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Ⅲ. 研究成果の刊行物・別冊

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REVIEW ARTICLE

Reactivation of hepatitis B virus following systemic chemotherapy for malignant lymphoma

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Abstract Reactivation of hepatitis B virus (HBV) has been reported not only in HBsAg-positive patients undergoing systemic chemotherapy, but also in a proportion of HBsAg-negative patients with HBc antibody and/or HBs antibody. Recently, rituximab-plus-steroid combination chemotherapy (R-CHOP, etc.) has been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma. Prophylaxis with antiviral drugs is essential for preventing HBV reactivation in HBsAg-positive patients, but there is little evidence on which to base the choice of drug or appropriate duration of prophylaxis. There are also few clinical data on HBsAg-negative patients and no established standard of care for such patients with HBV reactivation. Based on the limited number of previous reports, preemptive therapy, guided by serial HBV-DNA monitoring, is a reasonable strategy to prevent HBV reactivation in HBsAg-negative patients. However, clinical evidence alone is insufficient for determining optimal frequency of HBV-DNA monitoring during and after chemotherapy, or for determining when to stop preemptive therapy for HBV reactivation. Thus, well-designed clinical trials should be carried out to investigate the efficacy and safety of such preemptive therapy. Additionally, assessment of viral factors such as HBV genotypes and gene mutations may assist in the development of strategies to prevent the occurrence of severe hepatitis. In this review, we summarize the characteristics of HBV reactivation after systemic chemotherapy including rituximab, and propose a management strategy for malignant lymphoma patients suffering from HBV reactivation.

Keywords Reactivation · HBV · Rituximab · Malignant lymphoma · Chemotherapy

Abbreviations

HBV	Hepatitis B virus
HBsAg	Hepatitis B surface antigen
Anti-HBc	hepatitis B core antibody
Anti-HBs	Hepatitis B surface antibody
HBeAg	Hepatitis B e antigen
Anti-HBe	Hepatitis B e antibody
ALT	Aminotransferase
RTD-PCR	Real-time detection polymerase chain reaction
NAT	Nucleic acid amplification test
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisolone
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone

1 Introduction

Reactivation of hepatitis B virus (HBV) has been previously reported in hepatitis B surface antigen (HBsAg)-positive

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cancer patients receiving systemic chemotherapy. Anticancer therapy has progressed remarkably in the past decade. Especially for CD20-positive lymphoma patients, the introduction of rituximab (anti-CD20 monoclonal antibody) has improved treatment outcome dramatically. However, some HBsAg-negative lymphoma patients receiving chemotherapy incorporating rituximab have recently been reported to develop fatal hepatitis caused by HBV reactivation. Because it was thought that HBsAg-negative patients are at low risk for HBV reactivation following rituximab-containing chemotherapy, strategies to prevent this have not been developed. HBV reactivation in such patients may thus represent an important future clinical problem in lymphoma treatment.

In this review, we summarize the characteristics of HBV reactivation after systemic chemotherapy including rituximab, and propose a management strategy for malignant lymphoma patients with HBV reactivation.

2 Natural course of HBV infection and definition of HBV carriers

The natural course of acute HBV infection is shown in Fig. 1. First, the level of HBV-DNA is elevated after infection, and HBsAg becomes positive approximately five weeks later, as shown by experiments on chimpanzees. HBsAg levels then decrease to below the level of detection in most immunocompetent hosts over the next few weeks as anti-HBs antibody titers increase, reflecting seroconversion as in humans. Most acute hepatitis B completely resolves in adult patients. However, HBsAg positivity sometimes persists from several years to several decades when the virus is transmitted from mother to infant, or in immunocompromised hosts such as cancer patients. Under these conditions, HBV-infected individuals are likely to suffer from chronic hepatitis without eliminating the virus.

An individual is defined as an HBV carrier when HBsAg is detectable, or the serum contains HBV-DNA [>100 copies/mL by real-time detection polymerase chain reaction (RTD-PCR)]. In immunocompetent hosts, it is generally immunopathology that causes liver damage during acute hepatitis B. After a period of time, HBsAg becomes negative and serum HBV-DNA levels decrease to below the limit of detection (100 copies/mL by RTD-PCR). This is defined as "HBV carrier late phase", which has often been regarded as "resolved infection" or "past infection", because HBV replication no longer occurs.

However, re-initiated HBV replication and increasing levels of serum HBV-DNA have been reported following anticancer or immunosuppressive drug therapy, including the use of anti-CD20 monoclonal antibody (rituximab) in HBV carrier late phase patients. When a small amount of serum HBV-DNA is detected in HBsAg-negative patients, it is defined as "occult hepatitis B", and taken to indicate an increased risk of HBV reactivation, as is the case for HBsAg-positive patients.

3 Reactivation of hepatitis B following systemic chemotherapy

The natural course of chronic HBV infection is determined by the interplay between virus replication and the host's immune response [1]. HBV replication takes place in the liver and peripheral blood mononuclear cells over several weeks to several years from the onset of acute hepatitis B. This is the case even in patients with seroconversion of HBsAg, who are HBs antibody-positive and HBsAg-negative. Therefore, when patients with a history of HBV exposure receive therapy with immunological inhibitory activity, such as systemic chemotherapy, they are all at risk for HBV reactivation. Due to the immunosuppressive effect of chemotherapy, HBV is likely to rapidly increase and to infect many hepatocytes. Consequently, hepatitis B disease recurs because immunocompetent cells attack HBV-infected hepatocytes following immune recovery after chemotherapy.

Based on several previous reports [2–12] describing the characteristics of the clinical course of HBV reactivation after systemic chemotherapy, three main points can be made: (1) Most hepatitis B disease occurs after the patient has finished chemotherapy. However, in HBsAg-positive patients with high viral loads, hepatitis may even occur during chemotherapy. (2) Increased serum HBV-DNA prior to the onset of hepatitis is usually observed. (3) In addition to HBsAg-positive patients, HBV reactivation may occur in HBsAg-negative immunosuppressed patients as well.

4 Risk of HBV reactivation following anti-cancer therapy

In cancer patients receiving systemic chemotherapy, the risk of HBV reactivation varies according to the degree of immunosuppression due to the treatment, but also with the HBV infection status before treatment. In other words, HBV reactivation is influenced by the strength of immunosuppression (intensity of chemotherapy; treatment with drug combinations or not; type of disease) and by HBV status (viral load; genotype; HBV gene mutations). Important factors associated with immunosuppression were reported to be steroid combination chemotherapy, hematopoietic stem cell transplantation (allogeneic > autologous), organ transplantation and malignant lymphoma

