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Prevalence and Clinical Implications of Occult Hepatitis B Viral Infection in Hemophilia Patients in Japan

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The prevalence and clinical implications of occult hepatitis B virus (HBV) infection were investigated in the Japanese patients with hemophilia in whom a high prevalence of infection with transfusion-transmissible viruses has been reported. HBV DNA was detected in the sera of 22 of 43 (51.2%) patients with hemophilia who were negative for HBV surface antigen (HBs), indicating that these patients had occult HBV infection. No factor, including age, type or severity of hemophilia, presence of HBs or HBV core (HBc) antibody, or coinfection with hepatitis C virus (HCV) or human immunodeficiency virus (HIV) was associated with occult HBV infection, except for high anti-HBc titer and/or coinfection with HCV genotype 1 (1a or 1b). In general, occult HBV infection did not appear to have significant clinical implications. However, in patients in whom HBV was detected by PCR specific for the surface (S)-region, higher alanine aminotransferase levels were observed. The genotype of the occult HBV in the present study was exclusively the domestic type indigenous to Japan (genotype C), suggesting a different route of transmission for HBV in comparison to HCV and HIV in this population. ***J. Med. Virol. 73:195–199, 2004.*** © 2004 Wiley-Liss, Inc.

KEY WORDS: hemophilia; hepatitis B virus; occult infection; genotype; route of transmission

INTRODUCTION

Patients with hemophilia are at high risk of infection with parenterally transmissible viruses due to the frequent use of blood products. High prevalence of infection with hepatitis C virus (HCV) [Makris et al., 1990; Troisi et al., 1993], human immunodeficiency virus (HIV) [Tsuchie et al., 1985; Kroner et al., 1994], and GB virus C (GBV-C) [Hanley et al., 1998; Toyoda et al., 1998] has been reported. A few studies have also been carried out on hepatitis B virus (HBV) infection in this population by serological evaluation [Kumar et al., 1993; Goedert et al., 2002].

Recently, several studies have reported occult HBV infection in subjects without HBV surface (HBs) antigen (HBsAg), and its clinical implications are suggested [Cacciola et al., 1999; Brechot et al., 2001; Torbenson and Thomas, 2002]. In addition to a history of frequent use of blood products, a large number of hemophilia patients are infected with HIV, which can result in dynamic changes in the immune status. Reactivation of HBV in association with changes in the immune status can occur and can cause liver damage, which sometimes results in liver failure [Xunrong et al., 2001]. Thus, hemophilia patients are a population in which the assessment of occult HBV infection is important, especially for those patients with HIV.

In the present study, we attempted to clarify the prevalence and clinical importance of occult HBV infection in the Japanese hemophilia patients without HBs antigen. As a result, occult HBV infection was found in around one-half of the patients.

PATIENTS AND METHODS

Patients

Among 44 patients with hemophilia who had been followed-up as outpatients at Nagoya University Hospital and who were admitted regularly to the hospital during 2002, 1 patient had HBs antigen and the other

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43 patients were negative for HBs antigen. These 43 patients were enrolled in this study. All were males with a mean age of 34.0 ± 12.1-years-old. Thirty-four patients had hemophilia A, and the remaining 9 had hemophilia B. Thirty-seven patients had severe, 4 patients had moderate, and 2 patients had mild hemophilia. All 24 patients with HIV infection were receiving HAART therapy at the time of sampling serum. Eight of 38 patients with HCV infection had a history of interferon therapy, but no patients were treated with interferon at the time serum was sampled.

Evaluation for Coinfection With HIV and HCV

HIV infection was confirmed by anti-HIV1 antibody detected by particle agglutination (SERODIA-HIV, Fuji Rebio, Tokyo, Japan). Serum HIV RNA concentration was measured by the Amplicor HIV Monitor test (Roche Diagnostics K.K., Tokyo, Japan). The presence of HCV was confirmed by both an HCV antibody assay (2nd generation, Dinabot; Tokyo, Japan), and detection of HCV RNA by nested reverse transcription-polymerase chain reaction (RT-PCR) [Okamoto et al., 1990]. HCV genotypes, according to Simmonds et al. [1994] classification, were determined by RT-PCR with genotype-specific primers [Okamoto et al., 1996]. Serum HCV RNA concentrations were measured by Amplicor Monitor assay (Roche Diagnostics K.K., Tokyo).

