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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.

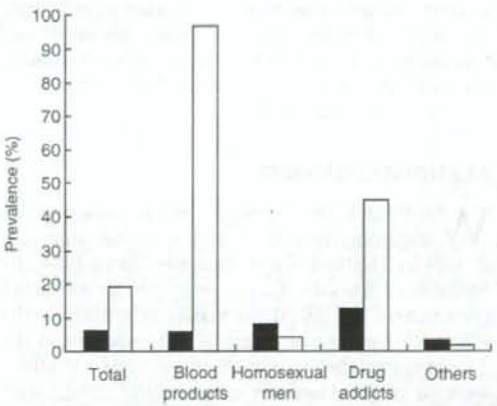

Figure 1 Prevalence rates of persistent hepatitis B virus and hepatitis C virus infections in the HIV-positive population sorted by the HIV risk group. (■), HBsAg, hepatitis B surface antigen; (□), anti-HCV, antibody to hepatitis C virus. *Prevalence rates of anti-HCV are obtained from Koike K *et al.*[16]

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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ORIGINAL ARTICLE

Spleen size of live donors for liver transplantation

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Abstract
Background Normal spleen size is not well defined for the adult population.
Methods Abdominal computed tomography (CT) scans of 238 consecutive living donors for liver transplantation were studied. Two methods for determining splenomegaly were applied. In Method N, a horizontal line was drawn to the left side from the most ventral point of the spleen. A perpendicular line was drawn from the central point of the aorta of the CT slice. The height of the cross point of the two lines was compared with the diameter of the aorta. In Method C, a perpendicular line was drawn from the most ventral point of the spleen. The distance between the posterior and anterior abdominal walls was partitioned in three parts, from dorsal to ventral and defined of Zones 1, 2, and 3, respectively. Donors were divided into two groups, those under age 40 and those over age 40.
Results The mean volume of the spleen was 123 ± 45 cm^3. Spleen volume was negatively correlated with age ($R = -0.32$, $p < 0.001$) and positively correlated with body mass ($R = 0.24$, $p < 0.001$). In donors under age 40, the most ventral point of the 96% of the spleens was below four times the diameter of the aorta (Method N). In Method C, 52% of the spleens were located in Zones 1 and 2. In donors over age 40, the most ventral point of the 96% of the spleens was below three times the diameter of the aorta (Method N). Totally 82% of the spleens were located in Zones 1 and 2 (Method C).
Conclusions Splenomegaly can be evaluated by the simple method on CT although the threshold must be changed by the age of the subject.

Keywords Splenomegaly · Live donor · Liver transplantation

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Introduction

The availability of computed tomography (CT) has facilitated the evaluation of live donors for liver transplantation [8]. A large spleen is sometimes considered to be splenomegaly in CT obtained to evaluate donor eligibility. Candidates with splenomegaly must be considered as contraindicated as live liver donors because it is related to hematopoietic and lymphopoietic, immunologic and circulatory disorders, as well as portal hypertension.

Although radiologists apply some simple methods to determine spleen size, normal spleen size is not well defined in the adult population [14]. Splenic index [9] and measurement volume using three-dimension CT [10] are devised for precise diagnosis of splenomegaly. Bezerra et al. [1] reported that correlation coefficient of a multiple by factors (length × width × thickness) was higher than single index each of length, width and thickness. However, measurement volume method is complicated. The simpler and easier method for judging the splenomegaly may be useful for a potential donor in the screening stage. We examined whether the conventional methods are appropriate for this aim using CT data of the live liver donors.

Methods

The subjects were 238 consecutive living donors evaluated for liver transplantation (135 men, 103 women; average age, 37 years; age range 17–66) that received CT between 1996 and 2005. Mean body mass (BM) was 61 ± 10 (standard deviation) kg. Criteria for donor selection were described elsewhere [17]. Serial abdominal transverse CT sections were taken at 0.5- or 1.0-cm intervals.

The most ventral point of the spleen was marked on the CT slice in which the spleen was shown at its maximum size. Two methods for determining splenomegaly were applied. A perpendicular line was drawn from the most ventral point of the spleen. In our method, Method N, a ventral line was drawn to the left side from the most ventral point of the spleen. A perpendicular line was drawn from the central point of the aorta in the CT slice. The height of the cross point of the two lines was examined (Fig. 1). The spleen was considered to be in Zone 1 when the cross point was below the ventral point of the aorta; in Zone 2 when the cross point was above the horizontal point of the aorta, but below two times the diameter of the aorta. Zones 3, 4, and 5 were defined similarly; that is, Zone 3 was the region below three times the diameter of the aorta, Zone 4 below four times the diameter of the aorta, and Zone 5 below five times the diameter of the aorta.