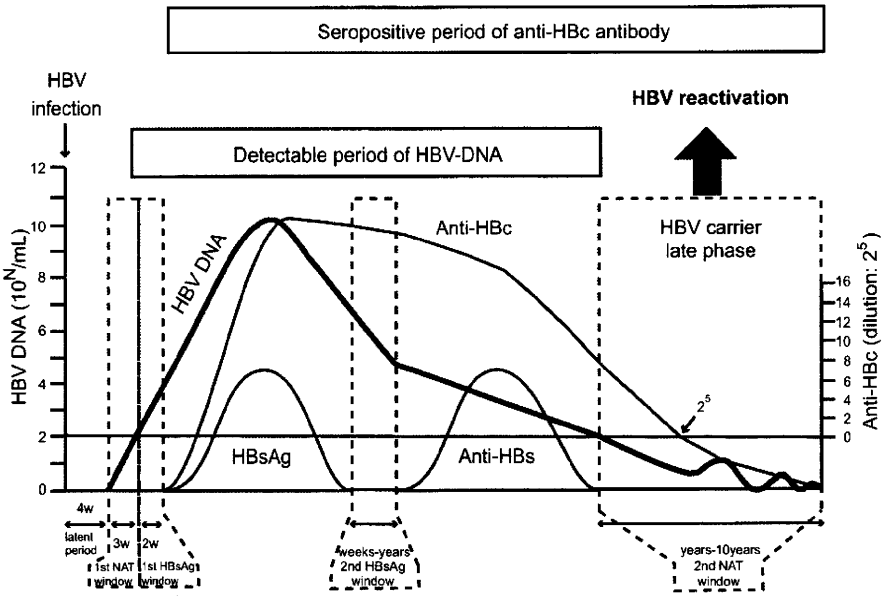

Fig. 1 Acute hepatitis B virus infection in the experimental chimpanzee model. When infected with HBV, HBV-DNA is elevated first, and HBsAg becomes positive approximately 5 weeks later, shown by experiments in the chimpanzee. Thereafter, HBsAg becomes undetectable in most immunocompetent hosts within several weeks and anti-HBs antibody emerges, completing seroconversion as in humans. Most acute hepatitis B resolves in adult patients, who are then referred to as being in the "HBV carrier late phase". Reactivation of hepatitis B virus has been reported when anticancer or immunosuppressive drugs are given to patients in the HBV carrier late phase. This figure is modified from Katatsumuri 2007 Jan No117 from the Miyakawa Memorial Research Foundation

[3, 5, 8, 13–16]. It was recently reported that a rituximab-plus-steroid combination regimen was another important risk factor for HBV reactivation [4, 11]. Conversely, levels of viral factors such as HBV-DNA viral load before treatment and HBV-related serum markers (presence or absence of HBsAg, HBe antigen, hepatitis B core (HBc) antibody, HBs antibody) have also been reported to be associated with HBV reactivation following anticancer therapy [11, 12, 15]. Based on these reports, the risk of HBV reactivation according to HBV markers and the strength of host immunosuppression can be classified as shown in Fig. 2.

5 HBV reactivation in HBsAg-positive patients with malignant lymphoma

Most cases of HBV reactivation in patients with malignant lymphoma were reported in HBsAg-positive patients before the rituximab era. Because this group was well recognized as representing patients at high risk for HBV reactivation, when they received chemotherapy, the standard of care in clinical practice was to give prophylactic antiviral drugs or to avoid steroid combination regimens during lymphoma treatment.

When an HBsAg-positive patient is given chemotherapy, the risk of HBV reactivation is reported to be 24–53%. Yeo et al. [10] reported HBV reactivation in 47 of 193 patients receiving lymphoma treatment (24%). Lok et al. [7] gave systemic chemotherapy to 27 malignant lymphoma patients, of which 13 (48%) developed hepatitis. Lau et al. [6] conducted a randomized study of HBV reactivation after chemotherapy in 30 HBsAg-positive malignant lymphoma patients assigned either to receive or not to receive prophylactic antiviral drug treatment. HBV reactivation occurred in 8 (53%) of the untreated groups, but in none of the prophylactically treated group.

6 HBV reactivation in HBsAg-negative patients with malignant lymphoma

Until recently, the HBsAg-negative group (including occult hepatitis B and resolved or past hepatitis B) was not recognized to be at risk of HBV reactivation when receiving conventional systemic chemotherapy. Lok et al. [7] also reported that the risk of HBV reactivation in the HBsAg-negative patient was only 2.7% (2 of 72), far lower than the 48% of HBsAg-positive patients. However, HBV reactivation has been sporadically reported to occur in HBsAg-negative patients receiving rituximab-containing chemotherapy, in the form of case reports or

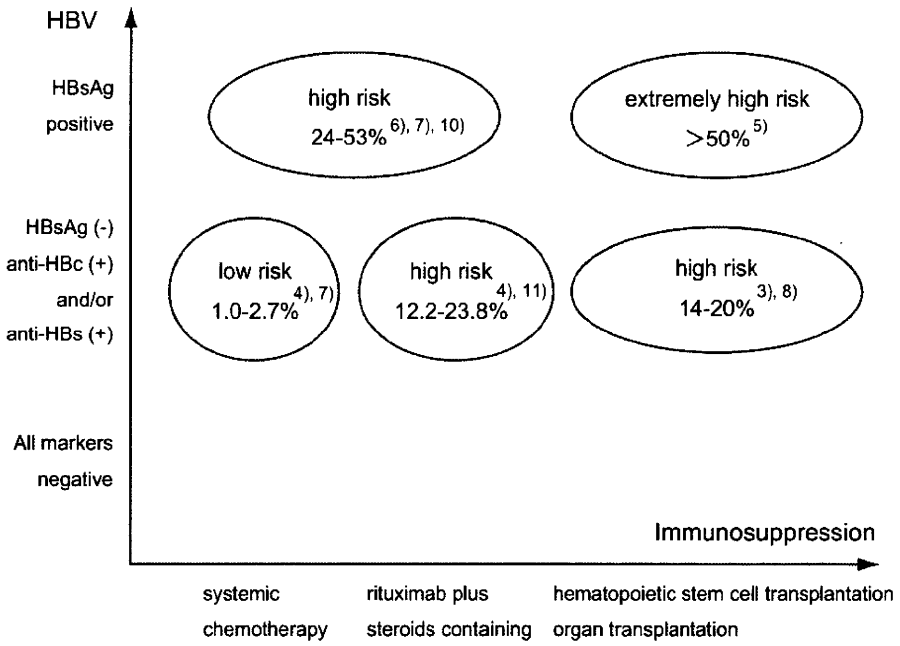

Fig. 2 Risk classification for HBV reactivation. The *vertical axis* shows the HBV infection status according to serum markers before systemic chemotherapy, and the *horizontal axis* shows the strength of immunosuppression caused by treatment. The risk of HBV reactivation in HBsAg-positive patients receiving systemic chemotherapy or hematopoietic stem cell transplantation is 24–53%, and >50%, respectively. In HBsAg-negative patients, the risk of HBV reactivation is higher in the group receiving rituximab-plus-steroid-containing chemotherapy (12.2–23.8%) and hematopoietic stem cell transplantation (14–20%), compared to those on conventional chemotherapy (1.0–2.7%)

small case series, the first of which was by Dervite et al. in 2001 [2]. In 2006, Hui et al. [4] reported that 8 of 244 HBsAg-negative lymphoma patients receiving systemic chemotherapy developed new-onset hepatitis B (3.3%). These 8 patients were seropositive for either HBc or HBs antibody. However, the incidence of HBV reactivation in this cohort in the rituximab-plus-steroid combination group was higher at 12.2% (6 of 49) compared to other combination therapy groups in which it was only 1.0% (2 of 195). Multivariable analysis demonstrated for the first time that rituximab-plus-steroid combination chemotherapy is a risk factor for HBV reactivation.

