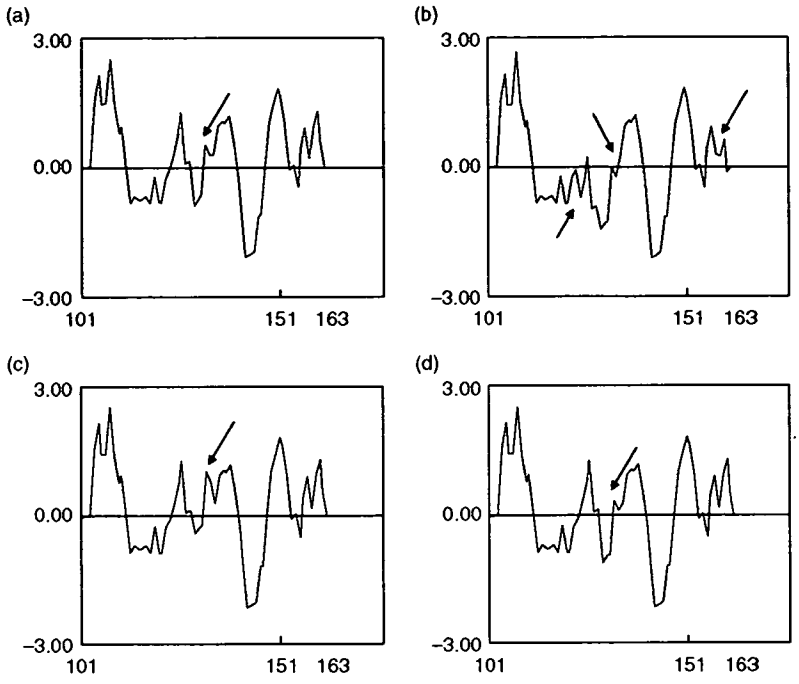

Figure 3 Hydropathy profile of the major hydrophilic region (MHR) of the *S* gene elaborated using the Kyte-Doolittle hydropathy index. Arrows show the positions of amino acids which are different among X01587, J02205, AB289701 (alanine-131) and AB289720 (proline-131). (a) X01587, (b) J02205, (c) AB289701 (alanine-131), (d) AB289720 (proline-131).

structure of the region. Therefore, the antibody produced against J02205 vaccines may not completely neutralize X01578 and vice versa. Indeed, previous studies showed that antibody profiles induced by recombinant vaccines produced from different genotypes are not identical with each other,[12] which suggests that antibodies produced by recombinant vaccines might not protect viral infection with different genotypes.

As shown in Figure 2, the aa sequences of our isolates classified into genotype A are very close to the aa sequence of J02205. Therefore, the transmission of genotype A HBV is prevented by Heptavax which is made from J02205.

The aa sequences of our isolates classified into genotype B are the same as the aa sequence of J02205 except for one substitution at aa131. This aa, which is asparagine and is located in the first stem loop structure of the MHR, was substituted with threonine in our genotype B isolates. Because asparagine and threonine have an uncharged side chain and similar polarity, genotype B HBV infection may be prevented effectively by Heptavax.

The aa sequences of our isolates classified into genotype C were the same as that of X01587 except for four isolates having a substitution at aa131. Bimmugen, which is produced from X01587, may be effective for preventing genotype C HBV infections caused by those four isolates. However, Heptavax may not be effective for preventing genotype C HBV infection because of the difference in eight amino acids as described above.

The four isolates have proline or alanine instead of threonine-131, which has never been reported before. The polarities of threonine and proline/alanine are quite different. The Kyte-Doolittle hydropathy analysis suggests that substituting threonine at aa131 with alanine or proline would increase hydrophobicity, which may then lead to a change in antigenicity. Hou *et al.* reported that some blood donors who were tested negative for serum HBsAg had a substitution of isoleucine for threonine at aa131 in the S region.[24] They suggested that the structure and antigenicity of HBV may be altered by this substitution.

The secondary structure of our isolate with alanine-131 predicted by Chou-Fasman analysis suggested an α-helix configuration instead of a β-configuration in the region from aa126 to aa135. The secondary structure of our isolate with proline-131 predicted by Robson analysis suggested that this change causes the loss of a turn structure between aa131 and aa134. Some changes in the secondary structure can affect the three-dimensional structure of the protein and thus affect antigenicity. These results suggest that the transmission of the four

isolates with an aa substitution at aa131 may not be prevented by either Heptavax or Bimmugen.

However, the protective immunity elicited by HBV vaccines, which is usually polyclonal in nature, may not be totally lost or severely affected *in vivo* by the alteration of only a single amino acid in the 'a' determinant region.[25] Also, antibodies against regions outside the 'a' determinant region may be protective.[26] The protectivity of current vaccines may be elucidated by *in vitro* binding studies using polyclonal antibodies.

It was reported that some individuals immunized with recombinant vaccines are infected with HBV with or without mutations in the 'a' determinant region.[11,27,28] HBV isolates with amino acid substitutions at aa144[29–31] or 145[11,27,28] are known to be transmitted despite vaccination. Indeed, some chronic HBV carriers are reported to have HBV with such amino acid substitutions.[32,33] We were unable to find patients who had these substitutions in the present study. However, large-scale studies are necessary to elucidate the prevalence of 'vaccine-escape mutants' in patients with acute hepatitis B.

In conclusion, we have shown that the aa sequence of the MHR in the *S* gene of HBV is different among isolates from patients with acute HBV infection. Current vaccination may prevent the transmission of these HBV isolates, which should be further investigated.

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Short Communication

Low serum level of hepatitis B core-related antigen indicates unlikely reactivation of hepatitis after cessation of lamivudine therapy

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Aim: The clinical significance of hepatitis B virus (HBV) core-related antigen (HBcrAg) in predicting the reactivation of hepatitis after halting lamivudine administration was analyzed.

Methods: A total of 34 patients with chronic hepatitis B were enrolled. Lamivudine was administered for at least 6 months before cessation, and reactivation of hepatitis was defined as elevation of alanine aminotransferase levels to more than 80 IU/L within 12 months of cessation.

Results: In total, 20 (59%) patients experienced hepatitis reactivation. Although concentrations of HBV DNA and HBcrAg in serum did not differ between the two groups of patients at the onset of lamivudine administration, HBcrAg serum levels were significantly higher ($P = 0.009$) in the reactivation patients (median 4.9, 25–75% range 4.7–5.9 log unit/mL) than the non-reactivation patients (median 3.2, 25–75% range <3.0–4.5 log unit/mL) post-lamivudine treatment. The concentration of HBV DNA did not differ between the two groups (median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the reactivation group vs. median <3.7, 25–75% range <3.7–<3.7 log copy/mL in the non-reactivation group). Receiver operating characteristic analysis of HBcrAg concentration showed an area under the curve of 0.764 in predicting patients without reactivation of hepatitis.

Conclusion: HBcrAg can be a useful marker to identify patients who are not at risk of reactivation of severe hepatitis after discontinuation of lamivudine administration.