In Method C [4, 6], the distance between the posterior and anterior abdominal walls was partitioned in three parts, from dorsal to ventral, and defined as Zones 1, 2, and 3, respectively. Donors were divided into two groups, those under age 40 (Group A) and those over age 40 (Group B).

The whole spleen was outlined on each slice using a digital pen and tablet device and the enclosed area was measured using image-analysis software (Adobe Photoshop, Adobe systems Inc., San Jose, CA, USA). We divide calculated number of pixels by number of pixels per a square centimeter. This process was repeated for every 1 cm of CT slice. Finally, calculated square centimeter converted to cubic centimeter. BM and body surface area (BSA) were recorded at the time of the CT examination. BSA was calculated using the Whitington formula [16] as follows: BSA (m^2) = BM $(kg)^{0.425}$ × body height $(cm)^{0.725}$ × 0.007184. The correlation between the calculated spleen volume and age, BM, or BSA was analyzed by simple regression analysis using a statistical software package (SPSS 11.0 J, SPSS Japan Inc., Tokyo, Japan).

The values are given as the mean ± standard deviation. The difference in spleen volume by gender and age (groups) was analyzed with unpaired t test. A $p < 0.05$ was considered statistically significant in each analysis.

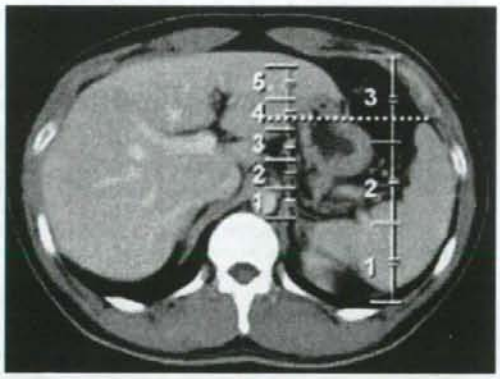

Fig. 1 Zones of Methods N and C. The most ventral point of the spleen was marked on the computed tomography slice in which the spleen was shown at its maximum size. A *broken line* was drawn ventrally from the most ventral point of the spleen

Results

The mean volume of the spleen was 123 ± 45 cm^3 (range 37–285 cm^3). The average BSA was 1.66 ± 0.18 m^2 (men 1.77 ± 0.14, women 1.53 ± 0.11 m^2). There was a significant difference between the mean spleen volume of men and women (132 ± 49 and 113 ± 38 cm^3, respectively, $p < 0.001$). Mean spleen volumes of Groups A and B were 137 ± 46 and 108 ± 41 cm^3, respectively. There was a significant difference between the groups, $p < 0.001$. Spleen volume was significantly correlated with age ($R = -0.32$, $p < 0.001$, Fig. 2). There was a significant correlation between spleen size and either BM or BSA ($R = 0.24$, $p < 0.001$; $R = 0.28$, $p < 0.001$).

In Group A (Table 1), 96% of the spleens were located in Zones 1–4 (Method N) and 52% in Zones 1 and 2 (Method C). In contrast, in Group B, 96% of the spleens were in Zones 1–3 (Method N) and 82% in Zones 1 and 2 (Method C).

Discussion

There are few reports on spleen size in the normal population [7]. The present study is new in that the subjects were live donors for liver transplantation who were considered to be completely healthy. In the present study, we determined the simple method for evaluating splenomegaly.

A previous study [11] revealed that normal spleen volume of the cadavers ranges from 26 to 250 cm^3 in Chinese population ($n = 30$, mean 110 cm^3, standard deviation 70 cm^3). Henderson and associates [6] reported a mean spleen volume of 219 cm^3 in a normal population, as calculated from CT, but the number of subjects was small ($n = 11$). Prassopoulos et al. [13] calculated a mean spleen volume from CT and reported that the mean value was