Serological Tests for HBV Infection and Detection of HBV DNA

HBV serum markers (HBs antigen, HBs antibody, and HBV core [HBc] antibody) were examined by means of commercial immunoenzyme assays (Abbott Laboratories, North Chicago, IL). For detection of HBV DNA, extracted DNA was amplified by nested touchdown PCR [Don et al., 1991] with three independent primer sets specific for HBV surface (s)–(sense: 5′-CTCTTGTCCTCCAATTTGTCCT-3′ and antisense: 5′-CAGCAAAGCCCAAAAGACCCAC-3′ for the first PCR, and sense: 5′-AGGTA-TGTTGCCCGTTTGTCCT-3′ and antisense: 5′-GGGTTTAAATGTATACCCA-3′ for the second PCR), core (c)–(sense: 5′-ACTGTTCAAGCCTCCAAGCT-3′ for the first and second PCR, antisense: 5′-GGAATACTAACATTGAGATTCCCGAG-3′ for the first PCR, and antisense: 5′-AGTGCGAATCCACACTC-3′ for the second PCR), and X-regions (sense: 5′-TGCCAAGTGTTTGCTGACGC-3′ for the first PCR, sense: 5′-CTGCCGATCCATACTGCGGAAC-3′ for the second PCR, and antisense: 5′-TTCCTGCAGTGGAGACCACCGTGAACG-3′ for the first and second PCR). HBV DNA was amplified from 100 ng of extracted DNA in a total volume of 50 μl, in the presence of 10 pmol of each primer, 125 μM dNTP, and 2.5 U Taq polymerase (Toyobo, Tokyo, Japan). PCR was performed in a DNA thermal cycler (Perkin-Elmer Cetus, Norwalk, CT). The PCR program consisted of 20 cycles of denaturation at 94°C for 30 sec, annealing at 65°C for 30 sec with a 0.5°C decrease per one cycle (55.5°C at final cycle), and extension at 70°C for 3 min with an initial denaturation at 94°C for 1 min, and a subsequent 20 cycles of denaturation at 94°C for 30 sec, annealing at 55°C for 30 sec, and extension at 70°C for 3 min with a final extension at 70°C for 10 min. The same PCR program was used for both the first and second PCR amplifications. Amplified PCR products were analyzed by electrophoresis on 1.0% agarose gel and transferred to a Hybond-N+ nylon membrane (Amersham-Pharmacia, Buckinghamshire, UK). The amplified products were detected by hybridization with a specific probe based on the entire HBV sequences. This probe was generated with a DIG DNA Labeling and Detection kit (Roche Diagnostics, Mannheim, Germany). Results were considered to be valid only if identical results were obtained in at least two separate experiments.

The patients who were positive for both the S- and C-regions were considered to be positive for HBV. The patients who were positive for only one of the two regions examined were then referred to the result of PCR specific for the X-region, and HBV infection was confirmed according to this result.

Genotyping of HBV DNA

Genotyping of occult HBV was performed in seven patients in whom HBV DNA was detected by PCR specific for the S-region. For genotyping of HBV DNA, the PCR product from amplification of the S-region was sequenced directly, and phylogenetic analysis was performed with the neighbor-jointing and bootstrap methods.

The entire protocol was approved by the hospital ethics committee and carried out in compliance with the Helsinki Declaration.

RESULTS

Serological Prevalence of Hepatitis B Viral Markers

Among the 43 patients with hemophilia but without HBs anitgen, HBs antibody was detected in 27 patients (62.8%), and HBc antibody was detected in 37 patients (86.0%). There was no correlation between the type and/or severity of hemophilia and serological prevalence of HBV. There was no association between coinfections with HIV and/or HCV and HBV serological status.

Detection of HBV DNA in Serum

HBV DNA was detected in 22 of the 43 hemophilia patients (51.2%). HBV was detected by PCR specific for the S-region in 7 patients and by PCR specific for the C-region in 19 patients. In 4 patients HBV DNA was detected by both methods. In the 18 patients in whom HBV DNA was detected by only one of the PCR methods, additional PCR targeting the X-region was performed and all patients were positive for this region.