Key words: chronic hepatitis B, hepatitis B virus core-related antigen, hepatitis B virus DNA, hepatitis reactivation, lamivudine

INTRODUCTION

LAMIVUDINE, A NUCLEOSIDE analog that inhibits reverse transcriptase, has been found to inhibit the replication of hepatitis B virus (HBV), reduce hepatitis, and improve histological findings of the liver in long-term treatment.[1,2] Furthermore, it has been shown that lamivudine treatment improves the long-term outcome of patients with chronic hepatitis B.[3,4] However, there are a number of problems with lamivudine therapy, including hepatitis relapse due to the appearance of YMDD mutant viruses and the reactivation of hepatitis after its discontinuation.[5,6]

During lamivudine administration, the concentration of serum HBV DNA decreases, and usually becomes undetectable to even high sensitivity HBV DNA assays. However, this undetectable level is an inadequate indicator for safely discontinuing lamivudine

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administration as active hepatitis often recurs in patients post-treatment.

Previously, a chemiluminescence enzyme immunoassay (CLEIA) was developed by our laboratory to detect of hepatitis B core-related antigen (HBcrAg).[7,8] This HBcrAg CLEIA simultaneously measures the serum levels of hepatitis B core (HBc) and e (HBe) antigens using monoclonal antibodies, which recognize common epitopes of these two denatured antigens because both proteins are transcribed from the precore/core gene and their first 149 amino acids are identical.[9–11] Although this assay reflects the viral load of HBV in a similar manner to HBV DNA assays during disease progression, HBcrAg CLEIA shows characteristics different from HBV DNA assays under lamivudine administration since HBcrAg levels decrease more slowly than HBV DNA after treatment begins.[12] In the present study, we analyzed the clinical significance of the HBcrAg assay in predicting the likelihood of non-reactivation of hepatitis after discontinuing lamivudine administration in HBV treatment.

METHODS

Patients

A TOTAL OF 34 patients with chronic hepatitis B who were treated with lamivudine for at least 6 months were enrolled in the present study. The patients comprised 20 men and 14 women with a median age of 46 years (range 23–65 years), and were selected retrospectively from five medical institutions in Japan (Shinshu University Hospital, Kyoto Prefectural University Hospital, National Nagasaki Medical Center, Toranomon Hospital, and Hiroshima University Hospital). Written informed consent was obtained from each patient.

Of the 27 patients whose HBV genotype was determined, 25 (93%) were genotype C and the remaining two (7%) were genotype B. Serum HBV DNA was detectable in all patients, and HBe antigen was positive in 16 (47%) of the 34 patients before lamivudine administration.

For treatment of HBV infection, daily doses of 100 mg lamivudine were administered for at least 6 months. Lamivudine administration was stopped when alanine aminotransferase (ALT) levels were reduced to 40 IU/L or less in at least three separate tests. Serum samples were taken at several time points during and after lamivudine administration, and patients were seen at least once a month for at least 12 months after cessation of lamivudine. Estimated duration of HBV DNA level <3.7 log copy/mL before stopping lamivudine was a median 10 months (range 0–29 months).

Reactivation of hepatitis was defined as elevation of ALT to more than 80 IU/L within 12 months of stopping lamivudine treatment.

Serological markers for HBV

Serum hepatitis B surface antigen, HBe antigen, and anti-HBe antibody were measured by commercially available CLEIA kits (Fujirebio, Tokyo, Japan). Six major genotypes (A–F) of HBV are detectable using the method reported by Mizokami *et al.*[13] in which the surface gene sequence is amplified by polymerase chain reaction (PCR) and analyzed by restriction fragment length polymorphism. Serum concentration of HBV DNA was determined using a transcription mediated amplification (TMA) assay kit (Chugai Diagnostics Science, Tokyo, Japan) which has a quantitative range of 3.7–8.7 log copy/mL.

Serum concentration of HBcrAg was measured using a CLEIA developed by Fujirebio, as described previously.[7] Briefly, 150 μL of serum was incubated with 150 μL of pretreatment solution containing 15% sodium dodecylsulfate at 60°C for 30 min. After incubation, 120 μL of pretreated specimen was added to a ferrite microparticle solution in an assay tube. Ferrite microparticles were coated with monoclonal antibodies (HB44, HB61, HB114) against denatured HBc and HBe antigens. After washing, two other monoclonal antibodies against denatured HBcAg and HBeAg (HB91 and HB110) labeled with alkaline phosphatase were added as secondary antibodies. After further washing, 200 μL of AMPPD (3-(2′-spiroadamantan)-4-methoxy-4-(3″-phosphoryloxy) phenyl-1, 2-dioxetane disodium salt; Applied Biosystems, Bedford, MA) solution was added as substrate, and the assay tube was incubated for 5 min at 37°C.

From this, the relative chemiluminescence intensity was measured, and HBcrAg concentration was determined by comparison with a standard curve generated using recombinant pro-HBe antigen (amino acids, 10–183 of the precore/core gene product). The HBcrAg concentration was expressed as units/mL (U/mL) and a immunoreactivity of recombinant pro-HBe antigen of 10 fg/mL was defined as 1 U/mL. In the present study, the cutoff value of HBcrAg concentration was set at 3.0 log U/mL.

Statistical analysis

The Mann–Whitney *U*-test was used to analyze quantitative data, and Fisher's exact test was used for

qualitative data. Receiver operating characteristic (ROC) curve analysis was used to analyze cut-off levels of HBcrAg concentration for prospective recurrence of hepatitis. Statistical analyses were performed using the SPSS 14.0 J statistical software package (SPSS, Chicago, IL, USA), and a *P*-value of less than 0.05 was considered to be statistically significant.

RESULTS

TWENTY (59%) OF the 34 patients enrolled in the present study showed reactivation of hepatitis within 12 months after discontinuing lamivudine administration, with 15 (75%) showing reactivation within 6 months. The peak serum ALT levels in the 20 reactivation patients ranged from 103 to 1019 IU/L, with a median of 308 IU/L. After lamivudine cessation, the maximum serum HBV DNA was significantly higher ($P < 0.001$) in the reactivation patients (median 7.8, 25–75% range 7.4–8.1 log copy/mL) than in the non-reactivation patients (median 4.8, 25–75% range 4.1–5.9 log copy/mL).

Table 1 shows a comparison of the clinical backgrounds at the onset and completion of lamivudine administration between the two groups of patients. Although backgrounds were similar between the two groups just prior to lamivudine administration, HBcrAg levels were significantly higher in the reactivation patients after treatment. Both HBV DNA levels and positive rates of HBe antigen were similarly low between the two groups. The duration of undetectable HBV DNA before stopping lamivudine administration was also similar ($P > 0.2$) between the two groups (reactivation patients, median 11 months, 25–75% range 8–13 months vs. non-reactivation patients, median 6 months, 25–75% range 5–13 months).

In 23 patients who were negative for HBe antigen after treatment, HBcrAg levels were significantly higher ($P = 0.011$) in the reactivation patients ($n = 12$, median 4.8 log U/mL, 25–75% range 4.0–5.0 log U/mL) than in non-reactivation patients ($n = 11$, median 3.0 log U/mL, 25–75% range 2.5–4.4 log U/mL). In contrast, levels were similar ($P > 0.2$) between the two groups in 11 patients who were positive for HBe antigen after treatment (reactivation patients $n = 8$, median 5.9 log U/mL, 25–75% range 5.1–6.1 log U/mL vs. non-reactivation patients $n = 3$, median 5.6 log U/mL, 25–75% range 2.5–8.0 log U/mL).