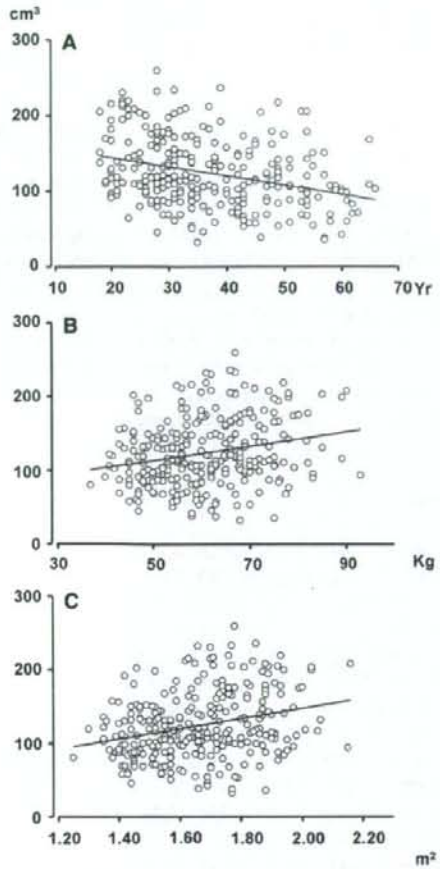

Fig. 2 Scatterplot of the spleen volume and age (**a**), body weight (**b**) and body surface area (**c**). The correlation was significant in each relation. A regression line is superimposed

Table 1 Distribution of the most ventral point of the spleen

Group	Zone in Method N					Zone in Method C		
	1	2	3	4	5	1	2	3
A								
n (%)	12(8)	45(30)	64(43)	21(14)	6(4)	5(3)	72(49)	71(48)
B								
n (%)	19(21)	52(57)	16(18)	4(4)	0(0)	14(15)	60(66)	17(19)

215 cm^3 in a normal population ($n = 140$). De La Grandmasion et al. [3] weighed the spleens of 684 postmortem Caucasian subjects. The subjects were selected from those that died of injury with a short survival time. They reported that mean spleen weight was 156 ± 87 g in males ($n = 355$) and 140 ± 78 g in females ($n = 329$). Spleen size on CT is relatively larger than that measured on postmortem. This may be due to the measurement method. Spleen size is measured after blood had been removed from spleen.

We found that spleen volume estimated by CT was significantly correlated with not only age, but also with BM and BSA. The results were consistent with previous data [2], but were discrepant with the data from another study [14] of autopsy cases. A weak correlation between spleen size and BM or BSA was also reported also by Prassopoulos and associates [13] who calculated the spleen volume by CT. One of the reasons for the discrepancy between our and Prassopoulos et al.'s results might be the subject age distribution. In our study, the ages ranged from 20 to 65 years and the ratio of those under 40 years of age was 62%; in their study, the ages ranged from 20 to 80 and the ratio of those under 40 years of age was only 20%. Meier and associates [12] reported that volume of adult spleen did not change significantly with age. In their report [12], a mean spleen volume age of 18–40 was 234 ml and age of 50–81 was 213 ml although the number of the subjects was small ($n = 57$).

We found the difference of spleen size by gender in Japanese population. The results were consistent with previous data [5]. However, previous data [13, 15] indicated no difference by gender in spleen size of 141 human fetuses and 153 children. The difference of spleen size by gender could be explained by the difference of the body size.

Conclusion

Spleen volume was measured by CT in 238 healthy donors for liver transplantation. Spleen size was significantly correlated with age and body size. Splenomegaly can be evaluated by the simple method on CT although the threshold must be changed by the age of the subject.

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Original Article

Double-dose double-phase use of second generation hepatitis B virus vaccine in patients after living donor liver transplantation: Not an effective measure in transplant recipients

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Aims: Post-transplant active immunization for chronic hepatitis B patients has been attempted in several studies with controversial results. We assessed the effect of a double-dose double-phase vaccination regimen among partial living donor liver recipients.

Methods: Eighteen patients who underwent liver transplantation (LT) for chronic hepatitis B and two non-hepatitis B virus (HBV)-infected patients who received hepatitis B core antibody (HBcAb)-positive donor organs were recruited 18–78 months after LT. All were on hepatitis B immunoglobulin (HBIG) mono-prophylaxis before and throughout vaccination, to maintain hepatitis B surface antibody (HBsAb) titers of more than 100 IU/mL. Recombinant hepatitis B surface antigen vaccine (40 μg) was administered intramuscularly during weeks 0, 4, 8, 24, 28 and 32.

Results: The patients consisted of 15 males and five females with a median age of 52 (39–59) years. None developed a sufficient HBsAb titer above 500 IU/mL by week 48. In two patients whose maximum HBsAb titer increased to above 300 IU/mL, we attempted to skip HBIG, but shortly thereafter the titer dropped below 100 IU/mL and HBIG administration was resumed. Although the HBIG dose was reduced during and after vaccination, cessation of administration was not achieved.

Conclusion: Double-dose double-phase use of second generation recombinant vaccine was not effective in this study population. The selected population should be targeted for a conventional vaccine regimen, and different approaches, such as strong adjuvant or pre-S containing protein, should be further tested in a larger number of patients after LT for chronic hepatitis B.

