapy, adjuvant; DNA, viral; Hepatitis B virus; Lymphoma, non-Hodgkin; T-lymphocytes, cytotoxic

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New knowledge added by this study

In patients with occult hepatitis B who receive chemotherapy:

- at baseline, serum hepatitis B virus (HBV) DNA is quite commonly detected;
- transient elevation in serum hepatitis B virus DNA levels does not predict biochemical reactivation; and
- HBV DNA elevation ($\geq 1 \log_{10}$ IU/mL) persisting 4 weeks or longer predicts liability to biochemical reactivation.

Implications for clinical practice or policy

- In these patients, regular monitoring of serum HBV DNA and liver biochemistry is important.
- Antiviral treatment should be considered for patients having a persistent increase in serum HBV DNA of $\geq 1 \log_{10}$ IU/mL.

Introduction

In patients with acute or chronic hepatitis B virus (HBV) infection, the loss of hepatitis B surface antigen (HBsAg) does not necessarily lead to a complete clearance of virus. Hepatitis B viral deoxyribonucleic acid (DNA) can still be detected in serum, liver, and peripheral blood mononuclear cells several decades after apparent recovery from acute HBV infection.¹ Occult hepatitis B infection (OBI) is defined as the presence of HBV DNA in the liver (with detectable or undetectable HBV DNA in the serum) of individuals with HBsAg test negative by currently available assays.² When detectable, the amount of HBV DNA in the serum is usually very low (<200 IU/mL) in healthy people.² Patients with OBI can be classified as seropositive and seronegative according to the presence of anti-hepatitis B core antibody (anti-HBcAb) and/or anti-hepatitis B surface antibody (anti-HBsAb). Some patients are infected by HBV variants, which are not detected by some or all of the commercial assays. Their serum HBV DNA levels are comparable to those usually detected in different phases of serologically positive HBV infection and they are defined as patients with 'false' OBI.

Hepatitis B viral reactivation with reappearance of HBsAg and elevation of serum HBV DNA level may occur when patients with OBI become immunocompromised.³ The reactivation rate is between 3.3% and 4%,^{3,4} and is more common after rituximab plus other conventional chemotherapeutic agents and can be fatal.⁵⁻⁷ Hui et al⁴ suggested a 100-fold rise in serum HBV DNA level could predict a biochemical flare-up.

To date, there is not enough information to recommend routine prophylaxis in patients with OBI while they are given chemotherapy.⁸ However, in this group of patients it is advisable to monitor liver enzymes during chemotherapy. In patients with elevated alanine aminotransferase (ALT) levels, further diagnostic work-up is required. Initiation of antiviral therapy is necessary when the diagnosis of HBV reactivation is established.¹ However, frequency of virological rebound and whether virological rebound will be followed by biochemical or clinical flare-up remains unknown.

In this prospective study, we aimed to evaluate the baseline viral load, viral kinetics, and clinical outcome of OBI in lymphoma patients undergoing chemotherapy.

Methods

From 1 April 2007 to 31 March 2008, all patients newly diagnosed with lymphoma in the United Christian Hospital were checked for the presence of HBsAg, anti-HBsAb, anti-HBcAb and anti-hepatitis C antibodies. The HBV vaccination history was obtained.

前瞻性評估接受化療的淋巴瘤患者中的血清陽性隱匿性乙型肝炎病毒感染

目的 評估感染隱匿性乙型肝炎病毒的淋巴瘤患者的乙型肝炎病毒的動力學，然後研究所得結果與臨床結果的相關性。

設計 一年隨訪期的病例系列。

安排 香港一所分區醫院。

患者 2007年4月1日至2008年3月31日期間，新被診斷患有淋巴瘤所有患者會接受乙肝表面抗原（HBsAg）、抗乙肝表面抗體（anti-HBsAb）、抗乙肝核心抗體（anti-HBcAb）的測試。隱匿性乙型肝炎病毒的患者被界定為血清中檢測不到HBsAg但仍可檢測到有anti-HBsAb及/或anti-HBcAb，及從未有乙肝疫苗注射。在開始化療時（基線值）、以及化療期間及化療後的12個月內（或直至患者死亡為止），每四個星期為患者檢查血液HBsAg、anti-HBsAb、anti-HBcAb、HBV DNA水平及肝功能。如果患者出現與病毒學反跳（即HBV DNA水平比基線值升高十倍並持續四星期）有關的肝功能指標升高，會為病人處方恩替卡韋（entecavir）。研究並找出與乙型肝炎病毒有關的肝炎發病率和過程，以及與病毒動力學及肝炎的時間關係。