The ability of HBcrAg concentration to predict non-recurrence of hepatitis was analyzed using a ROC curve (Fig. 1), and the area under the curve was as wide as 0.764. The point at which specificity was 0.8 and sensi-

Table 1 Comparison of clinical characteristics at the onset and cessation of lamivudine administration between patients with and without reactivation of hepatitis

Characteristics	Reactivation of hepatitis		*P*-value†
	Positive ($n = 20$)	Negative ($n = 14$)	
Demographics			
Age (years)	44 (38–51)	50 (35–59)	NS
Sex (male/female)	13/7	7/7	NS
HBV genotype (B/C)	0/16	2/9	NS
At onset of lamivudine administration			
ALT (IU/mL)	103 (57–234)	211 (76–515)	NS
HBeAg (positive)	12 (60%)	4 (29%)	NS
HBV DNA (log copy/mL)	7.1 (6.1–8.1)	6.0 (5.3–7.4)	NS
HBcrAg (log unit/mL)	6.2 (5.6–7.7)	6.4 (5.0–6.6)	NS
At cessation of lamivudine administration			
Duration of lamivudine (months)	12.7 (10.4–16.3)	10.3 (6.4–17)	NS
ALT (IU/mL)	30 (15–36)	21 (15–24)	NS
HBeAg (positive)	8 (40%)	3 (21%)	NS
HBV DNA (log copy/mL)	<3.7 (<3.7–<3.7)	<3.7 (<3.7–<3.7)	NS
HBcrAg (log unit/mL)	4.9 (4.7–5.9)	3.2 (<3.0–4.5)	0.009

†Analysis of continuous variables performed using Mann–Whitney *U*-test; analysis of dichotomous variables performed using Fisher's exact test. Values shown as median (25–75% range) or *n* (%).
ALT, alanine aminotransferase; HBcrAg, hepatitis B core-related antigen; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; NS, not significant.

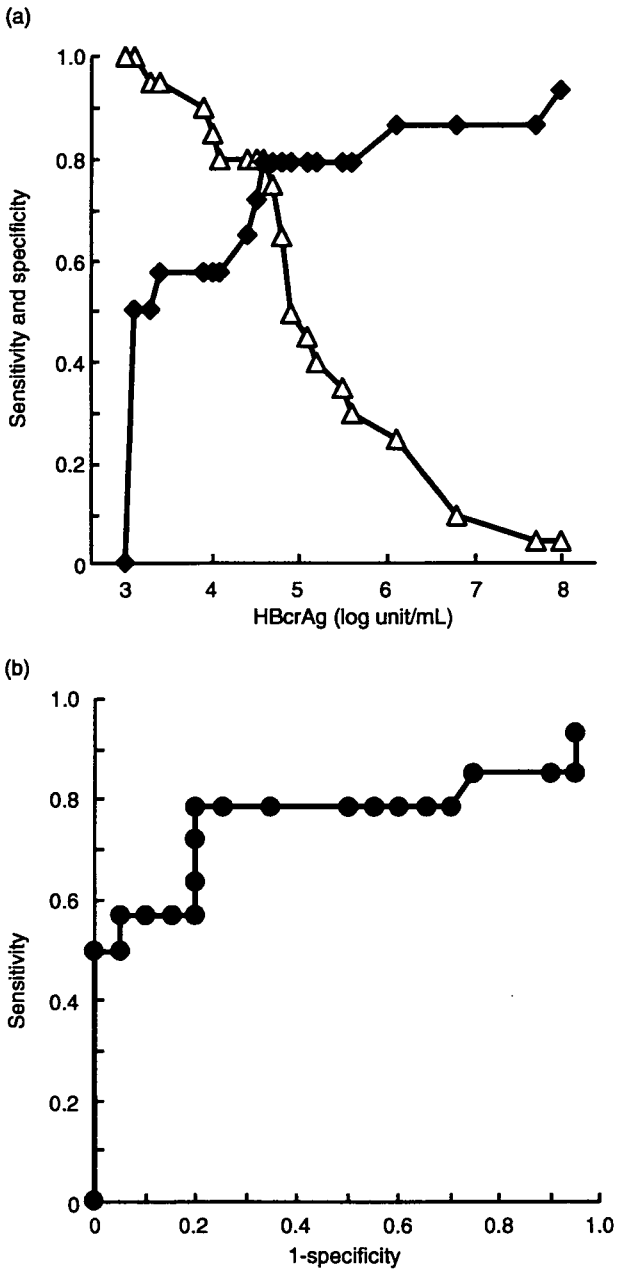

Figure 1 Receiver-operator characteristic (ROC) analysis of hepatitis B core-related antigen (HBcrAg) concentration for predicting patients without risk of reactivation of hepatitis within 12 months after halting lamivudine administration. (a) Sensitivity (■) and specificity (△) curves according to concentration of HBcrAg. (b) The ROC curve with the area under curve of 0.764.

tivity approximately 0.8 was deemed best for halting treatment without the risk of hepatitis recurrence. This point corresponds to an HBcrAg concentration of 4.1–4.6 log unit/mL.

DISCUSSION

THE REACTIVATION OF hepatitis following lamivudine administration was defined in the present study as an elevation of serum ALT level to more than 80 IU/L because we sought to find a more reliable indicator for safer discontinuation of lamivudine administration. Under these conditions, the majority (20/34) of patients showed reactivation of hepatitis within 12 months, as has been previously reported.[5,6] HBV DNA levels at the time of discontinuing lamivudine were similarly low between the two groups of patients, which is understandable as an undetectable reading typically indicates HBV remission following lamivudine therapy. However, HBcrAg levels were significantly higher in reactivation patients, implying that HBcrAg level is a better marker than HBV DNA level for predicting non-reactivation of hepatitis after discontinuing lamivudine administration especially in patients without HBe antigen.

In this study, ROC curve analyses showed a wide area under the curve of 0.764 in predicting the non-reactivation of HBV with HBcrAg level. If the corresponding cutoff is set at 4.5 logU/mL, then both specificity and sensitivity are as high as approximately 0.8. To obtain a higher specificity of 0.9, the cutoff value of HBcrAg concentration should be set at 4.0 log unit/mL. In this case, the sensitivity would still be nearly 0.6. The cutoff value of HBcrAg for predicting the non-relapse of hepatitis in our study is a little higher than that reported by Shinkai *et al.* (3.4 logU/mL).[14] Because numbers of patients analyzed were small in both studies, further studies are required to confirm the most appropriate cutoff value. It is noteworthy that this cutoff value may also differ among genotypes, which have been reported to be correlated with outcome of chronic HBV infection.[15] However, as over 90% of the patients had genotype C in this study, reactivation could not be analyzed in relation to HBV genotypes.

The HBV is an enveloped DNA virus containing a relaxed circular DNA genome which is converted into a covalently closed circular DNA (cccDNA) episome in the nucleus of infected cells and serves as transcriptional template for the production of viral RNA.[11,16,17] Reverse transcription of pregenomic RNA and second-strand DNA synthesis then occur in the cytoplasm within viral

capsids formed by the HBV core protein. Because lamivudine inhibits reverse transcription of pregenomic RNA, it directly suppresses production of HBV virions, and serum HBV DNA levels decrease rapidly after the initiation of lamivudine administration. However, the production of viral proteins is not suppressed by lamivudine as this process does not include reverse transcription. Furthermore, it has been reported that the amount of cccDNA, which also serves as a template for mRNAs, decreases quite slowly after commencement of administration of nucleoside analogs.[18,19] Thus, it is possible that serum HBcrAg levels reflect the cccDNA level in hepatocytes more accurately than serum HBV DNA. High levels of cccDNA are considered to be associated with hepatitis reactivation because they preceed reactivation of viral replication and consequent elevation of HBV DNA level in serum.