Key words: Hepatitis B vaccine, HBcAb positive donor, HBIG, lamivudine, liver transplantation, prophylaxis

INTRODUCTION

THE LONG-TERM use of hepatitis B immunoglobulin (HBIG) and/or nucleos(t)ide analog prophylaxis has dramatically improved survival rates after liver transplantation for hepatitis B virus (HBV)-related liver disease.[1] Historically, hepatitis B (HB) recurs in approximately 80% of liver transplant recipients with HBV-related liver diseases. The use of HBIG mono-prophylaxis has improved the rate of recurrent hepatitis to 35%.[2] Long-term use of HBIG therapy, however, is costly and there is insufficient evidence regarding the optimal length of administration. Lamivudine (LAM) mono-prophylaxis is less costly and is useful for decreasing the rate of hepatitis recurrence to less than 40%.[3,4] Emerging resistant strains are a concern in long-term follow-up for transplant recipients, however, and additional nucleos(t)ide analogs such as adefovir and entecavir are required.[5,6] The combination of HBIG and antiviral agents has decreased the rate of hepatitis B recurrence.[7,8]

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Active immunization against hepatitis surface antigens has been attempted in patients after liver transplantation for HB-related liver diseases.[9–11] Repeated vaccination effectively initiates the production of anti-hepatitis B antibodies and is thus followed by HBIG administration withdrawal.[9–11] This idea is theoretically a more economical and simple method compared to passive immunization or nucleos(t)ide analog administration. Contradictory results have been reported, however, and suitable candidates for this type of vaccination have not been determined.[12–15]

At the University of Tokyo, we use HBIG monoprophylaxis.[16] The ultimate goal of vaccination is to achieve sufficient production of anti-hepatitis B immunoglobulin and to discontinue further prophylaxis against recurrent hepatitis B. In the present study, we report the results of an active immunization protocol in chronic hepatitis B-related living donor liver transplantation (LDLT) recipients.

METHODS

Subject selection

PATIENTS WHO UNDERWENT LDLT at least 18 months before for HBV-related end-stage liver disease or received hepatitis B core antibody (HBcAb)-positive donor livers were enrolled if they were being treated with an HBIG prophylaxis protocol, free of nucleos(t)ide analogs, without co-infection with hepatitis C virus or human immunodeficiency virus, and if they had no evidence of HBV reactivation. Twenty Japanese patients provided informed consent to the study protocol. There were 15 men and five women with a median age of 52, ranging from 39 to 59 years. Etiologies of end-stage liver disease included chronic hepatitis B in 16, fulminant hepatic failure with a history of chronic hepatitis B in two, and end-stage liver disease due to autoimmune hepatitis and primary biliary cirrhosis in one each. Two non-HBV patients and one chronic hepatitis B patient received core antibody-positive donor organs. Nine patients with hepatocellular carcinoma were free from post-transplant recurrence with a median follow-up period of 43 (18–90) months.

The donors were 13 men and seven women ranging in age from 18 to 54 years and weighing 43–75 kg. Their relationship to the patients included 10 children, five spouses, three nephews, one sibling and one cousin. Right liver graft was performed in eight, extended right graft in one, right lateral graft in two, left lobe with caudate graft in seven and left lobe graft in two. Three donors were positive for both HB surface antibody (HBsAb) and HBcAb.

Pre- and post-LDLT follow-up protocol

The post-transplantation immunosuppression regimen consisted of steroid induction with tacrolimus, or cyclosporine in case of tacrolimus intolerance, for maintenance.[17] Among the 18 patients with hepatitis B virus infection, LAM 100 mg/day was given orally prior to LDLT. One patient received LAM for more than 1 year, one for 3 months, and 15 received LAM for less than 4 weeks. Of the 17 patients on LAM, a negative HBV-DNA load was confirmed preoperatively in nine cases. In eight patients whose HBV-DNA was detectable pre-transplant, LAM therapy was continued for 4 weeks after LDLT, and discontinued after confirming negative HBV-DNA. Postoperatively, HBIG (Mitsubishi Tanabe Pharma, Hebsbulin-IH, Tokyo, Japan) was administered to HBV-infected patients and those who received HBcAb-positive donor organs. Details of the HBIG administration protocol and doses are described elsewhere.[16] In brief, HBIG was administered to maintain the anti-HB surface antibody (HbsAb) levels at greater than 1000 IU/L for patients with HBV and greater than 500 IU/L for patients that received HBcAb-positive donor organs. After 1 year, 1000–2000 U was given intravenously indefinitely to maintain HbsAb levels of greater than 100 IU/L.[16,18]

Vaccination protocol

After obtaining informed consent, baseline laboratory tests were performed in all patients. Table 1 shows the baseline patient characteristics and laboratory findings. HBV-DNA was undetectable in all patients. Increased dose, namely "double-dose", of recombinant anti-HB vaccination (40 μg/2 mL; Heptavax II, Banyu Pharm, Tokyo, Japan) was injected bilaterally into the deltoid muscles (half dose each side). HBsAb titers were measured at week 0 and every 4 weeks thereafter. Vaccination was scheduled for two phases of three administration cycles at weeks 0, 4, 8, 24, 28 and 32.

