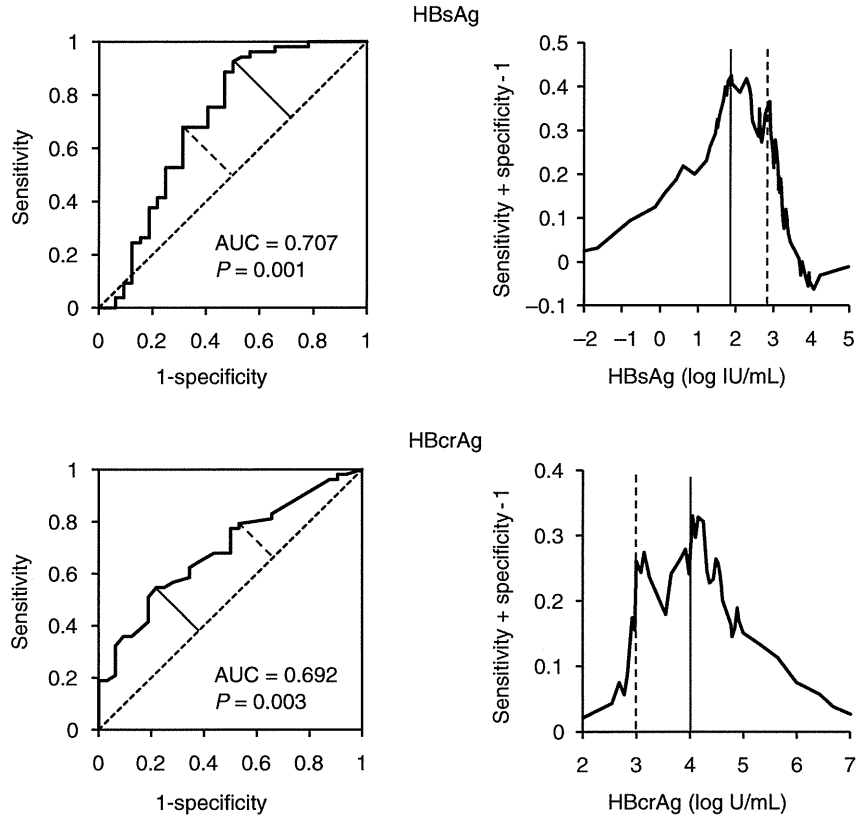

Figure 5 Receiver operating characteristic curve (ROC) analysis of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) to discriminate between patients with and without hepatitis relapse. The existence of two inflection points is suggested for both HBsAg and HBcrAg. Short diagonal lines indicate main inflection points and short broken diagonal lines indicate second inflection points. Vertical lines indicate actual values of antigens that correspond to the main inflection points and vertical broken lines indicate actual values of antigens that correspond to the second inflection points.

patients with and without relapse (Fig. 5). Thus, we set cut-off values as 1.9 and 2.9 log IU/mL for HBsAg and 3.0 and 4.0 log U/mL for HBcrAg in our model for predicting hepatitis relapse.

We tentatively defined three groups using the sum of the scores for HBsAg and HBcrAg levels at the time of NA discontinuation for our model. Conversions were made by assigning a score of 0 for an HBsAg level lower than 1.9 log IU/mL, 1 for a level from 1.9 to 2.8 log IU/mL, and 2 for a level equal to or higher than 2.9 log IU/mL. HBcrAg was scored as 0 for a level lower than 3.0 log U/mL, 1 for a level from 3.0 to 3.9 log U/mL, and 2 for a level equal to or higher than 4.0 log U/mL. Overall, group 1 consisted of patients with a total score of 0, group 2 of patients with a total score of 1 or 2, and group 3 of patients with a total score of 3 or 4.

Patients whose HBV DNA was lower than 3.0 log copies/mL and in whom HBeAg was negative at the time of NA discontinuation were assigned to one of the three groups. Figure 6 shows the comparison of non-relapse rates among the three groups using Kaplan–Meier analysis, which differed significantly. The non-relapse rate was approximately 90% in group 1, as low as 10% in group 3, and intermediate in group 2. When factors associated with relapse were analyzed in group 3 patients, an age of over 40 years at the time of discontinuation was calculated as a significant factor (hazard

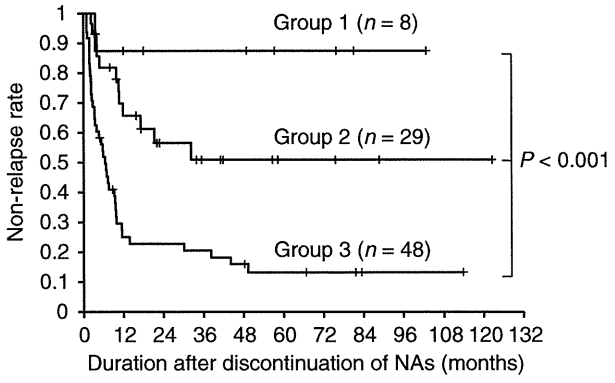

Figure 6 Comparison of non-relapse rates using the Kaplan–Meier method among three groups classified by the sum of the scores of hepatitis B surface antigen (HBsAg) and hepatitis B core-related antigen (HBcrAg) levels at the time of nucleo-s(t)ide analog (NA) discontinuation.

ratio = 5.25, range 2.37–11.65, $P < 0.001$). No significant factors were associated with relapse in group 2 patients.

DISCUSSION

THE EUROPEAN ASSOCIATION for the Study of the Liver recommends continuation of NA treatment until HBsAg is cleared.[25] Liu *et al.* came to a similar conclusion in their study of chronic hepatitis B patients treated with LVD.[14] Indeed, the clearance of HBsAg is a reliable marker for the safe discontinuation of NAs, but the rate of patients who can clear HBsAg is relatively low (1–3%/year).[26–28] Thus, additional factors associated with relapse of hepatitis B after discontinuation of NAs were analyzed in the present study to better identify candidates who could achieve drug-free status. Such studies are relatively few, possibly because patients who discontinue NAs prematurely often experience severe complicating relapse and hepatic failure.[9] Although prospective studies are desirable to obtain accurate results, retrospective studies, such as ours, are also necessary to minimize the risk of adverse complications.

Since HBV cannot be completely eradicated in hosts, the primary goal in treating chronic hepatitis B is to convert symptomatic patients into inactive carriers in whom HBeAg is negative (usually anti-HBe-positive), serum HBV DNA is low, and serum ALT is normal.[1,2,18,29] Thus, we set the clinical conditions of a successful discontinuation of NAs as serum HBV DNA level below 4.0 log copies/mL and ALT below 30 IU/L following NA cessation. Patients who satisfy these conditions are not recommended for treatment by the Japanese guidelines for hepatitis B,[18] and it is also widely accepted that the risk of developing cirrhosis or complicating hepatocellular carcinoma is very low in such patients.[30,31] We used our cohort's mean and maximal values of HBV DNA and ALT for relapse analyses. Mean values were useful for evaluating relapse of hepatitis as a whole since parameter levels often fluctuated after discontinuation, and maximal values were used to evaluate relapse in a real-time fashion during the follow-up period. It is noteworthy that the mean and maximal values correlated very closely for both HBV DNA and ALT. The mean HBV DNA value of 4.0 log copies/mL corresponded to the maximal HBV DNA value of 5.7 by ROC analysis, and similarly the mean ALT value of 30 IU/L corresponded to the maximal ALT value of 79 IU/L. Thus, relapse of hepatitis B was judged to occur when serum ALT became higher than 79 IU/L or when serum HBV DNA surpassed 5.7 log copies/mL after the time of NA discontinuation. Such criteria may also be useful for physicians to detect relapse at an early phase and avoid the occurrence of severe reactivation or unnecessary discontinuation of NAs.