Lamivudine has already been eliminated from first line therapy in naïve chronic hepatitis B patients due to a higher incidence of developing resistant mutations than new antiviral agents, such as adefovir dipivoxil and entecavir.[20] However, the distinct characteristic of the HBcrAg assay under lamivudine therapy that is different from other HBV DNA assays is that lamivudine suppresses production of HBV virions by inhibiting reverse transcription of pregenomic RNA, but does not suppress the production of viral proteins, in which reverse transcription is unnecessary. Thus, it is possible that the HBcrAg assay may also useful for patients undergoing entecavir or adefovir dipivoxil administration because the main mechanism of suppressing HBV replication is similar between lamivudine and other antiviral agents. As a considerable number of patients who started lamivudine administration in the past are still taking this treatment now, the present study may be valuable for such patients when they consider changing therapies in the future. Additionally, further studies are required to determine whether the HBcrAg assay is indeed applicable to antiviral agents other than lamivudine.

In conclusion, significant markers that can predict reactivation of hepatitis after discontinuing lamivudine administration are clinically valuable because the reactivation of hepatitis is a fundamental problem in lamivudine therapy. Our results suggest that patients with an HBcrAg level of less than 4.5 log unit/mL may stop lamivudine administration with a lower risk of reactivation. The present study is a preliminary one because the patients enrolled were selected retrospectively without standardized criteria for stopping lamivudine and the number of patients enrolled was not large; however, the results may be valuable for patients with hepatitis B undergoing lamivudine therapy as such a diagnostic marker has rarely been reported. Further studies are required to establish the clinical significance of the HBcrAg assay in the treatment of hepatitis B.

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Short Communication

Prevalence of hepatitis B virus infection in Japanese patients with HIV

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Patients with HIV infection are frequently infected with hepatitis viruses, which are presently the major cause of mortality in HIV-infected patients after the widespread use of highly active antiretrovirus therapy. We previously reported that approximately 20% of HIV-positive Japanese patients were also infected with hepatitis C virus (HCV). Hepatitis B virus (HBV) infection may also be an impediment to a good course of treatment for HIV-infected patients, because of recurrent liver injuries and a common effectiveness of some anti-HIV drugs on HBV replication. However, the status of co-infection with HIV and HBV in Japan is unclear. We conducted a nationwide survey to determine the prevalence of HIV–HBV co-infection by distributing a questionnaire to the hospitals belonging to the HIV/AIDS Network of Japan. Among the 5998 patients reported to be HIV positive, 377 (6.4%) were positive for the hepatitis B surface antigen. Homosexual men accounted for two-thirds (70.8%) of the HIV–HBV co-infected patients, distinct from HIV–HCV co-infection in Japan in which most of the HIV–HCV co-infected patients were recipients of blood products. One-third of HIV–HBV co-infected patients had elevated serum alanine aminotransferase levels at least once during the 1-year observation period. In conclusion, some HIV-infected Japanese patients also have HBV infection and liver disease. A detailed analysis of the progression and activity of liver disease in co-infected patients is needed.

Key words: co-infection, hepatitis B, HIV, liver disease.

INTRODUCTION

HEPATITIS B VIRUS (HBV) infection is a major public health problem worldwide, along with hepatitis C virus (HCV) and HIV infections. In the USA, the estimated prevalence of HBV is less than 1%, but approximately 1 million people are persistently infected.[1] The prevalence of HIV in the USA is also <1%, and the virus is estimated to have infected approximately 800 000 people.[2] Because of the common transmission routes, that is, parenteral transmission routes, many people with HIV infection are also infected with HBV. Among the HIV-positive people in the USA, the prevalence of HBV co-infection is 6–14%.[1,2] Before the introduction of highly active antiretroviral therapy (HAART) in 1996, most patients with HIV infection died of HIV-associated opportunistic infections, such as *Pneumocystis jiroveci* pneumonia and cytomegaloviral infection. Since the widespread use of HAART, the mortality associated with HIV infection has declined. However, the reduction in mortality due to opportunistic infection, has left patients co-infected with HIV and hepatitis viruses faced with the menace of progressive liver diseases due to HBV infection,[3,4] in addition to HCV infection.[5]

HBV co-infection or superinfection of HIV-infected patients leads to several problematic situations. First, HBV infection tends to develop into persistent infection in HIV-infected patients,[1,6,7] which is a rare event in healthy adults, although it substantially depends on the genotype of HBV.[8] It results in the acceleration of the development of cirrhosis and eventually hepatocellular carcinoma. Second, some nucleoside reverse transcriptase inhibitors (NRTI) used in HAART also have

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inhibitory effects on the replication of HBV.[9–12] A careless administration or discontinuation of NRTI on HIV–HBV co-infected patients may cause reactivation and/or aggravation of hepatitis B. In addition, the administration of anti-HBV drugs in HIV–HBV co-infection may lead to the development of drug resistance.[11,12] Third, liver injury occurs more frequently in patients on HAART who are co-infected with HIV and HBV than those infected with HIV only.[9,10]

Importantly, co-infection with HIV and HCV increases the morbidity and mortality of HIV-infected patients in Japan,[13] where the prevalence of HIV infection is increasing linearly, and is exceptionally high among developed countries.[14] There are more than 14 000 HIV-positive people in Japan as of 2006, according to the AIDS National Survey in Japan,[14] and approximately 0.8 million chronic HBV carriers.[15] However, the prevalence of co-infection with HIV and HBV in Japan has not been clarified to date. Therefore, we conducted a nationwide study by distributing a postal mail-based questionnaire to the hospitals belonging to the HIV/AIDS Network of Japan.

PATIENTS AND METHODS

IN THE QUESTIONNAIRE, the following information was obtained from the hospitals regarding the number of patients who visited the hospitals at least once between January and December in 2006: (i) the number of HIV-positive patients; (ii) the number of hepatitis B surface antigen (HBsAg)-positive patients among (i); (iii) the number of patients among (ii) who were determined at least once to have a serum alanine aminotransferase (ALT) level higher than 100 IU/L; (iv) the number of HIV-positive patients that contracted HIV from blood products; (v) the number of HBsAg-positive patients among (iv), (vi) the number of patients among (v) who were determined at least once to have a serum ALT level higher than 100 IU/L; (vii) the number of HIV-positive patients among homosexual men, (viii) the number of HBsAg-positive patients among (vii), (ix) the number of patients among (viii) who were determined at least once to have a serum ALT level higher than 100 IU/L; (x) the number of HIV-positive patients that contracted HIV through intravenous drug use (xi) the number of HBsAg-positive patients among (x), (xii) the number of patients among (xi) who had at least one determination of a serum ALT level more than 100 IU/L; (xiii) the number of HIV-positive patients whose transmission routes were classified as "others"; (xiv) the number of HBsAg-positive patients among (xiii); and (xv) the number of patients among (xiv) who were determined at least once to have a serum ALT level higher than 100 IU/L.