Most recently, Yeo et al. [11] reported that 5 of 80 HBsAg-negative patients diagnosed as suffering from diffuse large B-cell lymphoma and receiving uniform systemic chemotherapy (R-CHOP or CHOP-like regimens) had reactivated HBV (6.25%). All 5 had received R-CHOP and all were HBc antibody-positive and HBs antibody-negative. Thus, of 21 anti-HBc-positive lymphoma patients receiving R-CHOP, 5 (23.8%) developed HBV reactivation. Therefore, not only HBsAg-positive patients, but also certain HBsAg-negative patients (HBc antibody-positive and/or HBs antibody-positive and/or serum HBV-DNA-positive) must be recognized as belonging to a group at high risk for HBV reactivation following rituximab-plus-steroid combination chemotherapy.

7 Summary of the characteristics of 111 patients developing hepatitis B after systemic chemotherapy containing rituximab in Japan (ZENYAKU Company data)

From September 2001 to May 2008, 111 patients developed serious hepatitis B after systemic chemotherapy containing rituximab, according to data from the ZENYAKU Company (including information gleaned retrospectively from medical practices, spontaneous reports to the company, reports at academic meetings and results of several investigational studies and clinical trials in Japan). Of these 111 hepatitis B patients, 47 (42%) were HBsAg-positive, and 50 (45%) were HBsAg-negative. The remainder were not available for assessing the seroprevalence of HBsAg before administration of rituximab. In the group of 50 HBsAg-negative patients who developed serious hepatitis B, 11 were available for assessing the seroprevalence of HBc antibody. All 11 were found to be HBc antibody-positive, of which 1 and 6 patients were HBs antibody-positive and -negative, respectively. The remaining 4 patients were not informative for HBs antibody. 11 patients were also available for assessing the seroprevalence of HBs antibody, of which 4 and 7 were positive and negative, respectively.

As shown in Table 1, from the viewpoint of the association between HBsAg status and systemic chemotherapy,

Table 1 Association between HBsAg status and systemic chemotherapy in 111 patients who developed serious hepatitis B after systemic chemotherapy containing rituximab (ZENYAKU Company data)

Systemic chemotherapy	HBsAg-positive[a] ($n = 47$)	HBsAg-negative[a] ($n = 50$)
Rituximab monotherapy	7	2
Containing steroids (R-CHOP etc.)	24	40
Not containing steroids (R-CHO, R-cladribine, etc.)	15	4
PBSCT	0	3

R-CHOP indicates rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone combination chemotherapy

R-CHO indicates rituximab, cyclophosphamide, doxorubicin and vincristine combination chemotherapy without prednisolone

PBSCT peripheral blood stem cell transplantation

[a] One patient each without information on chemotherapy was excluded

Table 2 HBsAg status and clinical outcomes in 111 patients who developed serious hepatitis B after systemic chemotherapy containing rituximab (ZENYAKU Company data)

HBsAg	Hepatitis B ($n = 111$)	Recovery/relief	Non-recovery	Death	Sequelae	Unconfirmed
Positive	47 (10)	27 (1)	6	13 (9)	0	1
Negative	50 (20)	22 (1)	1	25 (19)	1	1
Not examined	1	1	0	0	0	0
No information	13 (4)	7	1 (1)	4 (2)	0	1 (1)

The number of patients suffering from fulminant hepatitis is given in parentheses

seven of the 47 HBsAg-positive patients, but only two of the 50 HBsAg-negative patients, respectively, had been treated with rituximab monotherapy. Similarly, 24 versus 40 patients in these two groups had been treated with a steroid-containing regimen (R-CHOP, etc.), 15 versus 4 with a regimen not including steroids (R-CHO, R-cladribine, etc.), and zero versus 3 with autologous peripheral blood stem cell transplantation. One patient in each group was excluded because there was no information on chemotherapy. It is notable that HBV reactivation was observed even in the setting of rituximab monotherapy or steroid-free regimens within the HBsAg-negative group. Of these 111 patients, 8 of 47 HBsAg-positive patients, but only one of 50 HBsAg-negative patients, had been given the antiviral drug lamivudine prophylactically.

The clinical outcomes of these 111 patients with hepatitis caused by HBV reactivation were" recovery in 33 cases, relief in 24, one liver cirrhosis, 8 did not recover, 3 uncomfirmed and 42 deaths. Thus, mortality was high at 37.8% in this cohort. Furthermore, as shown in Table 2, the incidence of fulminant hepatitis (20 of 50, 40.0%) and the mortality (25 of 50, 50.0%) among HBsAg-negative patients was higher than in the HBsAg-positive patients (10 of 47, 21.3% and 13 of 47, 27.7%, respectively) (Fig. 3). The question why the outcome of hepatitis in HBsAg-negative patients is worse cannot be answered presently. Possibly, these patients were not recognized as a reactivation high-risk group, because their physicians considered lymphoma involvement in the liver or drug-induced hepatitis a more likely cause, leading to an underestimation of hepatitis due to HBV reactivation. Therefore, antiviral treatment may have begun too late, rather than immediately as hepatitis developed.

To analyze the time of onset of hepatitis, we compared 44 HBs-negative patients with the same number of HBsAg-positive patients (23 patients were excluded due to lack of information). The onset time is defined as the period between the last treatment and development of hepatitis; the last treatment day is defined as the date closest to hepatitis onset after the last administration either of rituximab or chemotherapy. It was found that median time to onset of hepatitis in HBsAg-positive and HBsAg-negative patients was 4.2 and 9.6 weeks, respectively. Most of the HBsAg-negative patients developed hepatitis after completion of systemic chemotherapy, as anticipated.

The characteristics of HBV reactivation in HBsAg-negative patients are summarized in Table 3. All patients were positive for anti-HBc and/or anti-HBs, although one patient did have less than the cutoff value of HBc antibody (in whom the titer may have been reduced by immunosuppression). Interestingly, mortality following HBV reactivation ranged from 12.5 to 50%. However, there may be differences in the clinical course of HBV reactivation between Asian and Western countries, which may be associated with age, immune response, environmental factors and HBV genotypes as well as gene mutations. Eight genotypes have been detected by sequence divergence of >8% in the entire HBV genome of about 3,200