結果 47位淋巴瘤患者中，10人（21%）為血清陽性隱匿性乙型肝炎病毒帶菌者，他們的HBV DNA基線中位數水平為89 IU/mL（介乎<34至807 IU/mL）。其中一人有病毒學反跳，其HBV DNA水平比化療前高出十倍，後來出現肝炎，病人服食恩替卡韋後未有出現肝衰竭。至於其餘血清陽性隱匿性乙型肝炎病毒攜帶者的血清HBV DNA水平有波動，但並未出現肝炎。

結論 接受化療的隱匿性乙型肝炎患者經常會被檢測到低水平HBV DNA。血清HBV DNA水平有短暫升高的現象並不能預測肝炎，但如果病毒學反跳的情況持續出現，便需要考慮為病人制定抗病毒治療。

Patients who had evidence of seropositive OBI (negative HBsAg but positive anti-HBsAb and/or anti-HBcAb) were recruited after informed consent was obtained. Patients with a history of HBV vaccination or concomitant hepatitis C infection were excluded. The study was approved by the institutional board of the Kowloon East Cluster of the Hospital Authority. Serum HBsAg, anti-HBsAb, anti-HBcAb, HBV DNA levels, liver biochemistry results were obtained at baseline before commencement of chemotherapy and then at 4-week intervals until 12 months after cessation of chemotherapy or death. Serum HBV DNA assays used were real-time polymerase chain reactions (fluorescent hybridisation probe) with the range of detection from 34 to 1.7×10^8 IU/mL.⁹ Entecavir was started if there was evidence of a biochemical exacerbation as defined elevation of the

TABLE. Patient characteristics*

Patient No.	Sex/age (years)	Lymphoma/stage	Rituximab	Baseline anti-HBc/anti-HBs	Baseline HBV DNA (IU/mL)	Peak HBV DNA (IU/mL)	Virological rebound	Biochemical reactivation
1	F/76	DLBCL/IIA	+	+/-	790	36 082	-	-
2	M/78	DLBCL/IVA	-	+/-	721	4467	-	-
3	M/72	DLBCL/IVA	+	+/-	807	807	-	-
4	F/56	MBCL/IIEA	+	+/+	45	309	-	-
5	M/45	MBCL/IIEA	-	+/-	96	96	-	-
6	M/68	MBCL/IVB	-	+/+	43	670	-	-
7	F/67	ATCL/IVA	-	-/+	<34	189	-	-
8	M/55	Composite/IVA	-	+/-	<34	206	-	-
9	F/86	DLBCL/IIEA	-	+/-	<34	722	-	-
10	M/69	DLBCL/IIA	+	+/-	86	2 061 856	+	+

* anti-HBc denotes anti-hepatitis B core, anti-HBs anti-hepatitis B surface, HBV hepatitis B virus, DNA deoxyribonucleic acid, DLBCL diffuse large B cell lymphoma, MBCL malignant B cell lymphoma, ATCL angioimmunoblastic T cell lymphoma, and Composite composite lymphoma

ALT to more than 5 times of the upper limit of normal and more than twice the baseline value. Virological rebound was defined as an increase in serum HBV DNA $1 \log_{10}$ IU/mL in at least two determinations 4 weeks apart.

Results

In all, 47 patients were diagnosed to have lymphoma between 1 April 2007 and 31 March 2008, of whom 10 (21%) were found to have seropositive OBI—two patients were both anti-HBc and anti-HBs positive, and seven were anti-HBc positive but anti-HBs negative (Table). One of the patients was anti-HBs positive but anti-HBc negative; according to the history she provided, she had not received any HBV vaccination before. In all, nine patients had non-Hodgkin's lymphoma, and one had a composite lymphoma. All of them received the CEOP (cyclophosphamide, epirubicin, vincristine and prednisolone) regimen, whilst three also received rituximab. The median follow-up duration was 17 (range, 7–29) months.