It is generally understood that patients with a higher level of HBV DNA at the time of NA discontinuation are likely to relapse, but this cut-off value has not been analyzed sufficiently. Our findings using ROC analysis showed that patients with levels lower than 3.0 log copies/mL have a good possibility to achieve successful discontinuation. The presence of HBeAg is also generally accepted as a reliable factor to predict relapse of hepatitis. Our study showed that patients with detectable HBeAg at the time of NA discontinuation were likely to relapse, even if their HBV DNA levels were lower than 3.0 log copies/mL. Therefore, we next analyzed additional factors associated with a relapse of hepatitis after discontinuation of NAs by selecting patients who met both of these criteria.

Nucleos(t)ide analog treatment produces a rapid decrease in serum HBV DNA by suppressing reverse transcription of pregenomic HBV RNA. However, the key intrahepatic HBV replicative intermediate, covalently closed circular DNA (cccDNA), tends to remain and is capable of reinitiating replication once NAs are ceased.[32] Measurement of HBV cccDNA has been reported to be useful for monitoring and predicting responses to antiviral treatments.[33] However, its measurement is difficult in the clinical setting as it requires a liver biopsy. Due to the mechanism of action of NAs mentioned above, serum HBV DNA does not reflect intrahepatic HBV cccDNA in patients undergoing NA treatment.[34] To address this, quantitative measurement of HBV antigens has been reported to be useful for predicting the effect of antiviral treatment in patients with chronic hepatitis B. Although HBsAg is usually used as a serum marker for the diagnosis of HBV infection, several groups have shown that HBsAg levels can also be reflective of the response to peg-interferon in chronic hepatitis B.[28,35,36] The HBcrAg assay measures serum levels of HB core and e antigens simultaneously using monoclonal antibodies that recognize the common epitopes of these two denatured antigens. Since the assay measures all antigens transcribed from the pre-core/core gene, it is regarded as core-related.[37] Serum HBcrAg has been reported to accurately reflect intracellular levels of HBV cccDNA even during NA treatment,[24,34,38] and was found to be useful for identifying patients who were likely to show relapse of hepatitis after the discontinuation of NAs.[39,40] It is possible that levels of HBsAg and HBcrAg have different roles in

monitoring antiviral effects because the transcription of these two antigens are regulated by alternative enhancer-promoter systems in the HBV genome.[3] Therefore, we analyzed both of these antigens to elucidate their ability to predict relapse of hepatitis after discontinuation of NAs.

Multivariate analysis demonstrated that levels of HBsAg and HBcrAg at the time of NA discontinuation were independent factors significantly associated with relapse of hepatitis. Thus, we believe these factors can also be applied for predicting relapse in patients whose HBV DNA is lower than 3.0 log copies/mL and whose HBeAg is negative at NA discontinuation. HBV DNA levels were further analyzed using a highly sensitive assay based on real-time polymerase chain reaction (PCR). However, even the level of a negative signal did not ensure successful discontinuation of NAs. The results obtained here indicate that the combined use of HBV-related antigens are useful makers for monitoring the effect of anti-viral treatment in ways different from HBV DNA. Finally, since prolonged NA administration was also a significant factor associated with safe discontinuation, physicians are advised to continue patient treatment for at least 16 months for the best possible outcome.

From our data, a tentative model for predicting relapse of hepatitis after discontinuation of NAs was constructed using levels of HBsAg and HBcrAg at discontinuation. A negative result for HBeAg and HBV DNA lower than 3.0 log copies/mL at the time of NA discontinuation are the essential conditions in this system. Levels of HBsAg and HBcrAg were each converted into scores from 0 to 2 partly because two cut-off values were needed for each antigen and partly because a scoring system may be more convenient for clinical use. The sum of the two scores, which ranged from 0 to 4, was used to prospect relapse. We found that group 1 patients who had a low score (0) could be recommended to discontinue NAs because nearly 90% of this group achieved successful discontinuation. Further analysis of factors associated with relapse are needed for group 2 patients who had middle range scores (1 or 2), since the odds of achieving successful discontinuation were approximately 50%. Continuation of NA treatment is recommended for group 3 patients having high scores (3 or 4) because nearly 90% of this group relapsed. However, this recommendation may be reconsidered in patients younger than 40 years; such cases tended to have a lower relapse rate in group 3. It is also noteworthy that relapse occurred mainly during the first and second years following NA discontinuation in all groups, similarly to a report by Liu *et al.*[14] Thus, clinicians should be vigilant in the early phase after discontinuation.

This study has several limitations. The patients who discontinued NAs were recruited retrospectively, and thus the decision to halt NA treatment was made by individual physicians without uniformly established criteria. Based on this, prospective studies are required to confirm our results. Furthermore, as over 90% of the patients we enrolled had genotype C and over 90% of cases were treated with LVD until discontinuation, the results obtained here can not be applied directly to other HBV genotypes or other types of NAs.

In conclusion, the present study showed that maximal levels of serum ALT and HBV DNA were useful for defining relapse patients after discontinuation of NAs. Along with serum HBV DNA of less than 3.0 log copies/mL and negative serum HBeAg, serum levels of HBsAg and HBcrAg at the time of NA discontinuation were able to predict relapse of hepatitis B and should therefore be considered when establishing uniform guidelines regarding the safe withdrawal of NA treatment. To this end, NA administration of more than 16 months is advisable to achieve successful discontinuation.

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Special Report

Prevention of hepatitis B virus reactivation in patients receiving immunosuppressive therapy or chemotherapy

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With the increasing use of potent immunosuppressive therapy, reactivation of hepatitis B virus (HBV) in endemic regions is becoming a clinical problem requiring special attention. A recent annual nationwide survey clarified that HBV reactivation related to immunosuppressive therapy has been increasing in patients with malignant lymphoma, other hematological malignancies, oncological or rheumatological disease. In the survey, rituximab plus steroid-containing chemotherapy was identified as a risk factor for HBV reactivation in hepatitis B surface antigen (HBsAg) negative patients with malignant lymphoma. In this setting, HBV reactivation resulted in fatal fulminant hepatitis regardless of the treatment of nucleoside analog. The Intractable Hepatobiliary Disease Study Group and the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis jointly developed guidelines for preventing HBV reactivation. The essential features of the guideline are as follows. All patients should be screened for HBsAg by a sensitive method before the start of immunosuppressive therapy. Second, hepatitis B core antigen (HBcAb) and hepatitis B surface antibody (HBsAb) testing should be performed in HBsAg negative patients, especially those receiving intensive immunosuppressive therapy. Prophylaxis with nucleoside analogs is essential for preventing HBV reactivation in HBsAg positive patients. In contrast, HBsAg negative with HBcAb and/or HBsAb positive patients should be monitored monthly for an increase in serum HBV DNA during and 12 months after completion of chemotherapy. Nucleoside analogs should be administrated immediately when HBV DNA becomes positive during this period. This strategy facilitates commencement of nucleoside analogs at an early stage of HBV reactivation and results in prevention of severe hepatitis.