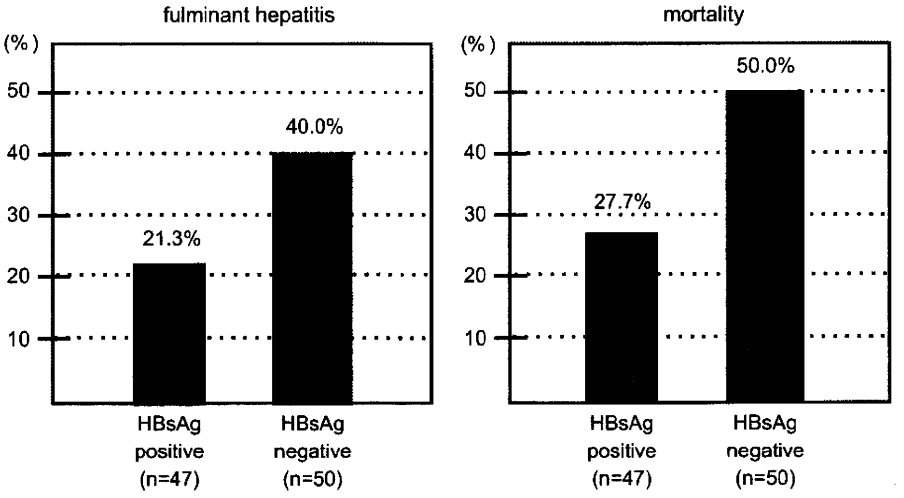

Fig. 3 Incidence of fulminant hepatitis and mortality among 111 patients who developed hepatitis B (according to ZENYAKU Company data). Dividing patients into HBsAg-positive and HBsAg-negative groups reveals that the incidence of fulminant hepatitis (20 of 50, 40%) and of mortality (25 of 50, 50%) among the latter is higher than in the former (10 of 47, 21.3% and 13 of 47, 27.7%, respectively)

Table 3 Characteristics of HBV reactivation causing hepatitis in HBsAg-negative patients

	n	Incidence	Modified incidence[a]	Data collection period	Anti-HBc	Anti-HBs	Predisposing factor	Mortality
Hui et al. [4] (Hong Kong)	8	3.3% (8 of 244)	12.2% (8 of 49)	Single institution, January 2001 to May 2005	7 of 8	4 of 8	Rituximab plus steroids	12.5% (1 of 8)
Yeo et al. [11] (Hong Kong)	5	6.25% (5 of 80)	23.8% (5 of 21)	Single institution, January 2003 to December 2006	5 of 5	0 of 5	R-CHOP	20.0% (1 of 5)
ZENYAKU Company data (Japan)	50	NA	NA	Multicenter, September 2001 to May 2008	11 of 11[b]	4 of 11[b]	Rituximab plus steroids[c] (80%, 40 of 50)	50.0% (25 of 50)

NA not available

R-CHOP rituximab, cyclophosphamide, doxorubicin, vincristine and prednisolone combination chemotherapy

Anti-HBc seropositivity for hepatitis B core antibody

Anti-HBs seropositivity for hepatitis B surface antibody

[a] The modified incidence is calculated from the number of patients with seropositivity for either HBc or HBs antibody regarded as one population in each cohort

[b] Of 50 HBsAg-negative patients, only 11 were available for assessing the seroprevalence of HBc antibody. All 11 were found to be HBc antibody-positive, of which 1 and 6 patients were HBs antibody-positive and -negative, respectively. The remaining 4 patients were not informative for HBs antibody. 11 patients were also available for assessing the seroprevalence of HBs antibody, of which 4 and 7 were positive and negative, respectively

[c] Among 50 HBsAg-negative patients with HBV reactivation, 40 (80%) received rituximab-plus-steroid-containing chemotherapy

nucleotides (nt). These are designated by capital letters from A to H in the order of their documentation [17]. They have distinct geographical distributions, i.e., genotype A is found mainly in Western countries, while genotypes B and C are prevalent in Asia and are associated with severity of liver disease as well as response to antiviral therapies [18].

According to a cross-sectional study which compared 23 patients with reactivation and 529 acute hepatitis B patients in Japan, marked differences in the distribution of HBV genotypes were seen; genotype A occurred less frequently, and genotype B more frequently among patients with HBV reactivation [19].

Another cross-sectional study from Japan found that an influence of HBV genotypes/subgenotypes was evident in a comparison of 40 patients with fulminant and 261 with acute self-limited hepatitis [20]. Remarkably, none of the 33 patients infected with subgenotype Ae developed fulminant hepatitis, whereas, in sharp contrast, 12 of the 22 (55%) patients infected with subgenotype Bj did so. Furthermore, both precore (G1896A) and core-promoter (A1762T/G1764A) mutations were detected significantly more frequently in patients with fulminant than acute self-limited hepatitis. These mutations are very frequent in patients with fulminant hepatitis in Asia [21] and the

Middle East [22]. It is very likely that the failure to detect these mutations in Western countries [23] would have been due to subgenotype Ae being frequent, but Bj is rare in the West because the precore stop-codon mutation (G1896A), accompanied by a C-to-T substitution at nt 1858 forming a base pair with it, was found mainly in HBV/Bj/B1 and not in HBV/Ae/A2. It is suggested that HBV genotypes as well as gene mutations could be associated with the onset of fulminant hepatitis caused by HBV reactivation.

8 Prevalence of HBV infection and definition of a high-risk group for HBV reactivation

Most clinical trials and case reports on HBV reactivation following systemic chemotherapy are from Asian countries such as Hong Kong, Taiwan and Japan. For accurate and extensive information on this topic, the reader is referred to the research papers of Hui et al. and Yeo et al. mentioned above. When interpreting data from other countries, it must be borne in mind that the prevalence of HBV infection varies greatly from country to country and in different areas. The frequency of HBsAg, HBc and HBs seropositivity in Hong Kong was reported to be 12, 62–76 and 58–65%, respectively (Table 4) in the literature [4, 15, 24]. According to a study of 3,874 specimens collected consecutively for screening of viral infections before blood transfusion for patients at Nagoya City University Hospital, Japan, over the two years 2005 and 2006, these values were 1.5, 20 and 22%, respectively.

For lymphoma treatment in the rituximab era, we should reconsider the level of risk for therapy-related viral reactivation in anti-HBc or anti-HBs-positive patients who are HBsAg-negative, and who were previously thought to be at low risk for HBV reactivation before the introduction of rituximab (Fig. 2). If these anti-HBc or anti-HBs-positive patients (i.e., the so-called resolved hepatitis B or past hepatitis B) are actually at high risk for HBV reactivation following systemic chemotherapy containing rituximab-plus-steroids combination for malignant lymphoma, it is mandatory to follow up this group of patients (20–23.2%) as a high-risk population. This implies that the number of patients at risk is >10-fold that of HBsAg-positive patients (1.5%), which represent the conventional high-risk group in Japan.

Next, we summarized the current evidence from clinical trials for the management of HBV reactivation after systemic chemotherapy and raised questions that should be the focus of future clinical studies.

9 Management of HBV reactivation after systemic chemotherapy

The timing of initiating antiviral treatment for hepatitis caused by HBV reactivation may be too late to result in the eradication of the virus. Yeo et al. [10] reported that of the 32 patients who received lamivudine as a therapeutic measure at the time of HBV reactivation, 5 (16%) died and 22 (69%) needed their chemotherapy modified, whereas only 5 managed to complete chemotherapy as planned. Umemura et al. reported that the rate of fulminant hepatitis and mortality following HBV reactivation is high compared to acute hepatitis B in Japan [19, 25]. Therefore, it is necessary to identify a high-risk group in advance before chemotherapy, and it is crucial to start antiviral drug treatment immediately on HBV reactivation, before hepatitis develops. Measures to prevent the onset of HBV reactivation, based on current evidence, include (1) prophylaxis with antiviral drugs, and (2) preemptive therapy starting with the detection of serum HBV-DNA.