Baseline liver biochemistry was normal in all the patients. Seven patients had detectable baseline HBV DNA, in whom the median level was 89 (range, <34–807) IU/mL. Only patient 10 who received rituximab and the CEOP regimen developed asymptomatic biochemical reactivation, which was preceded by virological rebound. In this patient, amino acid sequencing of HBV showed five mutations at pre-S1, five at pre-S2, and 23 at the S region. Six amino acid mutations were located in the 'a' determinant, which might account for the low sensitivity of commercial HBsAg assays.¹⁰ The serial changes of liver enzymes and HBV DNA levels in this patient are summarised in Figure 1. The virological rebound occurred at week 2 after the completion of chemotherapy, when the HBV DNA rose from 86 IU/mL to 2750 IU/mL and gradually increased to 22 336 IU/mL 4 weeks later.

In patients 1 to 9, there was fluctuation in their HBV DNA levels, but their increased levels were transient and not associated with a biochemical flare-up (Fig 2).

Discussion

In our cohort, 70% of patients had a detectable baseline serum HBV DNA, the median level was 89 (range, <34–807) IU/mL and appeared to be higher than those previously reported. The baseline HBV DNA level in lymphoma patients with biochemical flare-up after chemotherapy ranged from 4 to 14 IU/mL in the study by Hui et al,⁴ in which it was retrospectively determined only when de-novo HBV-related hepatitis developed. Hence, in the majority of the patients who did not develop biochemical reactivation, the baseline HBV DNA and its serial changes were not studied. In our prospective study, we observed that although a transient elevation of serum HBV DNA level was not uncommon, in the majority it was less than 1 log and not persistent. None of those with transient/fluctuating levels developed a biochemical flare-up. These may represent normal fluctuations in HBV DNA level in OBI patients. Different HBV DNA assays may contribute to some of the variations. However, the quantitative HBV DNA assays used in our study was validated with two EUROHEP HBV DNA standards (ad and ay subtypes) and exhibited low inter-assay (<16%) and low intra-assay (<6%) variation for both subtypes over a range spanning seven orders of magnitude.⁹

We encountered one virological rebound out of 10 patients, ie 10%. This was the only patient who had virological rebound and subsequently developed biochemical flare-up. In line with findings by Hui et al,⁴ a 2-log increase in serum HBV DNA level preceded the biochemical flare-up in patient 10. In patient 1 who had a transient increase in HBV DNA to 36 080 IU/mL from a baseline level of