The questionnaire was sent to the 372 hospitals belonging to the HIV/AIDS Network of Japan by mail. Answers were mostly returned by mail and in some cases by fax. The list of the hospitals in the HIV/AIDS Network of Japan can be viewed at http://www.acc.go.jp/mLhw/mLhw_frame.htm.

RESULTS

THE QUESTIONNAIRE WAS sent to all 372 hospitals that were on the list of the hospitals in the HIV/AIDS Network of Japan in January 2006. Two hundred and seven hospitals (55.6%) responded within the indicated period. In total, 5998 patients were reported to be HIV positive. The collection rate of 55.6% was higher than that (47.8%) for a questionnaire HIV–HCV co-infection study carried out in 2003.[15] It may appear rather low, particularly considering the number of reported HIV-positive people in 2006, which was approximately 14 000, according to the AIDS National Survey in Japan.[14] However, not all of the HIV-positive people were going to hospitals, and the answers to the questionnaire were obtained from most of the major hospitals in the HIV/AIDS Network in big cities around Japan. This suggests that not all, but a majority of HIV-positive Japanese patients were enrolled in the study.

Among the 5998 patients reported to be HIV positive, 377 (6.3%) patients were positive for HBsAg (Table 1). Of these 377 patients, 122 (32.4%) had elevated serum ALT levels at least one time during the 1-year observation period.

The HBV prevalence rates, when fractionated by the routes of transmission, were as follows: among the 508 HIV-positive patients who contracted HIV from blood products, such as unheated concentrated coagulation factors, only 30 (5.9%) were HBsAg positive, which shows a marked contrast to the prevalence of HCV in this cohort (Fig. 1).[16] Among the 23 intravenous drug users, three (13.0%) were HBsAg positive. Among the 3213 HIV-positive patients who were homosexual men, 267 (8.3%) were HBsAg positive. In the remaining 2254 patients who were HIV-positive and whose route of HIV transmission was classified as "others", most contracted HIV heterosexually. This number (2254) showed a substantial increase from the 1316 obtained in the questionnaire for the HIV–HCV co-infection study in 2003, while the total number of HIV-positive patients increased from 4877 to 5998.[16] Among these, 77 (3.4%)

Table 1 Prevalence rates of hepatitis B virus infection among HIV-positive patients

Routes of transmission	No. patients	HBsAg positive (% in HIV positive according to route)	ALT >100 IU/L (% in HBsAg positive according to route)
Blood products	508 (5.9%)	30 (40.0%)	12
Homosexual men	3213 (8.3%)	267 (32.2%)	86
Drug addicts	23 (13.0%)	3 (66.7%)	2
Others (heterosexual etc.)	2254 (3.4%)	77 (28.6%)	22
Total	5998	377 (6.3%)	122 (32.4%)

ALT, serum alanine aminotransferase; HBsAg, hepatitis B surface antigen.

were HBsAg positive. In terms of the route of HIV infection, 267 (70.8%) of the 377 patients were homosexual men among the HIV–HBV co-infected patients. This shows a contrast to the status of HIV–HCV co-infection, in which the majority of HIV–HCV co-infected Japanese patients contracted both viruses from blood products.[16]

There were one or more HIV-positive patients in 154 (74.4%) of the 207 hospitals in the HIV/AIDS Network of Japan (Table 2). Twenty four (11.6%) of 207 hospitals had 20–49 HIV-positive patients, and 16 (7.7%) hospitals had 50 or more HIV-positive patients. There were one or more patients who were co-infected with HIV and HBV in 64 (30.9%) of the 207 hospitals. There were 10 or more HIV–HBV co-infected patients in nine (4.3%) hospitals, all of which had 50 or more HIV-positive patients (Table 2). HIV–HBV co-infected patients were concentrated in specific hospitals in big cities around Japan. In particular, in the Kanto area, HIV–HBV co-infected patients were concentrated in the HIV/AIDS Network hospitals in the Tokyo city area.

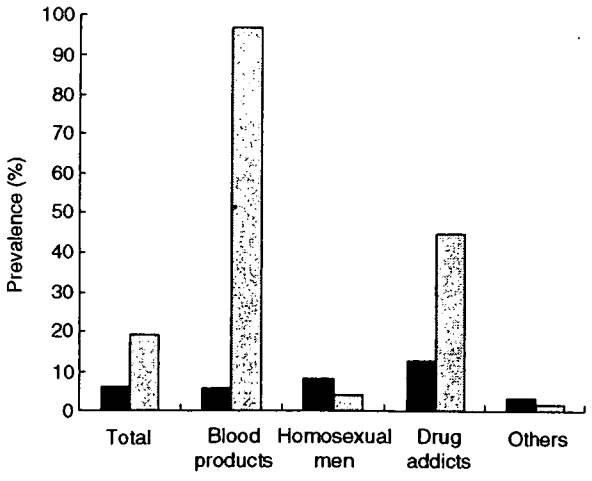

Figure 1 Prevalence rates of persistent hepatitis B virus and hepatitis C virus infections in the HIV-positive population sorted by the HIV risk group. (■), HBsAg, hepatitis B surface antigen; (⊡), anti-HCV, antibody to hepatitis C virus. *Prevalence rates of anti-HCV are obtained from Koike K *et al.*[16]

DISCUSSION

ALONG WITH THE increase in the number of HIV-infected patients in Japan, co-infection with HIV and hepatitis viruses has become a major medical issue. HBV infection of HIV-positive patients raises several difficult problems: HBV infection tends to develop into persistent infection, even in adults; some NRTI used in HAART also have inhibitory effects on the replication of HBV, the improper administration, or discontinuation of which may lead to drug resistance; and HIV–HBV co-infected patients on HAART have liver injuries more frequently than HIV-monoinfected patients. It is important to determine the status of HBV infection in HIV-positive patients.

According to the statistics of the Ministry of Health, Labor, and Welfare of Japan, the number of reported HIV-positive people was slightly over 14 000 in 2006.[14] In the present study, 6.4% of HIV-positive patients were positive for HBsAg, the most reliable marker for ongoing HBV infection. It might have been advantageous if

Table 2 Number of hospitals categorized according to the number of patients infected with HIV and those co-infected with HIV and hepatitis B virus (HBV)

No. HIV (+)/ HBV (+)	No. HIV(+)				
	0	1–19	20–49	50+	Total
0	53	76	13	1	143
1–9	0	38	11	6	55
10+	0	0	0	9	9
Total	53	114	24	16	207

serum HBV-DNA levels were determined, but unfortunately, HBV-DNA level determination was not a routine laboratory test in most hospitals. In addition, considering that the antibody to the hepatitis B core antigen might be the only marker of ongoing HBV infection in some immuno-compromised patients, it would also be advantageous if this viral marker were available. These issues should be investigated in future studies. Comments from hospitals to the questionnaire included one indicating that not all HIV-positive patients underwent a test for serum HBsAg, suggesting the actual prevalence of HBsAg in HIV-infected patients might be higher than 6.4%.