During the protocol period, HBIG was administered 2 weeks before and after vaccination if necessary; either 1000 or 2000 IU was administered intravenously according to the previously measured HBsAb titer, as long as it was maintained above 100 IU/mL.

Study end-points and ethical considerations

The primary end-point of this study was the vaccine response. A significant increase, that is >500 IU/mL, in

Table 1 Patient characteristics

Factors	Values†
Age	52 (39–59) years
Men, women	15, 5
HBV-related hepatitis	18
HBcAb positive donor	3
Immunosuppression (Tac + CS), (CyA + CS)	15, 5
Months from LDLT (median)	45 (17–90) months
Pre-LDLT LAM	17
Duration of LAM administration before LDLT: <1 month, 1–12 months, >12 months	15, 1, 1
HBV-DNA positive at LDLT‡	8
HBsAg titer at entry	138.5 (92–302) IU/mL
Aspartate aminotransferase	18.5 (7–30) IU/mL
Alanine aminotransferase	16 (5–30) IU/mL
Alkaline phosphatase	197 (103–528) IU/mL
γ-glutamyl transferase	33.5 (11–187) IU/mL
Lactate dehydrogenase	178 (99–315) IU/mL
Total bilirubin	0.9 (0.1–1.5) mg/dl

†Values are number or median (range). ‡HBV-DNA measured by transcription-mediated amplification methods. Lower detectable limit is 3.7 LEG/mL.
CS, corticosteroid; CyA, cyclosporine; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBV, hepatitis B virus; LAM, lamivudine; LDLT, living donor liver transplantation; Tac, tacrolimus.

HBsAb 12 weeks after the last vaccination was considered effective. Secondary end-points were changes in the required HBIG dose.

The study protocol was approved by the institutional review board (No. P2005015-11X) and informed consent was obtained from each patient.

Statistical analysis

The number of units of HBIG administered before and during the study protocol was recorded, and the sum of the number of HBIG units administered at −24 to −1 weeks, 0–23 weeks, 24–47 weeks and 48–71 weeks was compared by the Friedman test.

RESULTS

ALL PATIENTS COMPLETED the full vaccination course. Double-dose vaccination was well-tolerated with the only side effect of local pain not requiring analgesics. During the study period, none of the cases developed active hepatitis or rejection. One patient developed kidney dysfunction during the study and was unable to complete the HBIG administration protocol (case #5).

Pre-, post- and maximum serum HBsAb titers are shown in Table 2. None of the cases developed a sufficient HBsAb titer level of >500 IU/mL (Fig. 1). None achieved cessation of HBIG administration.

Three cases (#2, #3, #20) developed maximum titers of 313, 408 and 469 IU/mL, and HBIG was thus discontinued in two of them (#2 and #3). The titers then decreased below 100 IU/mL in both cases, however, and HBIG administration was resumed (Fig. 2). Case #20 desired to continue HBIG administration despite the elevation of HBsAb titer to 469 IU/mL. Eventually, HBsAb titer dropped to 214 IU/mL and thus the vaccination was considered ineffective.

HBIG administration in four 24-week periods (−24 to −1, 0 to 23, 24 to 47, 48 to 71) in each case are shown in Table 2. In case 11, HBIG administration between weeks 48 and 71 was stopped due to the appearance of HBsAg and HBV-DNA. HBIG doses as a whole, excluding case 11, decreased over time ($P = 0.006$), as illustrated by the box plot (Fig. 3).

After the final vaccination, all patients were followed for a median of 17 (10–18) months. All patients are alive. In two cases (#11 and #13), serum HBsAg and HBV-DNA were observed at 4 and 8 months after the vaccination protocol, and 60 and 58 months after liver transplantation. In these two cases, the minimum HBsAb level was above 100 IU/mL even after our vaccination protocol. These cases are currently on antiviral therapy and are free from signs of active hepatitis.