Key words: de novo hepatitis, fulminant hepatitis, hepatitis B virus reactivation, immunosuppressive therapy, rituximab

INTRODUCTION

HEPATITIS B VIRUS (HBV) is the most frequently identified agent that causes acute or chronic hepatitis in Eastern Asia. In Japan, approximately 970 000 people are infected with HBV, as estimated by hepatitis B surface antigen (HBsAg) testing in blood donors.[1] Chronic HBV carriers have a 15–40% lifetime risk of developing serious complications of chronic liver disease.[2] However, most carriers remain clinically silent for extended periods and some carriers will lose HBsAg over a long lifetime. In adults, most acute HBV infections are self-limited, and recovery occurs naturally. Seroconversion from acute HBV infection with HBsAg to antibody to hepatitis B surface antibody (HBsAb) is believed to represent viral clearance. Clearance of HBsAg and appearance of antibody to hepatitis B core antibody (HBcAb) with or without HBsAb provides evidence of resolved infection in patients. However, with the advent of sensitive polymerase chain reaction techniques for detecting HBV DNA in serum and liver, it has been shown that most HBsAb/HBcAb positive patients have HBV DNA in the liver and/or serum. It is estimated that 2 billion people worldwide have been infected with HBV.[3] In Japan, it is reported that 23.2% of blood donors are positive for HBcAb and/or HBsAb.[4]

Reactivation of HBV is a well-recognized complication in HBsAg positive patients who are undergoing immunosuppressive chemotherapy for cancer. The clinical manifestation ranges from subclinical hepatitis to severe, potentially fatal fulminant hepatitis. In this decade, HBV reactivation has been observed in patients

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with resolved infection (HBsAg negative and HBcAb and/or HBsAb positive) who have undergone intensive immunosuppressive chemotherapy such as rituximab plus steroid-containing chemotherapy. Previous reports have clarified that this combination therapy can lead to fulminant hepatitis and even death. For this reason, clinicians need to be aware of HBV reactivation not only in patients with current infection but also in those with resolved infection who are undergoing intensive immunosuppressive therapy.

This report summarizes the important issues related to HBV reactivation and suggests a guideline for preventing this condition in the clinical setting.

HBV REACTIVATION IN HBsAg POSITIVE CARRIERS

LIFE IN HBV carriers can generally be divided into four distinct phases: (i) immune tolerance phase; (ii) immune active phase; (iii) low-replication phase; and (iv) resolved phase. Inactive carriers in the low-replication phase are frequently associated with antibodies to hepatitis B e antigen (anti-HBe) seroconversion with a low viral load (<4.0 log copies/mL).[5] Sustained host immune control over viral replication in the low-replication phase may lead to HBsAg seroclearance. During the low-replication phase, 20–30% of patients may develop spontaneous HBV reactivation.[6] Patients with chronic HBV infection receiving immunosuppressive chemotherapy usually have impaired host immunity that may allow active HBV replication to occur. Following the completion of therapy, restoration of host immunity against HBV occurs, resulting in extensive cytotoxic T-cell-mediated lysis of the infected hepatocytes and clinical hepatitis flares. Some patients experience severe hepatitis with HBV reactivation, with fatality rates ranging 5–40%.[7–10]

The risk of HBV reactivation is mainly related to the underlying disease, intent of the immunosuppression and HBV replicative state. The risk is particularly high in patients with lymphoma.[11,12] Patients with other hematological malignancy such as multiple myeloma and B-cell chronic lymphocytic leukemia are also at risk.[13] Patients receiving intensive cytoreductive therapy and high-dose chemotherapy are highly susceptible to HBV reactivation. In non-hematological tumors, the rate of HBV reactivation is high in patients with breast cancer.[14,15] HBV reactivation can also occur in patients with non-malignant disease, such as rheumatological disease and collagen disease.[13]

The use of chemotherapy regimens containing corticosteroids or anthracycline increases the risk of HBV reactivation.[7,11,12,16,17] HBV DNA contains a glucocorticoid responsive element that has been suggested to facilitate HBV replication.[18,19] Anthracycline has also been demonstrated *in vitro* to stimulate HBV DNA replication.[20] Recently, the use of the anti-CD20 monoclonal antibody, rituximab, appears to be an independent risk factor of HBV reactivation.[21] This agent causes profound and long-lasting immunosuppression, reflecting a decrease in CD20 cells and HBsAb titer.[22] There are many reports of HBV reactivation following the use of rituximab as monotherapy[23] or in combination with other types of chemotherapy.[24–27]

REACTIVATION IN HBsAg NEGATIVE PATIENTS WITH HBcAb AND/OR HBsAb

IN PATIENTS WITH resolved HBV infection (HBsAg negative, HBsAb and/or HBcAb positive), HBV replication has been shown to persist in the liver and in peripheral blood mononuclear cells.[28,29] Cellular and humoral immune surveillance suppresses viral replication.[30,31] In the life cycle of HBV replication, covalently closed circular DNA (cccDNA) is formed in the nuclei of infected hepatocytes. cccDNA is the main template for the transcription of viral mRNA and has been shown to persist in the liver.[21] With impairment of the host defense system, cccDNA can evade host immunity and actively replicate again. This scenario of HBV reactivation in patients with resolved infection of so-called "de novo hepatitis B" appears to be a new clinical issue.

Hepatitis B virus reactivation has been reported in this setting after transplantation and allogenic and autologous hematopoietic stem-cell transplantation with the reappearance of HBsAg.[32–34] In recent years, the incorporation of rituximab with standard chemotherapy is associated with HBV reactivation in patients with non-Hodgkin's lymphoid malignancies. Hui *et al.* reported that the incidence of HBV reactivation with a combination of rituximab plus steroids was higher (12.2%, 6/49) compared with other combinations of therapy (1.0%, 2/195).[35] Recently, Yeo *et al.* reported that 23.8% of HBsAg negative/HBcAb positive lymphoma patients receiving rituximab plus steroid combination therapy developed HBV reactivation.[27] It is notable that there are a number of case reports of fatal hepatitis in HBcAb positive patients who received rituximab-containing chemotherapy for lymphoma.[24–27,36] Umemura *et al.* reported that the rate of fulminant hepatitis and

Table 1 Causes of HBV-related fulminant hepatitis and late-onset hepatic failure (LOHF)

	Total	Years					
		2004	2005	2006	2007	2008	2009
All patients	194 (9)	26 (2)	42 (2)	27	37 (1)	23 (2)	39 (2)
Transient infection	91 (1)	12 (1)	23	13	17	11	15
Carrier	72 (7)	9 (1)	11 (1)	9	14 (1)	11 (2)	18 (2)
Inactive carrier	35 (1)	6	7	3	5	3	11 (1)
Reactivation (inactive carrier)	20 (5)	2	3 (1)	1	4 (1)	5 (2)	5 (1)
Reactivation (resolved infection)	17 (1)	1 (1)	1	5	5	3	2
Undetermined	31 (1)	5	8 (1)	5	6	1	6

Data shown indicate the number of patients, and those in parentheses indicate the number of patients with LOHF.

mortality following de novo hepatitis B is high compared with acute hepatitis B in Japan.[37]

FULMINANT HEPATITIS CAUSED BY HBV REACTIVATION IN JAPAN

THE INTRACTABLE HEPATOBILIARY Diseases Study Group in Japan annually performs a nationwide survey of patients with fulminant hepatitis and late-onset hepatic failure (LOHF).[38] A recent annual nationwide survey from 2004 to 2009 revealed that HBV infection prevailed in 39.8% (194/488) of patients with fulminant hepatitis and LOHF. It is noteworthy that 19.1% (37/194) of HBV related-hepatitis was caused by HBV reactivation following immunosuppressive therapy or chemotherapy (Table 1). Furthermore, almost half of these patients have evidence of HBV reactivation from resolved infection (HBsAg negative before the start of therapy and HBsAg positive and HBcAb and/or HBsAb positive at the onset of hepatitis). The total number of patients with HBV reactivation has been increasing since 2004. We first compared the clinical features of 37 patients with HBV reactivation with those of transient infection and those with acute exacerbation (Table 2). The age of the patients was higher in the HBV reactivation group than that in the transient infection

Table 2 Clinical characteristics of patients with hepatitis B virus (HBV) reactivation, compared with those of patients with transient infection and HBV carriers who developed spontaneous acute exacerbation