In a previous questionnaire study of HIV–HCV co-infection, the prevalence of HCV infection among HIV-infected patients was 19.2%;[16] the prevalence of HBV infection (6.4%), is one-third of it. The lower positivity for HBsAg than for the anti-HCV antibody among those who contracted HIV through blood products accounts for this difference: almost all (96.9%) of the patients who contracted HIV through blood products were also anti-HCV antibody positive.[16] It should be noted that among the homosexual male patients who were HIV positive, 8.3% were HBsAg positive, which is twice as high as that of the anti-HCV antibody in these populations. A higher prevalence of HBV infection as a sexually transmitted infection than that of HCV[17] may explain the high prevalence of HBV infection in HIV-positive homosexual men. Similarly, a HBV prevalence of 3.4% in heterosexually transmitted HIV-positive patients is higher than that of the general Japanese population of the same age.[15]

Of the 377 patients who were HBsAg positive, 122 (32.4%) had elevated serum ALT levels at least once in the 1-year observation period. In this type of study using a questionnaire, it is difficult to obtain the details of patients' data, including age, body weight, and the degrees of liver injuries and fibrosis. If detailed items were included in the questionnaire, then the collection rate would be low. This time, to obtain a high collection rate, we asked whether the patients with HBsAg showed an elevated ALT level higher than 100 IU/L at least once during the 1-year observation period. We thereby do not have details on liver disease in HIV–HBV co-infected patients in the current study. Nonetheless, one-third of HIV–HBV co-infected patients have moderate liver injuries, either chronic hepatitis B or adverse effects of drugs, and are waiting for an aid for the amelioration of liver disease. A detailed analysis of the progression and activity of liver disease in HIV–HBV co-infected patients is expected.

The collection rate of the present questionnaire from the hospitals belonging to the HIV/AIDS Network was 55.6% (207 of 372). This was higher than that (47.8%) in the HIV–HCV co-infection questionnaire study carried out in 2003. The reason for this increase is not clear, but presumably the questionnaire conducted in 2003 has raised awareness among hospital staff regarding the relevance of hepatitis virus and HIV co-infection in clinical practice.

In the current study, both Japanese patients and those of other nationalities/ethnicities were included in the study. Although the ratio of newly diagnosed HIV-positive foreign people has been declining to approximately 10% in 2006, the one in total HIV positive still accounts for approximately 25% in Japan. Because the rates of the HBV carrier are different among countries, it is ideal to analyze the HBV prevalence separately according to the nationalities/ethnicities. However, in the current survey to the hospitals in HIV/AIDS Network of Japan, nationality/ethnicity was not itemized in order to make the questionnaire simple. If we would attempt to obtain such data under the approval of the ethical committee in each hospital, the response rate to questionnaire would be extremely lowered.

To establish measures that decrease the morbidity and mortality of HIV–HBV co-infected patients, it is essential to determine the current status of co-infection. In the present study, the number and transmission routes of HIV–HBV co-infected patients in Japan were determined for the first time, although detailed information on the severity and progression of liver disease in HIV–HBV co-infected patients has not been obtained yet. Undoubtedly, this will be the first step towards improving the prognosis and quality of life of Japanese patients co-infected with HIV and HBV.

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Clinical Transplantation

Histologic eosinophilia as an aid to diagnose acute cellular rejection after living donor liver transplantation

Kishi Y, Sugawara Y, Tamura S, Kaneko J, Matsui Y, Makuuchi M. Histologic eosinophilia as an aid to diagnose acute cellular rejection after living donor liver transplantation.
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Abstract: The significance of histologic eosinophilia in the diagnosis of acute cellular rejection (ACR) after living donor liver transplantation was evaluated. A retrospective analysis was performed on 185 liver biopsy specimens to determine the presence of eosinophil infiltration around the portal tracts. Data were collected and analyzed to determine whether there was a correlation between ACR and the maximum eosinophil counts per portal triad (Em) and the rate of portal triads that included at least one eosinophil (Er). A receiver operating characteristic curve revealed the best cut-off value of Em and Er as 2% and 8% respectively. The sensitivity and specificity of an Em of two to predict ACR were 54% and 84% respectively. The sensitivity and specificity of Er were 72% and 65% respectively. One-way analysis of variance revealed that both Em and Er correlated with ACR severity. Histologic eosinophilia can be a useful parameter for confirming the occurrence of ACR and for evaluating ACR severity.

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Key words: acute cellular rejection – histological eosinophilia – liver transplantation

Abbreviations: ACR, acute cellular rejection; AECb, absolute eosinophil count three d before biopsy; AECo, absolute eosinophil count on the day; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil; LDLT, living donor liver transplantation.

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Liver biopsy remains the gold standard for diagnosis of acute cellular rejection (ACR) after liver transplantation. No other serum markers are sufficiently specific. Blood eosinophilia was first formally described in kidney recipients as a diagnostic marker of acute rejection (1). Subsequently, several reports (2, 3) suggested the usefulness of blood eosinophilia as a predictor of ACR after liver transplantation. In our previous report (4), the blood eosinophil count three d before or on the day of ACR was related to the severity of ACR. The sensitivity of blood eosinophilia for predicting ACR, however, was low (5, 6). The presence of tissue eosinophils (histologic eosinophilia) as a diagnostic feature of ACR was recently validated, but only in one center (2, 7).

Furthermore, these previous studies were based on the subjects of deceased donor liver transplantation. The inflammatory cells differ between deceased donor liver transplantation and living donor liver transplantation (LDLT) because of the longer cold ischemic time followed by more severe reperfusion in deceased donor liver transplantation. One experiment showed local eosinophilic infiltration after ischemia/reperfusion injury of rat intestine (8). There might, therefore, be a difference in the eosinophil response between LDLT and deceased donor liver transplantation cold ischemic

time, postoperatively. Here we evaluated whether histologic eosinophilia is a useful indicator of ACR in LDLT.

Patients and methods

From January 1996 to June 2005, 334 living donor liver transplantations were performed at our institution. Among them, 398 biopsies were performed in 181 patients within six months after transplantation for suspected ACR on the basis of liver dysfunction. Blood chemistry was examined every day or every other day during hospitalization, and once every two wk or once a month in the outpatient clinics. If all liver function data (aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, and total bilirubin) were elevated compared with previous levels and bile duct complication was ruled out by ultrasound, biopsy was indicated. Protocol biopsy was not performed. Among the biopsies, 263 biopsy specimens from 139 patients [74 men, 65 women; mean age 39.6 ± 1.6 (standard error)] were available for review and these were the subjects of this study. The indications for LDLT included hepatitis C virus-related cirrhosis (n = 36), hepatitis B virus related cirrhosis (n = 13), cirrhosis of other etiologies (n = 8), biliary atresia (n = 26), primary biliary cirrhosis (n = 27), primary sclerosing cholangitis (n = 3), autoimmune hepatitis (n = 6), fulminant hepatic failure (n = 11), metabolic diseases (n = 5), and others (n = 4). Seventy-eight specimens were excluded because they contained fewer than five portal triads, which was not sufficient for a diagnosis of ACR (9).

In all specimens, the diagnoses of ACR was evaluated by highly experienced pathologists and graded into four classes according to the Banff scheme (6) [grade 0 (G0): no evidence of rejection; grade 1 (G1): mild rejection; grade 2 (G2): moderate rejection; and grade 3 (G3): severe rejection]. The degree of portal infiltration of lymphocytes (P0-3), bile duct inflammation or damages (B0-3), and venous endothelial inflammation (V0-3) in the Banff scheme were evaluated. Eosinophil infiltration was not considered for the diagnosis. These specimens were reviewed retrospectively to count the numbers of eosinophils that infiltrated around the glisson in a blind manner. To quantify the degree of eosinophilia, the maximum eosinophil counts per portal triad within the specimen (Em) and the rate of portal triads that included at least one eosinophil (Er, number of portal triads which include at least one eosinophil/total number of portal triads contained in the specimen) were calculated for each specimen.