In the following sections, we will describe the feasible strategies to prevent the onset of HBV reactivation in both HBsAg-positive and -negative patients after systemic chemotherapy.

10 Strategy to prevent HBV reactivation in HBsAg-positive patients: prophylaxis with antiviral drugs is essential

HBV reactivation after systemic chemotherapy is an "old and new" problem in clinical practice. HBsAg-positive patients have been recognized as a high-risk group for

Table 4 Prevalence of HBV infection in Hong Kong and Japan

	Hong Kong	Hong Kong	Japan (Nagoya)
HBsAg-positive	12% (78 of 626) [15]		1.5% (56 of 3,874)
Anti-HBc-positive	76% (94 of 124) [24]	62% (152 of 244) [4]	20% (764 of 3,874)
Anti-HBs-positive	65% (81 of 124) [24]	58% (142 of 244) [4]	22% (822 of 3,874)
Anti-HBc-positive and/or Anti-HBs-positive	79% (98 of 124) [24]	71% (173 of 244) [4]	23.2% (899 of 3,874)

HBsAg-positive seropositivity for hepatitis B surface antigen

Anti-HBc positive seropositivity for hepatitis B core antibody

Anti-HBs positive seropositivity for hepatitis B surface antibody

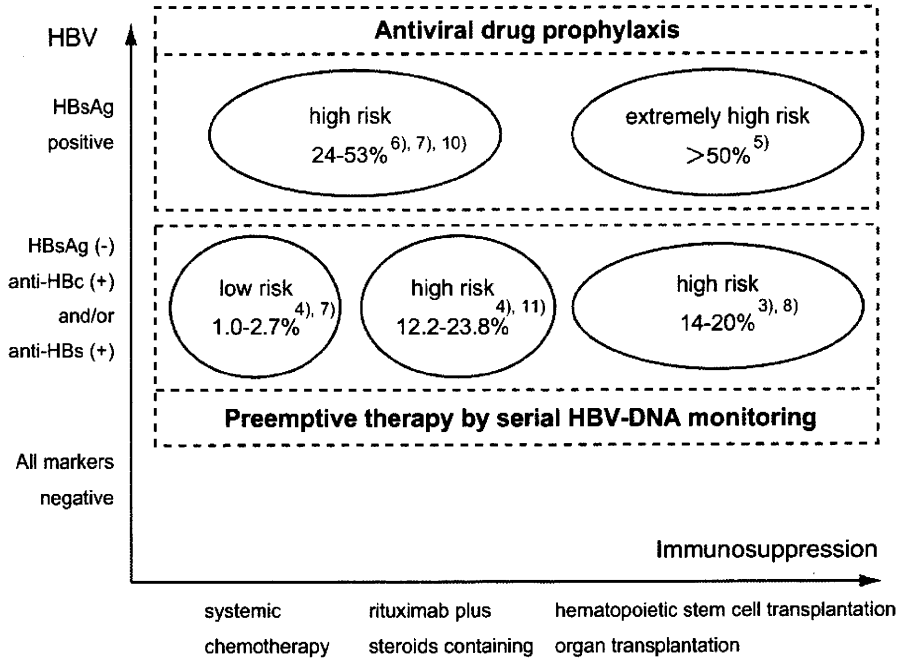

Fig. 4 Strategy based on risk classification proposed for preventing hepatitis due to HBV reactivation. Antiviral drug prophylaxis is essential for HBsAg-positive patients receiving systemic chemotherapy or hematopoietic stem cell transplantation. On the other hand, for HBsAg-negative patients seropositive for anti-HBc and/or anti-HBs on systemic chemotherapy including rituximab-plus-steroid combinations, or for patients immunosuppressed for organ transplantation, preemptive antiviral therapy starting as soon as serum HBV-DNA becomes detectable by monthly monitoring is recommended

many years. In the absence of antiviral drug prophylaxis, HBV reactivation was reported to occur in 24–53% of these patients. Some clinical trials of antiviral prophylaxis have been conducted by investigators in Hong Kong and Taiwan, which provide data on the efficacy of antiviral drug prevention of HBV reactivation in HBsAg-positive patients receiving systemic chemotherapy. Lau et al. [6] reported the results of a randomized controlled trial assigning 30 HBsAg-positive patients on systemic chemotherapy to two groups with and without lamivudine as antiviral drug prophylaxis. Treatment was from before, until 6 weeks after finishing chemotherapy. There was no case of HBV reactivation in the prophylactic group, but 53% in the other group. Yeo et al. [10] reported the results of a phase II study of 65 HBsAg-positive cancer patients on systemic chemotherapy receiving lamivudine prophylaxis (from one week before chemotherapy until 8 weeks after finishing) compared with historical controls ($n = 193$). HBV reactivation developed in 4.6% of this group compared with 24.4% of historical controls, demonstrating the efficacy of the antiviral drug as prophylaxis. In that study, despite lamivudine administration, it is notable that breakthrough hepatitis still occurred in three patients (4.6%). Recently, Loomba et al. [26] reported the results of a meta-analysis, concluding that preventive therapy with lamivudine for patients who test positive for HBsAg and are undergoing chemotherapy may reduce the risk of HBV reactivation and HBV-associated morbidity and mortality. Therefore, a consensus is emerging that prophylaxis with antiviral drugs is essential for HBsAg-positive patients undergoing systemic chemotherapy (Figs. 4, 5).

There is still insufficient data available on the optimal period for antiviral drug prophylaxis [27]. In our clinical practice, we start prophylaxis 1–2 weeks before systemic chemotherapy and aim to continue for at least 6 months after chemotherapy is finished. If we can postpone the start of lymphoma therapy, our strategy is to wait until the antiviral drug shows an effect before starting chemotherapy. Additionally, we avoid combining steroids with chemotherapy until the serum HBV-DNA becomes undetectable by RTD-PCR, because the risk of HBV replication would be high in patients positive for HBV-DNA. We might consider stopping antiviral treatment when serum HBV-DNA by RTD-PCR remains undetectable for 6 months, and serum HB core-related antigen [28], which correlates with the covalently closed circular DNA level [29], becomes undetectable or is considerably reduced. However, close monitoring of HBV-DNA and aminotransferase (ALT) is necessary after stopping prophylaxis to prevent severe ALT flare [27].