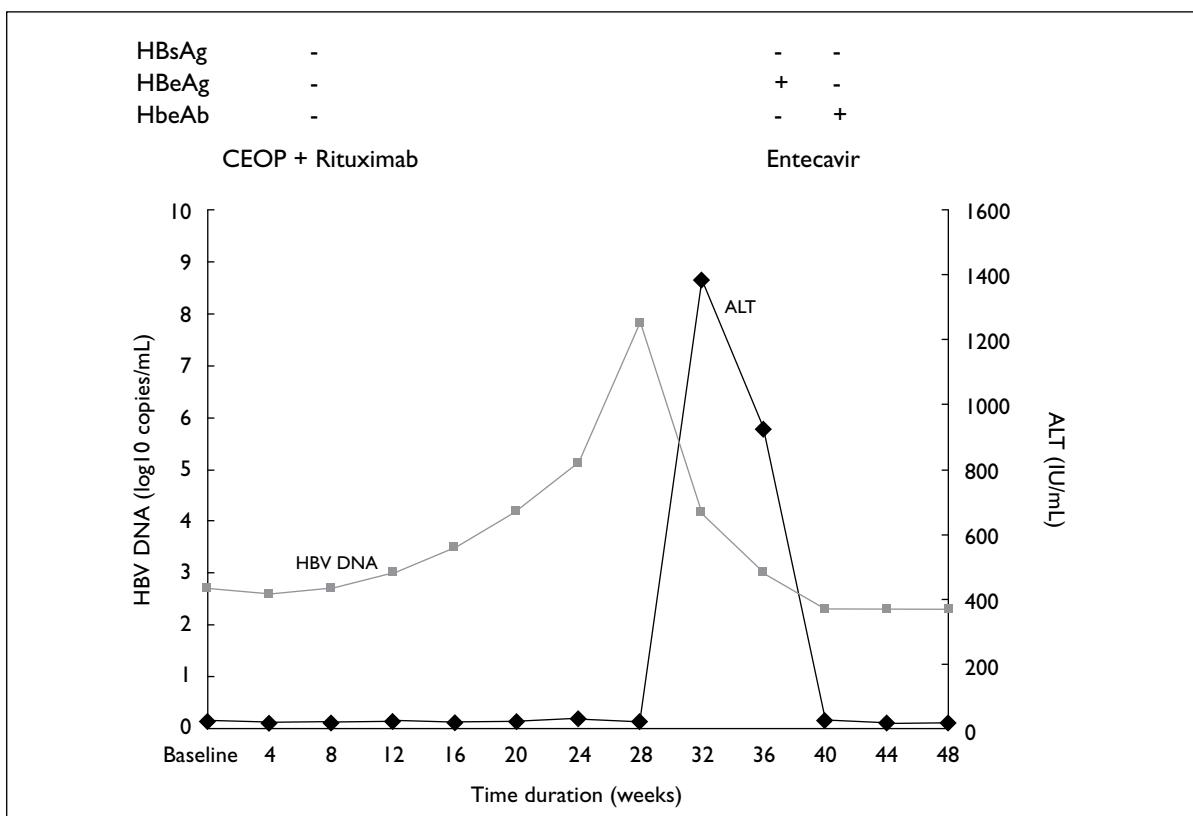


FIG 1. Serial changes of hepatitis B virus (HBV) DNA and alanine aminotransferase (ALT) in patient 10 who sustained a virological rebound and biochemical flare-up

CEOP denotes cyclophosphamide, epirubicin, vincristine and prednisolone; HBsAg hepatitis B surface antigen; HBeAg hepatitis B early antigen; and HBeAb hepatitis B early antibody

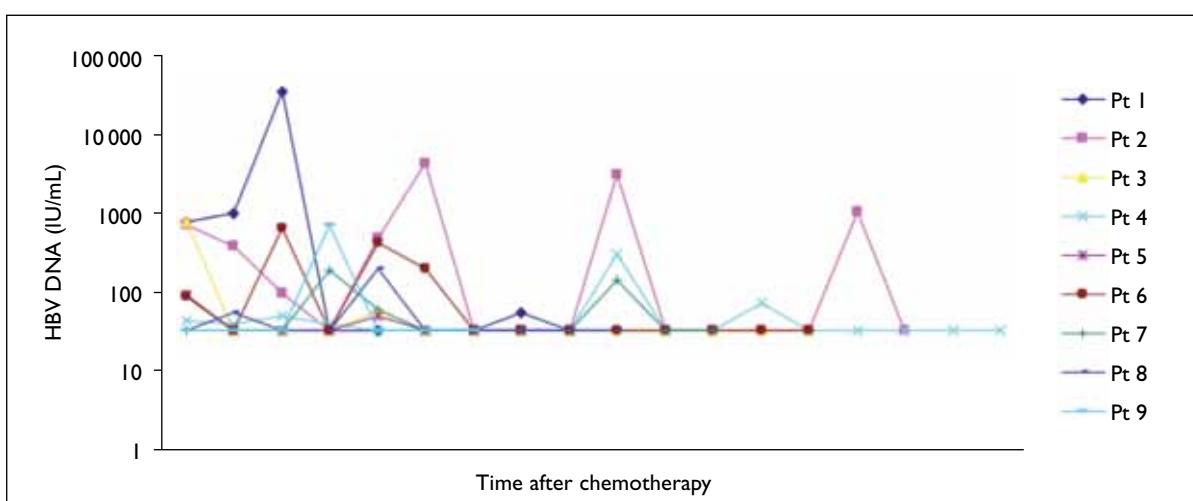


FIG 2. Hepatitis B virus (HBV) DNA trends in patients 1 to 9

790 IU/mL, there was no subsequent biochemical reactivation despite a transient 50-fold increase in HBV DNA. It appears that non-sustained increases in serum HBV DNA level are not necessarily followed by biochemical reactivation. Hence our findings do not support routine prophylactic use of antivirals in patients with transient increases of serum HBV DNA level. Interestingly, in the patient who sustained a biochemical reactivation, the HBsAg was persistently

negative as a result of pre-s/s-mutant and this illustrated the importance of HBV DNA monitoring.¹⁰