Tacrolimus and methylprednisolone were used for postoperative immunosuppression (10). As a first treatment for ACR, steroid recycle therapy, in which intravenous methylprednisolone at a dose of 20 mg/kg was tapered by half each day until reaching the same dose as before therapy, was administered. Here, if transaminase and bilirubin levels improved to normal levels and did not increase again during the following month, the ACR was defined as steroid responsive. In contrast, if liver dysfunction recurred again within one month, followed by biopsy-proven ACR, the ACR was defined as steroid-resistant (4).

The sensitivity and specificity of Em, Er, AECb and AECo to predict ACR were calculated to draw receiver operating characteristic curves. Biopsy episodes were divided to early (within 30 postoperative days) and late (over 30 postoperative days) period and the sensitivity and specificity stratified by the time after LDLT were calculated separately. The Em and Er values were compared with the degree of ACR to analyze their predictive values. Those values were then compared with the results of ACR treatment that was responsive or resistant to steroid recycle therapy. The correlation of blood and histologic eosinophilia was evaluated by comparing Em or Er rate with absolute eosinophil count three d before biopsy (AECb) or on the day of biopsy (AECo). The uni- and multivariate analysis were performed to evaluate the factors related with ACR among P, B, V scores, Em, or Er.

Statistics

Data are expressed as mean ± standard error. Sensitivity and specificity of eosinophilia to predict ACR or improvement of ACR was evaluated using a receiver operating characteristic curve to determine the best cut-off value of Em or Er. Comparisons among the different grades of ACR were performed by one-way analysis of variance. Correlations between blood (AECb or AECo) and histologic (Em or Er) findings were evaluated using Spearman's rank-correlation coefficient. For the evaluation of predictors of ACR, Wilcoxon signed rank test was used and multivariate analysis was performed by logistic regression test. A p-value of < 0.05 was considered statistically significant.

Results

Histologic eosinophilia for ACR prediction

The most appropriate cut-off value of Em to minimize the false positives and false negatives was two; however, the sensitivity and specificity of an

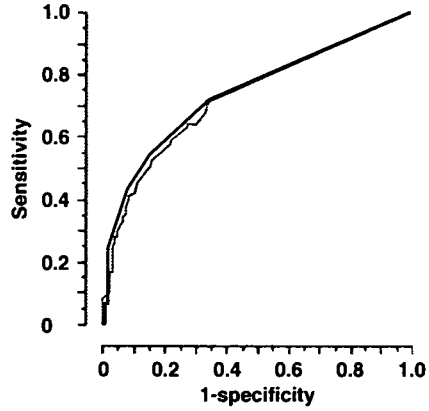

Fig. 1. Receiver operating characteristic curve to evaluate the sensitivity and specificity of histological eosinophilia. Black line, the maximum eosinophil counts per portal triad (Em); Grey line, the rate of portal triads that included at least one eosinophil (Er).

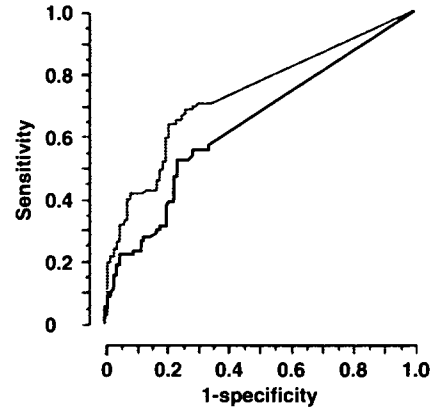

Fig. 2. Receiver operating characteristic curve to evaluate the sensitivity and specificity of blood eosinophilia. Black line, absolute eosinophil count three d before biopsy (AECb); Grey line, absolute eosinophil count on the day (AECo).

Table 1. Sensitivity and specificity of Em ≥ 2 and Er ≥ 8% to predict ACR and the impact of timing of ACR

	Em ≥ 2		Er ≥ 8%	
	Sensitivity	Specificity	Sensitivity	Specificity
Total (%)	54	84	72	65
Early (≥30 pod, %)	57	86	81	65
Late (>30 pod, %)	50	81	53	66
p-value (early vs. late)	0.65	0.79	0.01	>0.99

ACR, acute cellular rejection; pod, postoperative day; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil.

Table 2. Sensitivity and specificity of AECb ≥ 68 cells/mm^3 and AECo ≥ 82 cells/mm^3 to predict ACR and the impact of timing of ACR

	AECb ≥ 68 cells/mm^3		AECo ≥ 82 cells/mm^3	
	Sensitivity	Specificity	Sensitivity	Specificity
Total (%)	53	77	64	79
Early (≥30 pod, %)	50	73	73	75
Late (>30 pod, %)	57	84	43	88
p-value (early vs. late)	0.71	0.32	0.01	0.42

ACR, acute cellular rejection; pod, postoperative day; AECb, absolute eosinophil count three d before biopsy, AECo, absolute eosinophil count on the day.

Em of two to predict ACR was 54% and 84% respectively (Fig. 1). The most appropriate cut-off value of Er to minimize the false positives and false negatives was 8%, however, the sensitivity and specificity of an Er of 8% to predict ACR was 72% and 65% respectively. The sensitivity of Er ≥ 8% to predict ACR was higher in early period than that in late period (Table 1).

Blood eosinophilia to predict ACR

The most appropriate cut-off value of AECb and AECo was 68 cells/mm^3 (sensitivity 53%, specificity 77%) and 82 cells/mm^3 (sensitivity 64%, specificity 79%; Fig. 2). The sensitivity of AECo ≥ 82 to predict ACR was higher in early period than that in late period (Table 2). When eosinophilia was defined as > 400 cells/mm^3, the sensitivity and of specificity of AECb were 16% and 97%, respectively, and those of AECo were 21% and 97%.

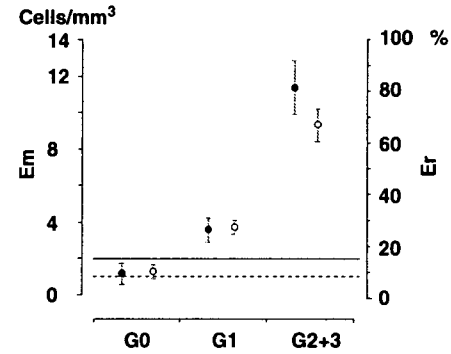

Fig. 3. Em (closed circle) and Er rates (open circle) values stratified by grade of rejection. Solid and dashed lines represented the cut-off value of Em (two cells/portal triad) and Er (8%) respectively.

Histologic eosinophilia and ACR severity

The number of specimens classified to G0, G1, G2, and G3 was 95, 76, 13, and 1 respectively (Fig. 3). There was a significant relationship between ACR

severity and Em and ACR severity and Er ($p < 0.0001$ for both comparisons). An ACR of G2 or G3 was more frequently resistant to steroid recycle therapy than an ACR of G1 ($p = 0.04$). There was no significant difference, however, in the Em ($p = 0.63$) or Er ($p = 0.38$) between ACR episodes that were responsive and resistant to steroid recycle therapy.