There is also little evidence on which to base a choice of which antiviral drug to use as prophylaxis against HBV reactivation, because mainly the drug lamivudine has been used in previous clinical studies. According to the 2007 guidelines for chronic hepatitis B, endorsed by the Ministry of Health, Labour and Welfare of Japan, if the patient is 35 years or older, entecavir is recommended as the first-line antiviral drug in view of its favorable efficacy and drug resistance qualities. Lamivudine resistance was reported to be 24% at 1 year for patients with chronic hepatitis B [30, 31]. Therefore, it is necessary to be aware of a high probability of acquired resistance causing flareups during

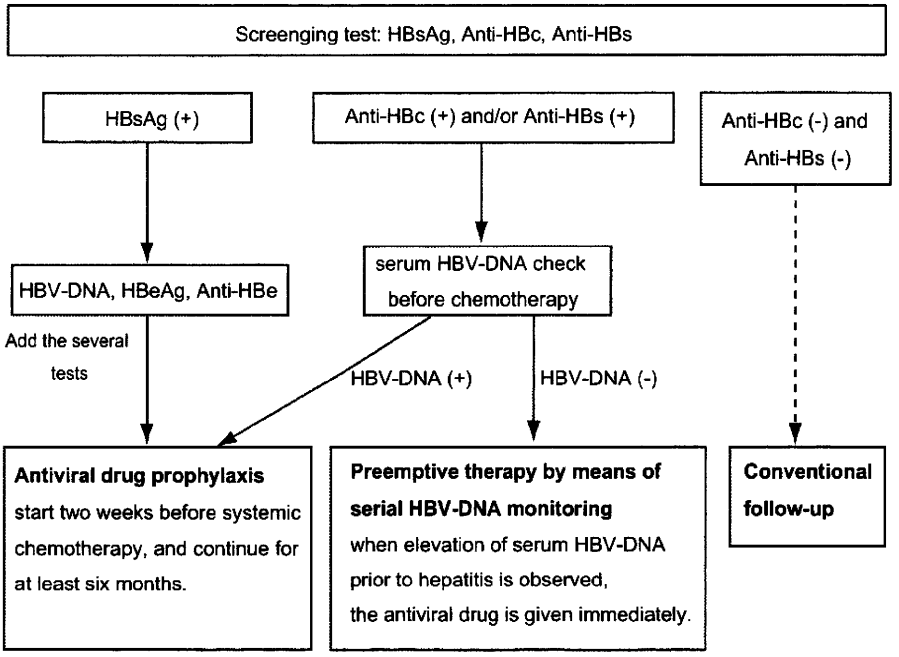

Fig. 5 Flowchart illustrating the proposed strategy based on risk classification for preventing hepatitis due to HBV reactivation. Before starting systemic chemotherapy, HBV-related markers (HBsAg, HBc antibody, HBs antibody) are screened by all available techniques. When the patient is found to be HBsAg-positive, to prevent HBV reactivation, we recommend prophylaxis with antiviral drugs starting 2 weeks before chemotherapy, and continuing for at least 6 months. If the patient is HBsAg-negative, but anti-HBc or anti-HBs-positive, we screen for serum HBV-DNA. If the patient is positive for serum HBV-DNA before systemic chemotherapy, we recommend prophylaxis with antiviral drugs as in the HBsAg-positive patients. If the patient is negative for serum HBV-DNA, but anti-HBc and/or anti-HBs seropositive, we recommend preemptive therapy, based on the results of serial HBV-DNA monitoring

prophylaxis with lamivudine. Entecavir will be used in future clinical trials for prophylaxis against HBV reactivation. However, entecavir drug resistance may also be induced by long-term treatment, and well-designed clinical trials are needed to evaluate the efficacy of the prophylaxis and the appropriate period of administration for prevention of HBV reactivation.

11 Strategy to prevent HBV reactivation in HBsAg-negative patients: well-designed clinical trials are needed to investigate the efficacy and safety of preemptive therapy by serum HBV-DNA monitoring

Clinical data for HBsAg-negative patients are extremely limited and standard management is not established to prevent HBV reactivation. Based on the previous reports reviewed above, rituximab-plus-steroid combination chemotherapy was found to be a new and important risk factor for HBV reactivation. HBsAg-negative, anti-HBc- and/or anti-HBs-positive patients, totaling 20–25% of hospitalized patients in Japan, represent a high-risk group (Table 4).

To establish an optimal strategy for hepatitis prevention and treatment in this setting, we can refer back to clinical data on the kinetics of HBV reactivation-related events in HBsAg-negative patients. Hui et al. [4] reported that the median time from the elevation of serum HBV-DNA to hepatitis onset was 18.5 weeks (range 12–28 weeks). Antiviral preemptive therapy is therefore recommended for the HBsAg-negative high-risk group starting when serum HBV-DNA becomes detectable by monthly monitoring (Figs. 4, 5). Based on the ZENYAKU Company data of 50 hepatitis B episodes developing in HBsAg-negative patients in Japan, the most delayed onset occurred 8.5 months after the end of chemotherapy. Therefore, we think that it is reasonable to assess serum HBV-DNA monthly until 1 year after the end of chemotherapy (if no additional chemotherapy or immunosuppressive therapy is required) (Fig. 4). However, clinical evidence to date is not informative for determining optimal frequency and duration of such HBV-DNA monitoring. Prospective clinical trials are therefore needed to establish the efficacy and safety of preemptive therapy by means of serial HBV-DNA monitoring.

On the other hand, although antiviral prophylaxis is one of the alternatives that may be investigated for HBsAg-negative, anti-HBc- and/or anti-HBs-positive patients, there are issues such as drug resistance and cost effectiveness, which also need addressing. It is theoretically

possible that the incidence of HBV reactivation would be decreased by applying systemic chemotherapy without steroids, but this would conceivably reduce the antitumor efficacy of the treatment. In fact, Cheng et al. [13] conducted randomized controlled trials in HBsAg-positive patients with malignant lymphoma, with and without inclusion of steroids in their combination chemotherapy. They reported that the incidence of HBV reactivation is significantly lower in patients not given steroids, but complete responses and overall survival tended to be lower than in patients on steroids.

12 Conclusion

It is necessary to revise the definition of patient groups at high risk of HBV reactivation during treatment for malignant lymphoma. Here, we have summarized current data on HBV reactivation both in HBsAg-positive and -negative patients during and after systemic chemotherapy, and proposed a strategy to prevent the onset of hepatitis due to HBV reactivation. Especially for the newly recognized high-risk group of HBsAg-negative patients, well-designed prospective clinical trials are required to investigate preemptive therapy by HBV-DNA monitoring.

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REVIEW

Reactivation of hepatitis B virus following rituximab-plus-steroid combination chemotherapy

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Abstract Reactivation of hepatitis B virus (HBV) has been reported as a fatal complication following systemic chemotherapy or other immunosuppressive therapy. The risk of HBV reactivation differs according to both the patient's HBV infection status prior to systemic chemotherapy and the degree of immunosuppression due to chemotherapy. For establishing an optimal strategy for hepatitis prevention and treatment, it is necessary to understand the characteristics, the clinical course and the risk factors for HBV reactivation and to recognize the difference between hepatitis B surface antigen (HBsAg)-positive and -negative patients with HBV reactivation. Among the important viral risk factors, HBV-DNA level and HBV-related serum markers have been reported to be associated with HBV reactivation in addition to cccDNA, genotypes and gene mutations. Rituximab-plus-steroid combination chemotherapy has recently been identified as a host risk factor for HBV reactivation in hepatitis B core antibody (anti-HBc)-positive and/or hepatitis B surface antibody (anti-HBs) positive—but nonetheless HBsAg-negative—lymphoma patients. For these patients with resolved hepatitis B, preemptive therapy guided by serial HBV-DNA monitoring is a reasonable strategy to enable early diagnosis of HBV reactivation and initiation of antiviral therapy. In this review, we summarize the characteristics of HBV reactivation following rituximab-plus-steroid combination chemotherapy, mainly in HBsAg-negative lymphoma patients, and propose a strategy for managing HBV reactivation.