DISCUSSION

ACTIVE IMMUNIZATION FOR chronic hepatitis B has been attempted in patients after liver transplantation. In early studies, commercially available recombinant vaccine was used in patients receiving HBIG mono-prophylaxis and was effective in 64–80% of patients.[9,19] This immune response was sustained for a longer follow-up period of 41 (31–85) months in 14 responders.[10] The use of a potential adjuvant in combination with recombinant vaccine is remarkably effective in 80% of post-transplant recipients, and the HBsAb titer was maintained for a long (8–27 months) follow-up period.[11] Studies by other groups, however, demonstrated unfavorable results in patients who were either on LAM prophylaxis or HBIG mono-prophylaxis.[12–14] Several factors are thought to

Table 2 Characteristics, HBsAb titer and dose requirement of HBIG

Case	Age	Sex	Etiology	Donor HBcAb	Pre-LT HBV-DNA	Pre-LT LAM	Timing of vaccination[1]	IS	Pre-HBsAb[2]	Max HBsAb[2]	End HBsAb[2]	HBIG administration				Outcome[1] (recurrence[4])
												−24–1 weeks[3]	48–71 weeks[3]	0–23 weeks[3]	24–47 weeks[3]	
1	52	F	PBC	+	Negative	NA	78	Tac	165	253	253	10	7	10	11	104, alive
2	46	F	AIH	+	Negative	NA	51	CyA	92	313	152	3	5	7	3	75, alive
3	54	M	B-FHF	−	Positive	Yes	48	Tac	122	408	136	12	5	8	5	65, alive
4	39	M	B-FHF	−	Positive	Yes	30	Tac	163	227	221	8	6	6	5	56, alive
5	50	M	BLC, HCC	+	Negative	Yes	90	Tac	175	79	48	6	5	6	7	115, alive
6	57	F	BLC	−	Positive	Yes	57	Tac	127	191	107	7	6	9	7	82, alive
7	47	F	BLC	−	Negative	Yes	56	Tac	140	186	132	7	6	6	6	81, alive
8	50	M	BLC, HCC	−	Negative	Yes	55	Tac	101	153	113	5	6	5	4	77, alive
9	50	M	BLC,	−	Positive	Yes	51	Tac	117	114	70	7	6	6	6	75, alive
10	51	M	BLC, HCC	−	Positive	Yes	49	Tac	111	293	138	8	5	6	5	74, alive
11	48	M	BLC, HCC	−	Positive	Yes	48	Tac	281	171	131	11	1	10	4	71, alive, rec 60
12	59	M	BLC	−	Positive	Yes	43	CyA	160	176	162	6	8	6	6	69, alive
13	52	M	BLC, HCC	−	Positive	Yes	43	Tac	209	210	132	8	7	6	6	67, alive, rec 56
14	57	F	BLC, HCC	−	Negative	Yes	41	CyA	109	220	220	12	7	10	8	67, alive
15	56	M	BLC, HCC	−	Negative	None	40	Tac	144	188	93	9	10	12	12	60, alive
16	54	M	BLC	−	Negative	Yes	40	Tac	137	190	190	8	6	5	5	60, alive
17	50	M	BLC	−	Negative	Yes	20	CyA	95	262	140	6	6	6	6	38, alive
18	51	M	BLC, HCC	−	Negative	Yes	19	CyA	135	179	115	11	8	10	9	44, alive
19	52	M	BLC, HCC	−	Negative	Yes	18	Tac	152	245	121	14	7	12	6	43, alive
20	53	M	BLC	−	Negative	Yes	17	Tac	302	469	214	14	10	12	12	37, alive

[1]Number indicates months after liver transplantation. [2]IU/mL. [3]Number indicates vials of HBIG (1V = 1000 Units) used during the period. [4]Number indicates months after liver transplantation, when HBV-DNA became positive.

AIH, autoimmune hepatitis; B-FHF, hepatitis B related fulminant hepatic failure; BLC, hepatitis B related liver cirrhosis; CyA, cyclosporine A; FHF, fulminant hepatic failure; HBsAb, hepatitis B surface antibody; HBcAb, hepatitis B core antibody; HBIG, hepatitis B immunoglobulin; HCC, hepatocellular carcinoma; IS, immunosuppression; LAM, lamivudine; LC, liver cirrhosis; LT, liver transplantation; PBC, primary biliary cirrhosis; Tac, tacrolimus.