Correlation between blood and histologic eosinophilia

The ρ-value of Spearman's rank-correlation coefficient between blood and histologic eosinophil counts ranged between 0.35 and 0.45 (Table 3).

Uni- and multi-variate analysis

In univariate analysis, all of the P, B, V scores, Em, and Er were significantly higher in ACR group than in non-ACR group. Multivariate analysis, however, revealed only P, B, and V scores remained as significant factor related with ACR (Tables 4 and 5).

Discussion

The ideal method of diagnosing ACR is based on suspected ACR with high sensitivity before biopsy and confirmation of the diagnosis with high specificity. Our criterion to perform liver biopsy is an increase in all the liver function data. With this criterion, ACR was correctly diagnosed in 41% of all the biopsy cases (164/398). Unfortunately, absolute blood eosinophil count is not an ideal predictor of ACR because of its low sensitivity. The present result failed to reveal the equivalent impact on diagnosis of ACR between histologic eosinophilia and P, B, V scores. Histologic eosinophilia, however, might be useful for evaluating the severity of ACR after LDLT or differential diagnosis with hepatitis (11).

This result is consistent with a previous study on deceased donor liver transplantation. The Royal Free Hospital group (6) used histologic eosinophilia in the diagnosis and grading of ACR. In their ACR grading system, the maximum eosinophil number in the portal tract was included in addition to the usual items, i.e. portal inflammation, endothelilitis, and bile duct damage. They were scored 0 to 3 according to the maximum eosinophil counts 0, 1–4, 5–9, 10, or more. Ben-Ari et al. (12) demonstrated that mean eosinophils per portal tract was different/depending on the degree of ACR; 0.41 in mild rejection specimens and 27.38 in moderate-to severe rejection specimens. Foster et al. (4) evaluated the average number of eosinophils per portal tract, which was 11.4 in the rejection group vs. 1.0 in the non-rejection group.

The present analysis revealed that eosinophilia did not correlate with the response to ACR. This might be due to the smaller numbers of subjects (52 responsive vs. 38 resistant) or the influence of steroids, which downregulate eosinophilia (13). The response of graft eosinophils to corticosteroids might be related to the role of various cytokines in the pathogenesis of rejection.

In conclusion, histologic eosinophilia is useful for confirming ACR after biopsy and for evaluating the severity of ACR after LDLT. Although the sensitivity of blood and histologic eosinophilia to predict ACR was low, the presence of eosinophilia can help the differential diagnosis of ACR. For this, clear quantification of eosinophilia with more appropriate cut-off value is needed by further analyses with a larger number of specimens.

Table 3. Spearman's rank-correlation coefficient value (ρ) and p-value

Items	ρ-value	p-value
AECb vs. Em	0.37	<0.0001
AECo vs. Em	0.45	<0.0001
AECb vs. Er	0.35	<0.0001
AECo vs. Er	0.44	<0.0001

Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil; AECb, absolute eosinophil count three d before biopsy; AECo, absolute eosinophil count on the day.

Table 4. Univariate analysis for the predictor of ACR

Factor	ACR	No ACR	p-value
P score	1.31 ± 0.06	0.48 ± 0.05	<0.0001
B score	1.16 ± 0.05	0.32 ± 0.05	<0.0001
V score	1.29 ± 0.05	0.26 ± 0.05	<0.0001
Em	4.8 ± 0.60	1.2 ± 0.6	<0.0001
Er	26.0 ± 2.5	46.8 ± 2.6	<0.0001

ACR, acute cellular rejection; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil.

Table 5. Multivariate analysis for the predictor of ACR

Factor	Odds ratio	95% CI	p-value
P score	0.001	0.000007–0.06	0.002
B score	0.004	0.0001–0.06	0.0003
V score	0.0003	0.00000–0.006	<0.0001
Em	0.81	0.004–195.1	0.94
Er	0.55	0.01–16.5	0.74

ACR, acute cellular rejection; Em, the maximum eosinophil counts per portal triad; Er, the rate of portal triads that included at least one eosinophil; CI, confidence interval.

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ELSEVIER

Blood Eosinophilia After Living Donor Liver Transplantation for Hepatitis C Virus–Related Cirrhosis

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ABSTRACT

Background. Differentiating between acute cellular rejection (ACR) and recurrent hepatitis C virus after liver transplantation in hepatitis C virus–positive patients is difficult, but vital for preventing graft loss.

Methods. The blood eosinophil counts 3 days before or on the day of biopsy were retrospectively reviewed to evaluate their value for predicting ACR in 91 biopsy samples from 45 patients.

Results. Eosinophil counts on the day of biopsy were significantly higher in the ACR group (n = 20) than in the non-ACR (n = 71) group, although the difference was negligible 3 days before the biopsy. A relative eosinophil count of 2% or an absolute eosinophil count of 200 cells/mm^3 predicted ACR with a specificity of 94% or 96%, respectively.

Conclusions. Blood eosinophil count on the day of biopsy can be helpful in the diagnosis of ACR in patients who underwent living donor liver transplantation for hepatitis C virus–related cirrhosis.

A RECENT study[1] of liver transplantation reported that hepatitis C virus (HCV) infection is associated with a 23% increase in mortality and a 30% increase in the rate of graft failure. HCV-induced graft hepatitis and fibrosis/cirrhosis occur in 75% to 80% and 10% to 30% of recipients, respectively, after 5 years.[2] Once liver cirrhosis is established, the cumulative probability of developing clinical decompensation is approximately 50% after 1 year, and survival after decompensation is extremely short.[3] Cholestatic hepatitis occurs in approximately 10% of patients infected with HCV and leads to accelerated graft failure and death.[4] A current debate is whether the recipients of living donor liver transplantation (LDLT) are at risk for increased severity of HCV recurrence.[5–10] Acute cellular rejection (ACR), however, is a major complication that can lead to mortality. Thus, early diagnosis via liver biopsy is necessary to determine the appropriate treatment.

Differentiating ACR from recurrent HCV is critical, but difficult, especially in the early postoperative period.[11] Recently, Barnes et al reported that HCV-positive patients with ACR are less likely to have eosinophilia than HCV-negative patients with ACR. Thus, proposed that the eosinophil response might be suppressed in HCV-positive patients with ACR, and that ACR might be overdiagnosed if based on histopathology in these patients with normal eosinophil levels.[12] In the present study, we evaluated the efficacy of measuring eosinophil levels to diagnose ACR in HCV-positive LDLT patients.

MATERIALS AND METHODS

From June 1996 to June 2005, 80 HCV-positive patients underwent LDLT at the University of Tokyo Hospital. A total of 146 biopsies were performed in 64 (80%) patients during the 6-month period following LDLT. Preemptive interferon and ribavirin therapy was administered after LDLT. Protocol biopsies were not performed.[13] All biopsies were performed under the suspicion of ACR due to liver dysfunction. Blood chemistry was examined everyday or every other day during hospitalization, and either once every 2 weeks or once a month in the outpatient clinics after hospitalization. The following measures of liver function were analyzed: aspartate aminotransferase, alanine aminotransferase, gamma-glutamyl transpeptidase, alkaline phosphatase, and total bilirubin. When all measures were elevated compared with previous levels and bile

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