	Transient infection ($n = 91$)	Acute exacerbation in HBV carriers ($n = 35$)	HBV reactivation ($n = 37$)
Age, years, median (range)	46 (17–72)	53 (15–89)	64 (29–86)**††
Male/female	58/33	23/12	22/15
Disease types (F-A/F-SA/LOHF)	80/10/1	14/20/1**	4/27/6**††
Prognosis (alive/died/LT)	40/36/15	7/18/10*	2/33/2**††
ALT, IU/L (mean ± SD)	4207 ± 2725	989 ± 1183**	902 ± 1380**
Total bilirubin, mg/dL (mean ± SD)	10.8 ± 8.2	15.2 ± 10.3*	15.8 ± 7.7**
Prothrombin time (%), median (range)	18.4 (3.1–58.6)	24.9 (2.2–58.1)**	29.8 (8.0–48.0)**
HBV DNA level, log copies/mL (mean ± SD)	5.6 ± 1.4	6.3 ± 1.7*	7.2 ± 1.4**†
Treatment			
Lamivudine	57 (63)	19 (54)	22 (59)
Entecavir	29 (32)	16 (46)	18 (49)
Interferon	28 (31)	11 (31)	11 (30)

Unless otherwise indicated, data indicate the number of patients, and those in parenthesis indicate percentages of patients.
Laboratory data are at the onset of hepatic encephalopathy of coma grade greater than II. HBV DNA levels are at the onset of hepatitis.
Significant difference among group was assessed by Student's *t*-test, Mann–Whitney *U*-test and χ^2-test.
Values significantly difference from patients with transient infection; *$P < 0.05$, **$P < 0.01$.
Values significantly different from patients with acute exacerbation in HBV carriers; †$P < 0.05$, ††$P < 0.01$.
ALT, alanine aminotransferase; F-A, acute type fulminant hepatitis; F-SA, subacute type fulminant hepatitis; LOHF, late-onset hepatic failure; LT, liver transplantation; SD, standard deviation.

and acute exacerbation groups. There was a tendency for the reactivation group to show clinical manifestation of the subacute type or LOHF. The reactivation group had lower alanine aminotransferase (ALT) levels and higher bilirubin and HBV DNA levels. Of the 37 cases of HBV reactivation, 33 (89%) resulted in liver-related death, two (5%) survived and two(5%) received living-donor liver transplantation. We then compared the clinical features of the 20 patients with HBV reactivation in HBsAg positive carrier status with those of the 17 patients with HBsAg negative resolved HBV infection status (Table 3). The resolved infection group was older than the carrier group. Most of the resolved infection group showed clinical manifestation as subacute type. The resolved infection group had lower ALT levels and higher bilirubin levels than those in the carrier group. It is noteworthy that all patients with resolved infection who developed HBV reactivation died despite nucleoside analog treatment. Concerning underlying disease, non-Hodgkin's lymphoma or mucosa-associated lymphoid tissue lymphoma was most prevalent in 50% of the carrier group and in 76% of the resolved infection group, respectively. In the carrier group, there were patients with oncological, rheumatological or collagen disease. HBV reactivation occurred more frequently after immunosuppressive therapy in patients with resolved infection, as previously reported.[39] Rituximab plus steroids combination chemotherapy was administrated to 35% of patients in the carrier group and to 59% of patients in the resolved infection group, respectively. Corticosteroid was used as monotherapy or in combination therapy in approximately three quarters of both groups. Methotrexate and anthracycline antitumor agent were given in 10% in the carrier group.

PREVENTION OF HBV REACTIVATION FOLLOWING IMMUNOSUPPRESSIVE CHEMOTHERAPY

HBsAg positive patients

BECAUSE VIRAL REPLICATION precedes clinical evidence of hepatitis, it is efficacious to use nucleoside analogs in a prophylactic manner before the start of chemotherapy. Previous retrospective and prospective studies have shown that the risk of HBV reactivation can be greatly reduced by the use of prophylactic nucleoside analog therapy for susceptible patients.[15,40–43] In Japan, currently, there are three oral nucleoside analogs approved for the treatment of chronic hepatitis B (lamivudine, adefovir and entecavir). Concerning lamivudine, the drug has proven efficacy and safety in preventing HBV reactivation related to chemotherapy. However, a major problem with its prolonged use is the possibility of viral breakthrough following the emergence of treatment-resistant HBV variants with YMDD mutations.[44] Given their high potency and extremely low rates of drug resistance, new generation oral nucleoside analogs, such as entecavir or tenofovir, are anticipated to be effective for HBV reactivation. The incidence of entecavir resistance in nucleos(t)ide analog-naive patients is reported to be 1.2% at 3 years.[45–47] A recent report demonstrated that entecavir is effective in the prevention of HBV reactivation in cancer patients.[48,49]

The American Association for the Study of Liver Disease (AASLD) guidelines recommend that if the anticipated duration of treatment is less than 1 year and baseline serum HBV DNA is not detectable, lamivudine or telbivudine are desirable and in other cases, entecavir or tenofovir are desirable.[50] The European Association for the Study of the Liver (EASL) guidelines also recommend the use of lamivudine for patients with low HBV DNA and entecavir or tenofovir for patients with high HBV DNA.[51] A consensus of the Japan Society of Hepatology recommends the use of entecavir as the first-line drug when patients with chronic hepatitis B are treated.[52]

Although the optimal time point for the initiation of antiviral prophylaxis has not been clearly established, nucleoside analogs should ideally be started as early as possible before chemotherapy.[53] This strategy can prevent any increase in viral replication, reduce the likelihood of drug resistance, allow chemotherapy to be completed and minimize the risk of hepatitis flare-up once chemotherapy is stopped.

Another concern with the use of lamivudine has been the occurrence of hepatic flares upon cessation of the antiviral compound. The optimal duration of antiviral prophylaxis in HBsAg carriers receiving immunosuppressive chemotherapy has only partly been clarified and is under active investigation. Several cases of HBV reactivation and even fatal fulminant hepatitis have been reported when lamivudine was stopped less than 3 months after the completion of chemotherapy.[54] AASLD guidelines recommend that prophylaxis is discontinued 6 months after completion of chemotherapy in patients with baseline HBV DNA of less than 2000 IU/mL, otherwise prophylaxis continues. EASL guidelines recommend the same treatment as that for AASLD guidelines for 12 months after completion of chemotherapy.

Table 3 Differences between HBV reactivation in patients who are HBsAg carriers and patients with resolved infection

	Inactive carrier HBsAg(+) ($n = 20$)	Resolved infection HBsAg(−) ($n = 17$)	*P*-value
Age, years, median (range)	60 (29–84)	67 (48–86)	0.035
Male/female	11/9	11/6	NS
Disease types (F-A/F-SA/LOHF)	4/11/5	0/16/1	0.025
Prognosis (alive/died/LT)	2/16/2	0/17/0	NS
ALT, IU/L (mean ± SD)	1114 ± 1602	653 ± 1057	NS
Total bilirubin, mg/dL (mean ± SD)	13.6 ± 8.2	18.5 ± 6.3	NS
Prothrombin time (%), median (range)	28.8 (8.0–48.0)	30.3 (19.0–38.0)	NS
HBV DNA level, log copies/mL (mean ± SD)	7.6 ± 1.2	6.6 ± 1.5	NS
Treatment			
Lamivudine	13 (65)	9 (53)	NS
Entecavir	9 (45)	9 (53)	NS
Interferon	5 (25)	6 (35)	NS
Underlying disease			
NHL/MALT lymphoma	10 (50)	13 (76)	NS
Other onco-hematological	1 (5)	3 (18)	NS
Oncological	3 (15)	1 (6)	NS
Collagen disease	2 (10)	–	NS
Rheumatological	4 (20)	–	NS
HBV reactivation			
Under immunosuppressive therapy	10 (50)	2 (12)	0.015
After immunosuppressive therapy	3 (15)	14 (82)	<0.001
Type of immunosuppressive therapy			
CHOP	1 (5)	–	NS
R-CHOP	7 (35)	10 (59)	NS
Other rituximab-containing-therapy	1 (5)†	3 (18)‡	NS
Fludarabine plus prednisolone	–	1 (6)	NS
Anthracycline plus cyclophosphamide	2 (10)§	1 (6)	NS
Prednisolone	4 (20)	–	NS
Methotrexate	2 (10)	–	NS
Others	3 (15)¶	2 (12)††	NS