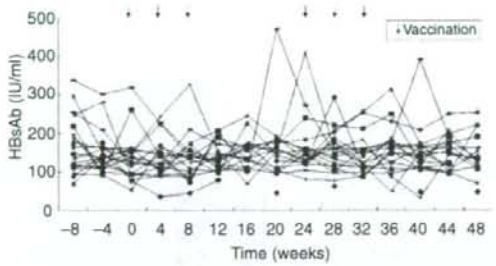

Figure 1 Change in serum hepatitis B surface antibody (HBsAb) titers before and during the vaccination protocol. Heptavax II (40 μg) was administered at weeks 0, 4, 8, 24, 28 and 32. HBsAb levels were between 100 IU/mL and 200 IU/mL in most cases. There was no significant response to vaccination throughout the study.

contribute to a good response, such as younger age, use of an adjuvant to the vaccine and negative HBV-DNA preoperatively. Use of LAM was once speculated to have a negative effect, and, in another study, Angelico *et al.* failed to reveal a favorable effect of mono-prophylaxis with HBIG.[12]

In our study, the double-dose double-phase use of a second generation recombinant hepatitis B vaccine was tested. Our institution applies an HBIG mono-prophylaxis protocol against hepatitis B recurrence. For non-replicate HBV disease, LAM is discontinued at the time of transplantation. During this study, HBIG was administered throughout the vaccination protocol according to Binzele's report,[11] and anti-hepatitis B antibody levels of greater than 500 IU/mL were determined to be effective. Unfortunately, none of the 20 patients developed an adequate anti-hepatitis B antibody titer after two vaccination cycles. Although the dose of HBIG administration decreased during and after the vaccination protocol, we did not consider it a significant change, since none achieved cessation of HBIG administration. The possible factors contributing to vaccination failure among the subjects are that 15 of the 20 patients were aged 50 years or older, eight of 18 chronic hepatitis B patients had a HBV replicative state at the

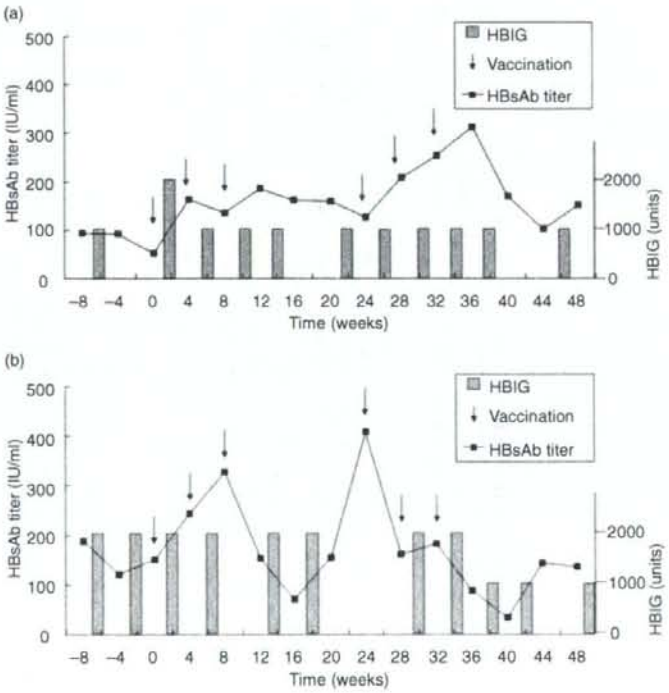

Figure 2 Hepatitis B surface antibody (HBsAb) titers in response to vaccination. (a) Case 2: 46-year-old female with auto-immune hepatitis (AIH), hepatitis B core antibody (HBcAb)-positive donor. (b) Case 3: 54 year-old male with B-fulminant hepatic failure. Hepatitis B immunoglobulin (HBIG) administration was skipped in response to elevated HBsAb level. The HBsAb titer then dropped and HBIG administration was resumed in both cases.

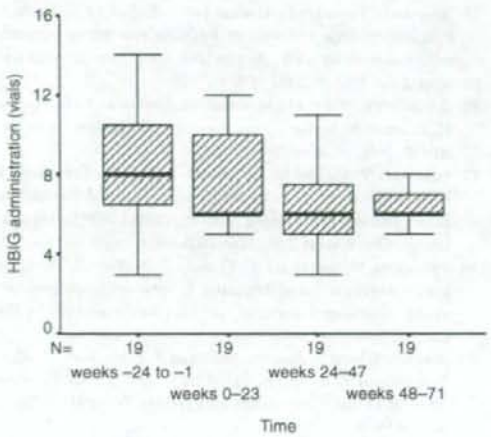

Figure 3 Hepatitis B immunoglobulin (HBIG) administration in four 24-week periods (−24 to −1, 0–23, 24–47, 48–71) is graphically shown by box plot. The dose of HBIG administration decreased over the time period ($P = 0.006$). Box plot explanation: upper horizontal line of box, 75th percentile; lower horizontal line, 25th percentile; horizontal bar within box, median; upper horizontal bar outside box, 90th percentile; lower bar outside box, 10th percentile.

time of transplantation, use of corticosteroids in combination with a calcineurine inhibitor, and one patient was receiving hemodialysis.[20] These factors might have affected the results of this study negatively.