In OBI patients who receive chemotherapy, there is no consensus on the criteria for initiation of antiviral treatment or the drug of choice. Recommendations from the American Association for the Study of Liver Diseases advise that patients who are HBsAg negative but anti-HBc and anti-HBs positive or with isolated anti-HBc antibodies

should be monitored and antiviral therapy should be initiated when the serum HBV DNA becomes positive.⁸ According to our observations, the baseline HBV DNA was positive in 70% of seropositive OBI patients, in whom virological rebound or biochemical reactivation ensued in only 10%, which does not seem to support the current recommendations. However, it is essential to monitor serum HBV DNA level serially and consider antiviral therapy if patients develop persistently elevated HBV DNA levels. More data are needed to decide the cutoff HBV DNA level above which the risk of flare-up is higher or whether the degree of HBV DNA increase matters. Both the guideline of the European Association for the Study of the Liver¹¹ and the Statement from the Taormina expert meeting on OBI² suggested that treatment should be initiated when HBV reactivation is established, but how to establish this has yet to be defined. In our study, only patients with sustained virological rebound was followed by biochemical reactivation, thus it is reasonable to

consider antivirals when virological rebound, ie an increase in serum HBV DNA of at least $1 \log_{10}$ IU/mL, is demonstrated in two determinations more than 4 weeks apart. Further study is needed to confirm this. In HBV-infected patients who receive chemotherapy, preemptive antiviral using lamivudine or telbivudine is recommended when patients have an undetectable baseline HBV DNA level and the duration of treatment is less than 12 months, otherwise tenofovir or entecavir are considered necessary.³ However, there is no consensus on the treatment of OBI patients who sustain reactivation. In our patients with biochemical flare-up, we planned for lifelong antiviral therapy and hence entecavir was chosen.

In conclusion, a detectable baseline serum HBV DNA is not uncommon in patients with OBI who receive chemotherapy. Transient elevations in HBV DNA do not predict biochemical reactivation, but antiviral treatment can be considered if there is evidence of persistent virological rebound.

References

1. Rehermann B, Ferrari C, Pasquinelli C, Chisari FV. The hepatitis B virus persists for decades after patients' recovery from acute viral hepatitis despite active maintenance of a cytotoxic T-lymphocyte response. *Nat Med* 1996;2:1104-8.
2. Raimondo G, Allain JP, Brunetto MR, et al. Statements from the Taormina expert meeting on occult hepatitis B virus infection. *J Hepatol* 2008;49:652-7.
3. Lok AS, Liang RH, Chiu EK, Wong KL, Chan TK, Todd D. Reactivation of hepatitis B virus replication in patients receiving cytotoxic therapy. Report of a prospective study. *Gastroenterology* 1991;100:182-8.
4. Hui CK, Cheung WW, Zhang HY, et al. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006;131:59-68.
5. Yeo W, Chan TC, Leung NW, et al. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* 2009;27:605-11.
6. Sarrecchia 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;11:189-91.
7. Law JK, Ho JK, Hoskins PJ, Erb SR, Steinbrecher UP, Yoshida EM. Fatal reactivation of hepatitis B post-chemotherapy for lymphoma in a hepatitis B surface antigen-negative, hepatitis B core antibody-positive patient: potential implications for future prophylaxis recommendations. *Leuk Lymphoma* 2005;46:1085-9.
8. Lok AS, McMahon BJ. Chronic hepatitis B: update 2009. *Hepatology* 2009;50:661-2.
9. Ho SK, Yam WC, Leung ET, et al. Rapid quantitation of hepatitis B virus DNA by real-time PCR using fluorescent hybridization probes. *J Med Microbiol* 2003;52:397-402.
10. Cheung WI, Chan HL, Leung VK, et al. Reactivation of hepatitis B virus infection with persistently negative HBsAg on three HBsAg assays in a lymphoma patient undergoing chemotherapy. *J Clin Virol* 2010;47:193-5.
11. European Association for the Study of the Liver. EASL Clinical Practice Guidelines: management of chronic hepatitis B. *J Hepatol* 2009;50:227-42.