Keywords Reactivation · HBV · Rituximab

Abbreviations

Anti-HBc	Hepatitis B core antibody
Anti-HBs	Hepatitis B surface antibody
cccDNA	Covalently closed circular DNA
CHOP	Cyclophosphamide, doxorubicin, vincristine, prednisolone
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HSCT	Hematopoietic stem cell transplantation
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone
RTD-PCR	Real-time detection PCR

Introduction

Reactivation of hepatitis B virus (HBV) has been reported not only in hepatitis B surface antigen (HBsAg)-positive patients following systemic chemotherapy, but also in a proportion of HBsAg-negative patients with anti-hepatitis B core antibody (anti-HBc) positivity and/or anti-hepatitis

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B surface antibody (anti-HBs) positivity [1–4]. Rituximab-plus-steroid combination chemotherapy has recently been identified as a risk factor for HBV reactivation in HBsAg-negative patients with malignant lymphoma [2, 3].

Rituximab is a chimeric human–mouse monoclonal antibody that targets the CD20 molecule [5]. Approximately 70–80% of malignant lymphomas are of B cell origin, and >90% of B cell lymphomas express CD20 on the cell surface. CD20 is an appropriate target molecule because it is not shed, modulated or internalized. The introduction of rituximab into the therapeutic regimen has dramatically improved the prognosis of CD20-positive lymphoma patients. The rituximab + cyclophosphamide, doxorubicin, vincristine, prednisolone (CHOP) combination chemotherapy, known as R-CHOP, is now the gold standard treatment for diffuse large B cell lymphoma worldwide [6]. The effectiveness of rituximab has recently been reported in refractory autoimmune diseases, including rheumatoid arthritis [7], thrombotic thrombocytopenic purpura [8] or idiopathic thrombocytopenic purpura [9], among others.

Many clinical trials have demonstrated the efficacy and safety of rituximab, as used in large numbers of patients, but it has also been recognized that attention must be paid to accompanying potential adverse events, which include late-onset neutropenia [10], progressive multifocal leukoencephalopathy [11] and HBV reactivation.

In this review, we summarize the characteristics of HBV reactivation following rituximab-plus-steroid combination chemotherapy, mainly in HBsAg-negative lymphoma patients, and propose a strategy for managing HBV reactivation.

Pathophysiology of HBV reactivation following systemic chemotherapy

In most immunocompetent hosts, HBV infection manifests as acute hepatitis [12]. Thereafter, HBsAg levels decrease to below the level of detection over some weeks as hepatitis B surface antibody (anti-HBs) titers increase. Most cases of acute hepatitis B completely resolve in adult patients. An individual is defined as having had a resolved HBV infection when he/she tests seropositive for anti-HBc and/or anti-HBs.

Because HBV replication mainly persists in the liver, even in patients with anti-HBs, for several years from the onset of acute hepatitis B [13], all individuals with a history of exposure to HBV are at risk of reactivation following systemic chemotherapy. Re-initiated HBV replication and increasing levels of serum HBV-DNA have been reported following systemic chemotherapy, especially those involving rituximab-containing regimens, even in patients with resolved HBV infections [1]. Under conditions of immunosuppression, HBV is more likely to increase rapidly and to infect many hepatocytes. With immune recovery after chemotherapy, immunocompetent cells attack the infected hepatocytes, causing recurrence of hepatitis B disease.

Risk factors associated with HBV reactivation: viral and host factors

As with chronic hepatitis B, the risk of HBV reactivation depends on the balance between replication of the virus and the immune response of the host. Thus, the risk of HBV reactivation differs according to the patient's HBV infection status prior to systemic chemotherapy as well as to the degree of immunosuppression due to chemotherapy.

Important viral factors, such as HBV-DNA viral load and HBV-related serum markers [presence or absence of HBsAg, hepatitis B e antigen (HBeAg), anti-HBc and anti-HBs] have been reported to be associated with HBV reactivation following anticancer therapy [3, 14–16] (Table 1). More recently, genotypes and mutations of HBV have been reported to be associated with reactivation [17, 18].

Important host factors associated with immunosuppression are steroid combination chemotherapy [19],

Table 1 Risk factors associated with HBV reactivation following systemic chemotherapy

Viral factors	Host factors
HBsAg	Combination therapy with steroid
HBeAg	Rituximab-plus-steroid combination therapy
Anti-HBc	Malignant lymphoma
Anti-HBs	Male gender
HBV-DNA levels	Absence of anti-HBs before chemotherapy
Covalently closed circular DNA	Decrease of anti-HBs titers during chemotherapy (in patients seropositive for anti-HBs before chemotherapy)
Occult HBV infection	
Genotype non-A (especially, genotype B)	
Gene mutation of precore and/or core promoter	

HBV Hepatitis B virus, *HBsAg* hepatitis B surface antigen, *HBeAg* hepatitis B e antigen, *anti-HBc* anti-hepatitis B core antibody, *anti-HBs* anti-hepatitis B surface antibody

rituximab-plus-steroid combination chemotherapy [2, 3], hematopoietic stem cell transplantation [20–23] (HSCT, allogeneic or autologous), as also shown in Table 1.

Viral risk factors: cccDNA, occult HBV infection, genotypes and gene mutations

Hepatitis B virus is known to be present in infected people as covalently closed circular DNA (cccDNA) located in hepatocyte nuclei and providing a stable template for replication [12]. It is this configuration that is believed to make HBV eradication so difficult. Therefore, the presence of cccDNA in the liver is considered to be an important risk factor in patients with HBV reactivation following systemic chemotherapy. The serum level of HBV core-related antigen (HBcrAg) has recently been reported to be correlated with the amount of cccDNA in the liver [24]. Consequently, quantification of this antigen would be expected to represent a predictive marker for HBV reactivation [25].

An HBsAg-negative individual is defined as having an occult HBV infection when HBV-DNA is nonetheless detectable in the blood using the real-time detection PCR (RTD-PCR) assay. Patients with occult HBV infection are thought to be at high risk for HBV reactivation, in the same way as HBsAg-positive patients [2, 26]. As a person with occult HBV infection is still anti-HBc-positive and/or anti-HBs-positive, the identification of such an occult HBV infection is possible by measuring HBV-DNA at screening when either marker is positive.

Ten genotypes have been detected with a sequence divergence of >8% in the entire HBV genome of about 3215 nucleotides (nt). These are designated by capital letters from A to J in the order of their documentation [27]. They have distinct geographical distributions, i.e., genotypes A and D are found mainly in Western countries, while genotypes B and C are prevalent in Asia, and they are associated with the severity of liver disease as well as response to antiviral therapy [28, 29]. Most recently, different genotypes were reported to be associated with different likelihoods of HBV reactivation [17, 18]. Thus, the non-A genotype, especially the B genotype, might represent an important risk factor in the setting of systemic chemotherapy.

Gene mutations in precore and core-promoter regions are very frequent in patients with fulminant hepatitis in Asia and the Middle East [30, 31]. These mutations, prevalent in genotype B and C viruses in Asia, might enhance viral replication, and thereby induce stronger immune responses, resulting in the development of fulminant hepatitis B. Most recently, these mutations have also been reported to be associated with the reactivation of HBV [18].