A second generation recombinant HB vaccine is used for prophylaxis in the healthy population. HBsAg-specific T and B cells are induced and a sufficient amount of HBsAb can be produced to neutralize circulating HB virus particles. The recombinant HB vaccines containing S, pre-S2 and/or pre-S1, the so-called the third generation vaccines, have immunogenic advantages over the second generation recombinant HB vaccines; they more efficiently induce an immune response than the second generation HB vaccines among a healthy population,[21,22] and induce not only anti-S antibodies, but also anti-pre-S2 antibodies. Such a vaccine containing S, pre-S1 and pre-S2 antigens was used among Chinese patients who underwent liver transplantation for chronic HB and were on LAM prophylaxis.[23] The authors of that study reported that the vaccine was effective in 10 of 20 patients. An earlier study by Karasu *et al.*,[13] however, failed to show the effectiveness of pre-S1, pre-S2 and S gene products.

Prevention of hepatitis B infection is equally important in HBsAg-negative patients receiving HBc-positive donor organs, as the risk for de novo HB is high.[24] The current standard of care for transplant recipients of HBcAb-positive donor organs is similar to that for patients with chronic HB, including long-term HBIG and/or nucleos(t)ide analog.[18,24–26] In our study, HB vaccine was administered to two HBV non-infected patients who received HBcAb-positive donor organs. Neither of these two patients, however, responded. Pediatric transplantation recipients who receive prior vaccination under an immunization program are likely to achieve a high anti-HB titer by active immunization after LDLT.[27] LAM prophylaxis was withdrawn after 2 years if an adequate anti-hepatitis B titer was achieved. Soejima *et al.*[28] recently reported that 6 of 11 Japanese patients receiving liver transplants for fulminant hepatitis B or for non-HBV diseases with HBcAb-positive donor organs had seroconversion. The tested patients were younger, with a median age of 33 years. These patients were on combination prophylaxis with LAM and HBIG, and HBIG was withdrawn after vaccination. The question remains, however, as to whether LAM therapy can be discontinued in the future among this population.

Vaccination may be promising in selected populations, such as in younger recipients or in those with fulminant hepatitis or HBsAg-negative recipients receiving HBcAb-positive donor organs. For patients older than 50 with vaccination failure or chronic hepatitis B patients, a different approach may prove optimal, such as the use of a pre-S containing vaccination. The research findings for the use of such vaccines are controversial, however, warranting further study.

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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

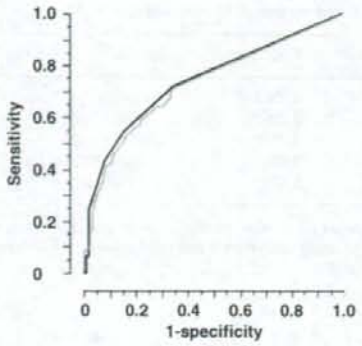

Fig. 1. Receiver operating characteristic curve to evaluate the sensitivity and specificity of histological eosinophilia. Black line, the maximum eosinophil counts per portal triad (Em); Grey line, the rate of portal triads that included at least one eosinophil (Er).

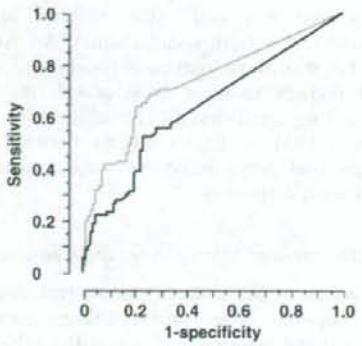

Fig. 2. Receiver operating characteristic curve to evaluate the sensitivity and specificity of blood eosinophilia. Black line, absolute eosinophil count three d before biopsy (AECb); Grey line, absolute eosinophil count on the day (AECo).

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%.

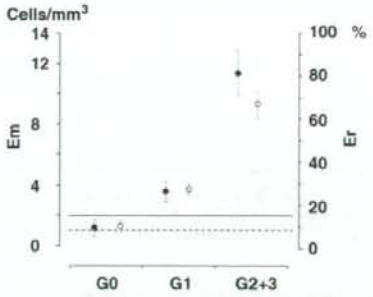

Fig. 3. Em (closed circle) and Er rates (open circle) values stratified by grade of rejection. Solid and dashed lines represented the cut-off value of Em (two cells/portal triad) and Er (8%) respectively.

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