Unless otherwise indicated, data indicate the number of patients, and those in parenthesis indicate percentages of patients.
Laboratory data are at the onset of hepatic encephalopathy of coma grade greater than II. HBV DNA levels are at the onset of hepatitis.
Significant difference among group was assessed by Student's *t*-test, Mann–Whitney *U*-test and χ^2-test.
†One patient: only rituximab.
‡One patient: rituximab, etoposide. One patient: rituximab, pirarubicin, cyclophosphamide, vincristine, prednisolone. One patient: rituximab, etoposide, mitoxantrone, carboplatin, prednisolone.
§One patient: adriamycin, cyclophosphamide. One patient: epirubicin, cyclophosphamide, fluorouracil, dexamethasone.
¶One patient: imatinib mesylate. One patient: carboplatin, paclitaxel, prednisolone. One patient: tacrolimus, etanercept, infliximab, methotrexate, prednisolone.
††One patient: cyclophosphamide, prednisolone. One patient: vincristine, doxorubicin, dexamethasone (for peripheral blood stem cell transplantation).
ALT, alanine aminotransferase; CHOP, cyclophosphamide, doxorubicin, vincristine, prednisolone; F-A, acute type fulminant hepatitis; F-SA, subacute type fulminant hepatitis; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; LOHF, late-onset hepatic failure; LT, liver transplantation; MALT, mucosa-associated lymphoid tissue lymphoma; NHL, non-Hodgkin's lymphoma; NS, not statistically significant; R-CHOP, rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone; SD, standard deviation.

HBsAg negative patients with HBcAb and/or HBsAb

The optimal screening and prophylactic strategy for prevention of HBV reactivation in patients with resolved infection remain unsettled. However, the most important first step in avoiding the serious morbidity associated with HBV reactivation is to identify patients at risk before the start of immunosuppressive therapy. In high HBV endemic areas, all patients should be screened for HBsAg. A highly sensitive assay is desirable to detect low levels of HBsAg and escape mutants of HBV.[55] In the next step, if HBsAg is negative, patients should be screened for HBcAb and HBsAb when they are receiving chemotherapy regimens that are associated with a high risk of reactivation. (e.g. intensive chemotherapy for hematological malignancies and hematopoietic stem cell transplantation [HSCT]). HBV reactivation during chemotherapy has been reported in HBsAb positive cases, HBcAb positive cases and cases positive only for HBcAb regardless of HBV DNA status.[33,35,56,57] In addition, while rare, there have been sporadic reports of cases in which HBV reactivation was observed after administration of rituximab in patients who were positive for HBsAb alone.[35,58] For the third step, if patients are HBcAb and/or HBsAb positive, they should be screened for HBV DNA. This screening can clarify the occult HBV infection.[58–60] Several reports have suggested an association between a decrease in HBsAb and HBcAb titers and risk of HBV reactivation, and monitoring these antibodies may provide an index for HBV reactivation.[61,62] However, this approach is not applicable to HBsAb negative and HBcAb positive patients. In addition, HBV reactivation has also been observed in patients with high HBsAb titers.[36] These may indicate that predicting reactivation only by monitoring HBsAb titers would be insufficient.

Using antiviral agents for patients with resolved infection in a prophylactic manner before the start of chemotherapy is probably as efficacious as in HBsAg positive carriers. However, there are issues such as obscure indications and cost-effectiveness. Alternatively, for patients with resolved infection, antiviral treatment can be deferred until seroconversion of HBsAg or detection of HBV DNA.[63–65] However, the appearance of HBV DNA in serum precedes HBsAg appearance, and there are cases of HBV reactivation without the appearance of HBsAg.[66] Therefore, periodic monitoring of HBV DNA may predict HBV reactivation, and it is therefore advantageous to combine these indices. Hui *et al.* reported that the median time from the elevation of serum HBV DNA to hepatitis onset was 18.5 weeks (range 12–28 weeks).[35] When monitoring for HBV reactivation, it is essential to identify HBV reactivation at an early stage. As previously shown in fulminant hepatitis cases, the start of nucleoside analogs after the onset of hepatitis cannot prevent fatal hepatitis. Therefore, commencement of nucleoside analogs at an early stage of HBV reactivation is important.[63,64]

The optimal duration of antiviral prophylaxis in HBsAg negative patients receiving immunosuppressive chemotherapy is not well understood. The intensity and duration of immunosuppression, as well as a number of host and viral factors, should be taken into consideration. In a previous study, in patients receiving rituximab plus steroid combination chemotherapy, discontinuation of lamivudine 4 weeks after completion of chemotherapy was followed by HBV reactivation, which occurred up to 6 months after treatment was withdrawn.[67] In the other setting of allogenic HSCT, HBV reactivation occurs later, in 40% at 2 years and 70% of patients at 5 years post-transplantation.[61]

GUIDELINES FOR PREVENTING HBV REACTIVATION

IN 2009, THE Intractable Hepatobiliary Disease Study Group in Japan and the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis developed guidelines for preventing HBV reactivation.[68] These guidelines underwent minor revision in 2011, as shown in Figure 1.

The essential features of the guidelines are as follows. All patients should be screened for HBsAg before the start of chemotherapy. If HBsAg is positive, HBeAg, hepatitis B e antibody (HBeAb) and HBV DNA should be checked. Regardless of the patient's HBeAg, HBeAb or HBV DNA status, prophylactic therapy with entecavir before initiation is recommended. If HBsAg is negative, HBcAb and HBsAb testing should be performed. If HBcAb and/or HBsAb is positive, HBV DNA should be checked. When HBV DNA is detectable, antiviral prophylaxis before initiation is recommended. When HBV DNA is not detectable, HBV DNA and aspartate aminotransferase/ALT levels should be monitored monthly during and 12 months after completion of chemotherapy. Nucleoside analogs should be administrated immediately when HBV DNA becomes positive during this period. The timing of termination of nucleoside analog treatment will be determined in accordance with the treatment for type B chronic hepatitis, if HBsAg is positive. If HBcAb and/or HBsAb is positive,