Host risk factors: loss of anti-HBs, use of rituximab, immune reconstitution

The anti-CD20 monoclonal antibody rituximab induces transient mature peripheral B cell depletion. In a multi-institutional phase II trial for patients with relapsed or refractory B cell lymphoma, rituximab was shown to almost completely deplete normal mature B cells for an average period of 6–9 months [5]. Recovery to normal levels may take 9–12 months. Because CD20 is not expressed by the antibody-forming plasma cells, hypogammaglobulinemia is rare, occurring in only 14% of patients receiving rituximab in clinical trials. Several large-scale randomized trials were subsequently conducted to assess the safety and efficacy of rituximab combination chemotherapy [6, 32]. In these trials, the addition of rituximab was not found to significantly increase infectious events. However, long-term treatment with rituximab, or HSCT together with rituximab, may result in the increased occurrence of hypogammaglobulinemia [33, 34] and may affect the clinical course, especially in terms of increased infectious events.

Decreased titers of anti-HBs have been reported to be closely associated with HBV reactivation [1, 35]. Reduced amounts of antibody are likely to contribute to reactivation in patients with resolved HBV infection. In a retrospective study on HBV reactivation in 80 HBsAg-negative lymphoma patients receiving R-CHOP-like or CHOP-like regimens, three important risk factors were identified, namely, male gender, absence of anti-HBs at diagnosis of lymphoma and the use of rituximab [3]. Multivariate analysis revealed that rituximab-plus-steroid combination chemotherapy was an important risk factor for HBV reactivation in 244 HBsAg-negative lymphoma patients who required chemotherapy [2].

HBV reactivation may also be associated with the immunological changes following either autologous or allogeneic HSCT [20–23, 36, 37]. The occurrence of acute or chronic graft versus host disease that requires the use of immunosuppressive drugs, such as steroids, cyclosporine or tacrolimus for long-term treatment, as well as delayed immune reconstitution may result in late onset HBV reactivation—especially in the allogeneic setting. In fact, some instances of HBV reactivation have been reported several years after allogeneic HSCT [37, 38]. It is therefore necessary to remain on the alert for such a late onset pattern.

Diagnosis and definition of HBV reactivation

The measurement of HBV-DNA and related serum markers, such as HBsAg, is essential for the diagnosis of HBV

reactivation. It remains important to rule out any other clinical conditions which may cause hepatitis.

As yet, there is no consensus on the definition of HBV reactivation. Among HBsAg-positive patients, it has been suggested that HBV reactivation should be defined as a one log or greater increase of HBV-DNA viral load compared to baseline, in addition to liver damage [39]. On the other hand, among HBsAg-negative patients, the definition of HBV reactivation has often been reported as the reappearance of HBsAg or the de novo detection of HBV-DNA in the blood [2, 3].

The clinician is advised to take the following precautions when considering a diagnosis of HBV reactivation:

1. A recent history of receiving a blood transfusion; consider a differential diagnosis of transfusion-transmitted hepatitis B.
2. Antibody titer may be decreased by immunosuppressive therapy or systemic chemotherapy [1, 35]; screen for HBV-related serum markers, such as anti-HBc and anti-HBs before initiating treatment.
3. Clinical course and prognosis of HBV reactivation are very different from acute hepatitis [40]; immediate initiation of antiviral therapy is extremely important for hepatitis due to HBV reactivation.

HBV reactivation in HBsAg-positive patients

Most cases of HBV reactivation associated with chemotherapy occur in HBsAg-positive patients, who are considered to be at high risk for HBV reactivation [41]. In particular, the use of steroids should be avoided in HBsAg-positive patients with lymphoma who are under chemotherapy and in those receiving HSCT. Prophylaxis with antiviral drugs for preventing HBV reactivation is important in these patients.

Before the rituximab era, 24–53% of HBsAg-positive patients on cancer chemotherapy experienced HBV reactivation. Accordingly, Yeo et al. [42] reported that HBV reactivation occurred in 47 of 193 (24%) of HBsAg-positive lymphoma patients who received systemic chemotherapy. Lok et al. [41] reported reactivation in 13 of 27 (48%) HBsAg-positive patients with malignant lymphoma following systemic chemotherapy. Lau et al. [43] conducted a randomized trial in 30 HBsAg-positive lymphoma patients either receiving or not receiving anti-viral drug prophylaxis during systemic chemotherapy. They found no reactivation in patients receiving prophylaxis, but eight of 15 (53%) patients without prophylaxis had HBV reactivation. More recently, Pei et al. [44] reported an extremely high incidence of HBV reactivation in HBsAg-positive lymphoma patients receiving rituximab-containing chemotherapy. Without prophylaxis in this setting, HBV reactivation occurred in eight of ten (80%) patients. The impact of rituximab on HBV reactivation in HBsAg-positive patients has not yet been studied. It remains necessary to collect clinical data on HBV reactivation in order to develop approaches to prevent it from occurring.

HBV reactivation in HBsAg-negative patients

Until recently, HBsAg-negative patients (including those with occult hepatitis B and resolved hepatitis B) were not recognized as being at risk for HBV reactivation when receiving conventional chemotherapy. This view is supported by the results of Lok et al. [41], who reported HBV reactivation in only 2.7% (2 of 72) of HBsAg-negative patients, which was far lower than the 48% of HBsAg-positive patients showing reactivation.

However, HBV reactivation has been sporadically reported in HBsAg-negative patients receiving rituximab-containing chemotherapy. The first case was reported by Devite et al. [1] in 2001. In 2006, Hui et al. [2] reported that of 244 HBsAg-negative lymphoma patients receiving systemic chemotherapy, eight (3.3%) developed HBV reactivation and all eight were either anti-HBc-positive and/or anti-HBs-positive. Moreover, the incidence of HBV reactivation in the rituximab-plus-steroid combination group was higher, namely, 12.2% (6/49 patients) than that in other combination therapy groups, in which it was only 1.0% (2/195 patients). Multivariate analysis demonstrated that rituximab-plus-steroid combination chemotherapy was a risk factor for HBV reactivation in HBsAg-negative patients. In that cohort, additional studies on archival samples showed a rising HBV-DNA viral load prior to hepatitis in all cases of reactivation, occurring at a median of 18.5 weeks (range 12–28) prior to overt hepatitis development. In 2009, Yeo et al. [3] also reported an HBV reactivation study in 80 HBsAg-negative patients with diffuse large B cell lymphoma receiving standard systemic chemotherapies, such as R-CHOP or CHOP-like regimens. HBV reactivation occurred in five (6.25%) of these patients, with four receiving antivirals and one patient dying of hepatitis. All 5 patients were anti-HBc-positive and anti-HBs-negative and had received R-CHOP. Thus, of 21 anti-HBc-positive patients receiving R-CHOP, five (23.8%) showed HBV reactivation. Therefore, not only HBsAg-positive patients, but also some HBsAg-negative patients, including anti-HBc-positive and/or anti-HBs-positive and/or HBV-DNA-positive patients, should be considered at high risk for HBV reactivation following rituximab-plus-steroid combination chemotherapy.

According to data collected by the Zenyaku Company of Japan, 111 Japanese patients developed serious hepatitis B