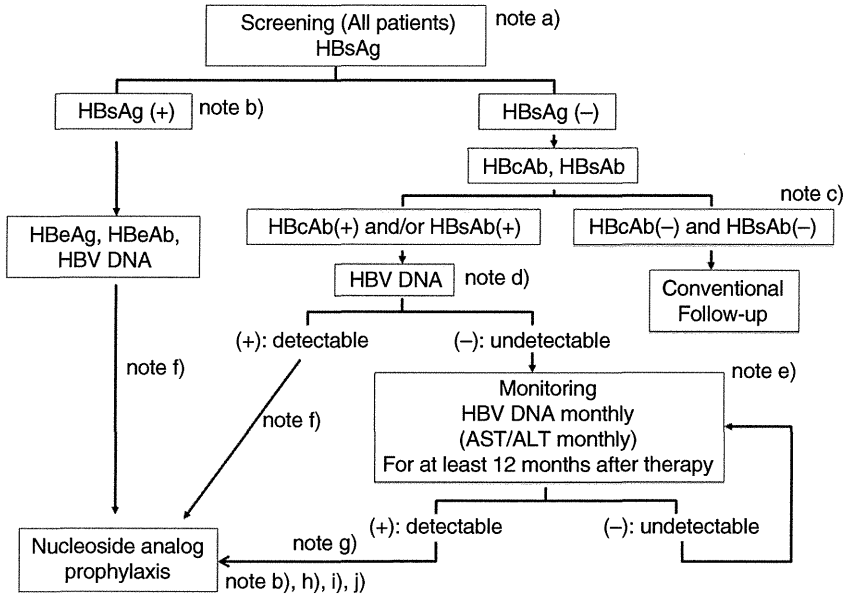

Figure 1 Guideline for preventing hepatitis B due to immunosuppressive therapy or chemotherapy (revised version). Reactivation of HBV can occur not only in HBsAg positive patients, but also in a proportion of HBsAg negative patients during and after intensive immunosuppressive therapy or chemotherapy of hematological malignancy. HBV reactivation deserves special attention because it can cause flare-up of hepatitis resulting in fulminant hepatitis. Appropriate measures are also necessary in patients receiving immunosuppressive therapy or chemotherapy for non-hematological malignancy in consideration of the risk of HBV reactivation. Because of a lack of evidence, there is no guarantee that prophylactic administration of nucleoside analog in these guidelines can prevent acute hepatic failure due to HBV reactivation. Notes: (a) HBV carriers and patients who have apparently recovered from HBV infection receiving immunosuppressive therapy or cytotoxic chemotherapy are at a risk of HBV reactivation. All patients should be screened for being HBV carriers by HBsAg. If results for HBsAg are negative, patients should be screened for evidence of previous infection by HBcAb and HBsAb. Highly sensitive detection methods for HBsAg, HBcAb and HBsAb are desirable. (b) HBsAg positive cases are subject to consultation with a hepatologist. Consultation with a hepatologist is desirable in all patients subject to administration of nucleoside analogs. (c) Detection of HBV DNA is desirable in those patients who have previously received immunosuppressive therapy or cytotoxic chemotherapy, and HBcAb and HBsAb are undermined before the start of the therapy. (d) Detection by PCR or real-time PCR is recommended. The sensitive real-time PCR method is desirable. (e) Patients receiving rituximab plus steroid combination therapy or hematopoietic stem cell transplantation are particularly at risk of HBV reactivation and deserve careful attention. Although there is a lack of evidence regarding the risk of HBV reactivation in patients receiving fludarabine, an intensive immunosuppressive agent, this still deserves careful attention in the future. (f) Prophylactic nucleoside analogs should be started as soon as possible before the start of immunosuppressive therapy or chemotherapy. (g) Nucleoside analogs should be administrated immediately when HBV DNA becomes positive during and after immunosuppressive therapy or chemotherapy. (h) Entecavir is recommended as the nucleoside analog. HBV DNA is monitored monthly during administration of nucleoside analogs. (i) Termination of nucleoside analog treatment is considered when the timing is as follows: If HBsAg is positive at screening, the timing of termination of nucleoside analog treatment will be determined in accordance to the treatment for type B chronic hepatitis. If HBcAb and/or HBsAb is positive at screening, nucleoside analog treatment will be discontinued when: (1) nucleoside analogs are administered for 12 months after the completion of immunosuppressive therapy or chemotherapy; (2) ALT levels are normal during the administration period, and (3) HBV DNA is negative during the administration period. (j) Patients should be closely observed for 12 months after treatment with nucleoside analogs. The follow up is according to the instruction method of each nucleoside analog. Nucleoside analogs should be re-administrated immediately when HBV DNA becomes positive during the observation period. These guidelines were jointly developed by the Intractable Hepatobiliary Disease Study Group in Japan and the Study Group for the Standardization of Treatment of Viral Hepatitis Including Cirrhosis in 2009. The guidelines underwent minor revision in 2011. ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBcAb, hepatitis B core antibody; HBeAg, hepatitis B e antigen; HBeAb, hepatitis B e antibody; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; PCR, polymerase chain reaction.

nucleoside analog treatment will be discontinued if HBV DNA is negative and ALT levels are normal. Patients are closely observed for 12 months after treatment with nucleoside analogs.

Although reactivation of hepatitis B commonly occurs in the setting of cancer chemotherapy, it may also follow the use of immunomodulatory therapy for non-malignant conditions, for example, infliximab therapy for inflammatory bowel disease and therapy for rheumatological diseases with corticosteroids, methotrexate,[69,70] anti-tumor necrosis factor-α alone[71,72] or in combination with other therapies.[73–75] However, for both HBsAg positive patients and HBsAg negative resolved infection patients, the data are currently insufficient to provide information on the incidence of HBV reactivation of these agents. Consequently, careful attention is necessary when using new immunosuppressive agents.

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Special Report

Etiology and prognosis of fulminant hepatitis and late-onset hepatic failure in Japan: Summary of the annual nationwide survey between 2004 and 2009

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Aim: To summarize the annual nationwide survey on fulminant hepatitis (FH) and late-onset hepatic failure (LOHF) between 2004 and 2009 in Japan.

Methods: The annual survey was performed in a two-step questionnaire process to detail the clinical profile and prognosis of patients in special hospitals.

Results: Four hundred and sixty ($n = 227$ acute type; $n = 233$ subacute type) patients had FH and 28 patients had LOHF. The mean age of patients with FH and LOHF were 51.1 ± 17.0 and 58.0 ± 14.4 years, respectively. The causes of FH were hepatitis A virus in 3.0%, hepatitis B virus (HBV) in 40.2%, other viruses in 2.0%, autoimmune hepatitis in 8.3%, drug allergy-induced in 14.6% and indeterminate etiology in 29.6% of patients. HBV reactivation due to immunosuppressive therapy was observed in 6.8% of FH patients. The short-term survival rates of patients without liver transplantation (LT) were 48.7% and 24.2% for the acute and subacute type, respectively, and 13.0% for LOHF. The prognosis was poor in patients with HBV reactivation. The implementation rate for LT in FH patients was equivalent to that in the previous survey. The short-term survival rates of total patients, including LT patients, were 54.2% and 40.8% for the acute and subacute type, respectively, and 28.6% for LOHF.

Conclusion: The demographic features and etiology of FH patients has gradually changed. HBV reactivation due to immunosuppressive therapy is problematic. Despite advances in therapeutic approaches, the prognosis of patients without LT has not improved.

Key words: acute liver failure, fulminant hepatitis, Japan, liver transplantation, viral hepatitis

INTRODUCTION

IN JAPAN, FULMINANT hepatitis (FH) is defined as having hepatitis, when grade II or worse hepatic encephalopathy develops within 8 weeks of the onset of disease symptoms, with a prothrombin time of 40% or less.[1,2] FH is further classified into two subtypes, acute and subacute types, in which encephalopathy occurs within 10 days and later than 11 days, respectively, of the onset of the disease symptoms. Patients showing a prothrombin time of 40% or less, with hepatic encephalopathy developing between 8 and 24 weeks of disease onset are classified as having late-onset hepatic failure (LOHF).[3] Etiologies with hepatitis present in the histology, such as viral infection, autoimmune hepatitis and drug allergy-induced liver injury are defined as causes of

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FH and LOHF. In contrast, acute liver failure due to other causes with the absence of hepatitis in the histology, such as drug toxicity, circulatory disturbance and metabolic disease, are excluded as causes of FH and LOHF. Recently, a novel diagnostic criteria for acute liver failure in Japan was established by the Intractable Hepato-Biliary Disease Study Group.[4,5] These criteria included other causes with liver damage without the absence of hepatitis in the histology in addition to the present criteria.

Among viral infection, hepatitis B virus (HBV) is a major cause of FH in Japan.[6,7] HBV infection is classified into transient HBV infection type and acute exacerbation in an HBV inactive carrier. With advances in cytotoxic chemotherapy and immunosuppressive therapy, reactivation of hepatitis B is becoming a clinical problem.[8] Moreover, recent introduction of rituximab plus steroid combination therapy for non-Hodgkin's lymphoma has been associated with HBV reactivation in transiently infected patients, namely, de novo hepatitis. However, the prevalence of HBV reactivation in patients with FH and LOHF is unknown.

Advances in therapeutic strategies for FH and LOHF have improved the prognosis. Since 1988, living-donor liver transplantation (LT) has been adopted in patients who are beyond the supportive care of a critical unit.[6] Recently, artificial liver support with high-flow or on-line hemodiafiltration (HDF) has been used. Since 2006, a nucleoside analog, entecavir, has been used as a substitute for lamivudine, as an antiviral agent for HBV. However, it is unknown whether these new treatments improve the prognosis of FH.

The Intractable Hepato-Biliary Diseases Study Group has annually performed a nationwide survey of patients with FH and LOHF since 1983.[6] Since 2000, approximately 600 hospitals have been enrolled in the survey. This report summarizes the results of the survey between 2004 and 2009 to addresses the trends in the etiology and prognosis of patients with FH and LOHF and compares them with the previous survey.[7]

METHODS

THE NATIONWIDE SURVEY was performed annually. The number of hospitals for survey has changed in each year. Maximum (608) was in 2007 and minimum (544) was in 2006, with active members of the Japan Society of Hepatology and the Japanese Society of Gastroenterology between 2005 and 2010. The survey was performed in a two-step questionnaire process to detail the clinical profile and prognosis of patients who were diagnosed as FH or LOHF in the previous year. The recovery rate of the first and second questionnaire was 39–59% and 60–100%, respectively. Patients who met the diagnostic criteria for FH or LOHF were entered into the survey. Patients under 1 year of age, those with alcoholic hepatitis, those with chronic liver diseases and those with acute liver failure with no histological features of hepatitis were excluded from the analysis.

According to criteria described in previous reports,[7,9] the etiology of FH and LOHF was classified into five categories: (i) viral infection; (ii) autoimmune hepatitis; (iii) drug allergy-induced liver injury; (iv) indeterminate etiology despite sufficient examinations; and (v) unclassified due to insufficient examinations. Patients with viral infection consisted of those with hepatitis A virus (HAV), HBV, hepatitis C virus (HCV), hepatitis E virus (HEV) and other viruses. The patients with HBV infection were classified into three subgroups according to serum markers of HBV, hepatitis B core antibody (HBcAb) and immunoglobulin (Ig)M-HBcAb: (i) transient HBV infection; (ii) acute exacerbation in HBV carriers; and (iii) indeterminate infection patterns. In the present study, we classified acute exacerbation in HBV carriers into three subgroups according to the new criteria:[4,5] (i) inactive carriers, without drug exposure; (ii) reactivation in inactive carriers by immunosuppressant and/or anticancer drugs; and (iii) reactivation in transiently infected patients by immunosuppressant and/or anticancer drugs (i.e. de novo hepatitis). Because not every patient was examined for serological markers of transient HBV infection before the onset of FH and LOHF (with HBcAb and/or hepatitis B surface antigen [HBsAg] in serum), we defined HBV reactivation as that occurring in transiently infected patients when they developed HBV-related hepatitis due to immunosuppressive therapy or cytotoxic chemotherapy with reappearance of HBsAg in the serum and did not conform to the criteria of transient HBV infection.

The statistical significance of differences between groups was assessed using Student's *t*-test, Fisher's exact test or Kruskal–Wallis one-way ANOVA. Data are shown as mean ± standard deviation. The study was conducted with the approval of the Ethical Committee of Kagoshima University Graduate School of Medical and Dental Sciences.

RESULTS

Demographic features and survival rates

FROM 2004–2009, 582 PATIENTS were enrolled in the survey. Ninety-four patients were excluded from

the survey according to the exclusion criteria. Consequently, 460 patients ($n = 227$ acute type; $n = 233$ subacute type) were classified as having FH and 28 as having LOHF (Table 1). The incidence of the acute and subacute types of FH was similar and the incidence of LOHF was one-sixteenth of FH. The male : female ratio was higher for the acute type and lower for the subacute type of FH and LOHF. The mean age of patients was significantly higher for the subacute type of FH and LOHF than that for the acute type of FH. Almost half of the patients with FH and LOHF had complications which preceded the onset of acute liver failure. Furthermore, approximately 60% of patients with FH had received daily medication. This tendency for receiving medication was more obvious in patients with the subacute type of FH and LOHF.

The survival rates of patients without LT were 48.7% for the acute type and 24.2% for the subacute type of FH, and 13.0% for LOHF. The survival rates of the subacute type of FH and LOHF was worse than that of the acute type. The prognosis of both the acute type and the subacute type of FH appeared to be equivalent annually. The survival rates of patients with LT were 79.6% for FH and 100% for LOHF, with no difference in these rates among the disease types.

Clinical profile

Symptoms, imaging findings and complications are shown in Table 2. Since 2006, diagnostic criteria of systemic inflammatory response syndrome (SIRS) for fever, tachycardia and tachypnea have been adopted in the survey.[10] Icterus, flapping tremor, ascites, hepatic fetor, tachycardia, tachypnea and pretibial edema were frequently found. The frequency of patients with ascites and pretibial edema was significantly greater in the subacute type of FH and LOHF than in the acute type of FH. In contrast, fever appeared more frequently in patients with the acute type of FH. The frequency of liver atrophy was greater in the subacute type of FH, and even higher in LOHF, than in the acute type of FH.

With regard to complications, disseminated intravascular coagulation, renal failure and bacterial infection were found in more than 30% of patients with FH and LOHF. Brain edema was less frequent in the subacute type than in the acute type of FH.

Causes of FH and LOHF

The cause of FH was identified as viral infection in 46.1% of the patients (Table 3). The frequencies of viral infection were highest for the acute type of FH. HAV infection was found in 3% of patients with FH. HBV infection was found in 40.2% of patients with FH and 32.1% of patients with LOHF. Transient HBV infection was more frequent in the acute type than in the subacute type of FH, whereas the frequency of acute exacerbation in HBV carriers was greater in the subacute type than in the acute type of FH. HBV reactivation in inactive carriers and in transiently infected patients were found in 3.3% and 3.5% of patients with FH, respectively. With regard to underlying diseases in patients with HBV reactivation, non-Hodgkin's lymphoma/mucosa-associated lymphoid tissue lymphoma was most prevalent in 50% of inactive carriers and in 76% of those with transiently infected patients. Among patients with HBV

Table 1 Demographic features and survival rates of patients with fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
Male/female	227/233	127/100	100/133**	9/19*
Age (years; mean ± SD)	51.1 ± 17.0	48.8 ± 16.9	53.4 ± 16.7**	58.0 ± 14.4**
HBV carrier (%)	13.1 (52/397)	10.5 (19/181)	15.3 (33/216)	22.2 (6/27)
Complications preceding acute liver failure (%)†	46.4 (208/448)	40.0 (88/220)	52.6 (120/228)**	50.0 (14/28)
History of medication (%)	59.9 (260/434)	51.2 (108/211)	68.2 (152/223)**	71.4 (20/28)*
Survival rates				
All patients	47.4 (218/460)	54.2 (123/227)	40.8 (95/233)**	28.6 (8/28)*
No LT	37.5 (132/352)	48.7 (93/191)	24.2 (39/161)**	13.0 (3/23)**
LT	79.6 (86/108)	83.3 (30/36)	77.8 (56/72)	100 (5/5)

*$P < 0.05$, **$P < 0.01$ vs acute type.
†Diseases such as metabolic syndrome, malignancy and psychiatric disorders.
Data in parenthesis indicate patient numbers.
HBV, hepatitis B virus; LT, liver transplantation; SD, standard deviation.

Table 2 Symptoms, imaging findings and complications of patients with fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
(a) Symptoms at diagnosis				
Fever†	13.0 (42/322)	17.5 (28/160)	8.6 (14/162)*	0 (0/23)*
Icterus	96.8 (427/441)	95.0 (208/219)	98.6 (219/222)*	96.4 (27/28)
Ascites	57.2 (237/414)	45.2 (88/204)	71.0 (149/210)**	81.5 (22/27)**
Convulsion	5.2 (22/422)	6.7 (14/210)	3.8 (8/212)	0 (0/27)
Tachycardia‡	36.7 (117/319)	39.5 (62/157)	34.0 (55/162)	47.8 (11/23)
Tachypnea§	34.5 (87/252)	39.1 (52/133)	29.4 (35/119)	31.6 (6/19)
Flapping tremor	79.0 (309/391)	75.8 (144/190)	82.1 (165/201)	80.8 (21/26)
Hepatic fetor	46.6 (146/313)	49.0 (73/149)	44.5 (73/164)	42.1 (8/19)
Pretibial edema	35.5 (127/358)	24.1 (42/174)	46.2 (85/184)**	75.0 (15/20)**,***
(b) Imaging findings				
Liver atrophy¶	58.8 (255/434)	45.6 (98/215)	71.7 (157/219)**	92.6 (25/27)**,***
(c) Complications				
Infection	34.8 (149/428)	32.9 (68/207)	36.7 (81/221)	51.9 (14/27)
Brain edema	18.5 (71/384)	24.1 (46/191)	13.0 (25/193)**	22.7 (5/22)
Gastrointestinal bleeding	13.2 (59/446)	11.0 (24/219)	15.4 (35/227)	20.0 (5/25)
Renal failure	38.9 (177/455)	40.9 (92/225)	37.0 (85/230)	39.3 (11/28)
DIC	34.6 (150/433)	35.7 (76/213)	33.6 (74/220)	53.8 (14/26)
Congestive heart failure	7.3 (31/427)	8.9 (19/214)	5.6 (12/213)	12.0 (3/25)

*$P < 0.05$, **$P < 0.01$ vs acute type, ***$P < 0.05$ vs subacute type.
†Temperature: >38°C or <36°C.
‡Heart rate: >90 beats/min.
§Respiratory rate: >20 breaths/min or $PaCO_2$: <32 Torr.
† ‡ § Cases between 2005 and 2009.
¶Liver atrophy detected by ultrasound and/or computed tomography imaging.
Data in parentheses indicate patient numbers.
DIC, disseminated intravascular coagulation.

reactivation, rituximab plus steroid combination chemotherapy was administrated to 35% of patients in inactive carriers and to 59% of those with transiently infected patients. HCV and HEV infection were less frequently found. In the survey, Epstein–Barr virus, herpes simplex virus and human herpes virus type-6 were found as other causes of viral hepatitis.

Autoimmune hepatitis was frequently observed in patients with the subacute type of FH and LOHF. Drug allergy-induced liver injury was observed in approximately 10–20% of patients irrespective of disease types. Anti-tuberculosis agents, non-steroidal anti-inflammatory drugs, anticancer agents, drugs for metabolic syndrome, and various herbal and natural remedies were the probable causative agents for this liver injury in the survey. Notably, the etiology was indeterminate in approximately 40% of patients with the subacute type of FH.

Therapies

For artificial liver support, plasma exchange and HDF were performed in most patients with FH (Table 4). Conventional HDF and continuous HDF (CHDF) were performed in 22.5% and 51.8% of patients with FH, respectively. A more powerful method, high-flow HDF (HF-HDF), high-flow CHDF (HF-CHDF) and on-line HDF were performed in 2.6%, 11.7% and 1.8% of the patients, respectively. The nucleoside analogs lamivudine and entecavir were used in approximately a quarter of patients with FH. Entecavir were used more frequently than lamivudine since 2007. Glucocorticosteroid, mainly as steroid pulse therapy, were administrated in more than 70% of patients with FH and LOHF. Anticoagulation therapy were performed in approximately 40–50% of patients with FH and LOHF. Glucagon/insulin, branched-chain amino acid-rich solution,

Table 3 Causes of fulminant hepatitis (FH) and late-onset hepatic failure (LOHF)

	FH			LOHF ($n = 28$)
	Total ($n = 460$)	Acute type ($n = 227$)	Subacute type ($n = 233$)	
Viral infection	46.1 (212)	62.6 (142)	30.0 (70)	32.1 (9)
HAV	3.0 (14)	5.7 (13)	0.4 (1)	0 (0)
HBV	40.2 (185)	54.2 (123)	26.6 (62)	32.1 (9)
(1) Transient infection	19.6 (90)	35.2 (80)	4.3 (10)	3.6 (1)
(2) Acute exacerbation in HBV carrier	14.1 (65)	7.9 (18)	20.2 (47)	25.0 (7)
(i) Inactive carrier, without drug exposure	7.4 (34)	6.2 (14)	8.6 (20)	3.6 (1)
(ii) Reactivation in inactive carrier†	3.3 (15)	1.8 (4)	4.7 (11)	17.9 (5)
(iii) Reactivation in transiently infected patient‡	3.5 (16)	0 (0)	6.9 (16)	3.6 (1)
(3) Indeterminate infection patterns	6.5 (30)	11.0 (25)	2.1 (5)	3.6 (1)
HCV	1.1 (5)	0.9 (2)	1.3 (3)	0 (0)
HEV	0.9 (4)	0.9 (2)	0.9 (2)	0 (0)
Other viruses	0.9 (4)	0.9 (2)	0.9 (2)	0 (0)
Autoimmune hepatitis	8.3 (38)	2.2 (5)	14.2 (33)	32.1 (9)
Drug allergy-induced liver injury	14.6 (67)	13.7 (31)	15.5 (36)	17.9 (5)
Indeterminate§	29.6 (136)	19.4 (44)	39.5 (92)	17.9 (5)
Unclassified¶	1.5 (7)	2.2 (5)	0.9 (2)	0 (0)

†Reactivation in inactive carrier by immunosuppressant and/or anticancer drugs.
‡Reactivation in transiently infected patients by immunosuppressant and/or anticancer drugs (de novo hepatitis).
§Indeterminate etiology despite sufficient examinations.
¶Unclassified due to insufficient examinations.
Data in parentheses indicate patient numbers.
HAV, hepatitis A virus; HBV, hepatitis B virus; HCV, hepatitis C virus; HEV, hepatitis E virus.

cyclosporin A and prostaglandin E_1 therapy were administrated less frequently compared with the previous survey.

Liver transplantation was performed in 23.5% and 17.9% of patients with FH and LOHF, respectively. Two patients received deceased-donor LT and 111 patients received living-donor LT. The frequency of LT was significantly greater in the subacute type than in the acute type of FH.

Prognosis

The prognosis of patients with FH and LOHF differed depending on the etiology (Table 5). Prognosis was good in patients with HAV infection. The prognosis was fair in patients with transient HBV infection. In contrast, the prognosis was poor in acute exacerbation in HBV carriers. The prognosis was extremely poor in patients with HBV reactivation, either from inactive carriers or transiently infected patients. Patients with the subacute type of FH and LOHF caused by autoimmune hepatitis, drug allergy-induced liver injury and indeterminate etiology also showed a poor prognosis.

The clinical features of the patients appeared to be associated with the prognosis. In the acute type of FH with no LT, the frequency of patients with SIRS (tachycardia or tachypnea) was greater in patients who died than in surviving patients ($P < 0.05$). Liver atrophy on ultrasound and/or computed tomography imaging was an important factor in predicting the prognosis of FH and LOHF with no LT. The frequencies were 25.0% and 64.5% in patients with the acute type ($P < 0.01$) and 55.6% and 78.1% in those with the subacute type of FH in surviving patients and those who died, respectively ($P < 0.05$).

Prognosis also appeared to be affected by complications. Any of the complications significantly decreased survival rate (data not shown). Furthermore, the number of these complications affected the prognosis. The survival rate of patients with the acute type of FH was greater than 80% in those with no complications, while it was less than 30% in those with two or more complications. The survival rate of patients with the subacute type of FH was decreased in proportion to the number of complications.