We compared the background, serological markers for HBV, and coinfection status between HBV DNA-positive and -negative patients (Table I). There were no significant differences in the background of patients, including age, and type or severity of hemophilia. Both

TABLE I. Characteristics of Patients With or Without Occult HBV Infection

	HBV DNA (−)	HBV DNA (+)
Number	21	22
Mean age	35.2 ± 10.5	32.8 ± 13.5
Type of hemophilia (A/B)	17/4	17/5
Severity of hemophilia (mild/moderate/severe)	1/1/19	1/3/18
HBs-antibody (+/−)	14/7	13/9
HBc-antibody (+/−)	17/4	20/2
HBc-antibody titer#	129.4 (39.1–897.8)*	291.3 (80.4–914.5)*
HIV (+/−)	13/8	11/11
HIV RNA concentration (copies/μl)##	7.6 (0.2–35)	0.4 (0.2–0.5)
Under limit of quantitation sensitivity	7 (53.8%)	9 (81.8%)
CD4+ cell count##	449.9 ± 300.1	577.7 ± 272.6
HCV (+/−)	20/1	18/4
HCV RNA concentration (copies/μl)###	383.5 (17–810)	498.8 (85–830)
HCV genotype (1a/1b/2a/2b/3a/4a)###	3/3/3/2/6/3**	10/3/1/2/2/0**
Serum ALT levels (IU/L)###	57.1 (12–208)	71.8 (10–209)

HBV, hepatitis B virus; HBs, hepatitis B viral surface; HBc, hepatitis B viral core; HIV, human immunodeficiency virus; HCV, hepatitis C virus; ALT, alanine aminotransferase.
#Only in patients with positive HBc-antibody.
##Only in patients with HIV coinfection.
###Only in patients with HCV coinfection.
*$P = 0.0476$ by Mann–Whitney U test.
**$P = 0.0230$ for 1a or 1b versus other genotypes by Chi-square test.

the rates of positive HBs antibody and HBc antibody were similar between HBV DNA-positive and -negative patients. The rates of both HIV and HCV coinfection were similar regardless of occult HBV infection. In addition, when compared in combination with HBs and HBc antibody, or in combination with HIV and HCV, there was no difference in the rate of patients with HBV DNA (Table II). In patients with positive HBc antibody, however, the antibody titer was higher in HBV DNA-positive patients than in HBV DNA-negative patients ($P = 0.0476$, Mann–Whitney U test). In patients with HCV, HCV genotype 1 (1a or 1b) was significantly more prevalent in patients with HBV DNA than in patients without HBV DNA ($P = 0.0230$, Chi-square test).

In all five patients without HCV coinfection, serum alanine aminotransferase (ALT) levels were continuously normal regardless of occult infection with HBV. In patients with HCV infection, there was no significant difference in serum ALT level which was calculated as the average value of four to six analyses over 1 year, between HBV DNA-positive and -negative patients. When this comparison was restricted to patients with HBV detectable by PCR specific for the S-region only, serum ALT levels in HBV DNA-positive patients were significantly higher than those in HBV DNA-negative patients (patients with HBV, 120.3 ± 66.6 vs. patients without HBV, 57.1 ± 44.0; $P = 0.0162$, Mann–Whitney U test).

Genotype of Occult HBV

HBV genotyping was carried out based on the sequence of the S-region in seven patients in whom HBV DNA was detected by PCR specific for the S-region. Genotype C, which is the major genotype observed in the Japanese patients with chronic hepatitis B without hemophilia, was detected in all seven patients.

DISCUSSION

The clinical significance of occult HBV infection for patients with chronic hepatitis C has been described in recent reports [Cacciola et al., 1999; Sagnelli et al., 2001] and remains controversial [Kao et al., 2002]. These reports consider the influence of HBV occult infection on advanced liver disease [Cacciola et al., 1999; Sagnelli et al., 2001], development of hepatocellular carcinoma [Sheu et al., 1992; Paterlini et al., 1993], and reduced response to interferon [Zignego et al., 1997; Cacciola et al., 1999]. The importance of HBV occult infection has been reported in immunosuppressive patients, even in those without HCV coinfection [Xunrong et al., 2001]. In these patients, reactivation of HBV caused liver damage and sometimes resulted in liver failure.

Patients with hemophilia are at high risk of exposure to transfusion-transmissible virus such as HIV, HBV, HCV, and GBV-C. The high prevalence of infection with HIV [Tsuchie et al., 1985; Kroner et al., 1994], HCV

TABLE II. Rate of Hepatitis B Virus DNA Detection (%)

HBsAb(+) HBcAb(+)#	HBsAb(+) HBcAb(−)	HBsAb(−) HBcAb(+)	HBsAb(−) HBcAb(−)
13/25 (52.0)	0/2 (0)	7/12 (58.3)	2/4 (50.0)
HIV(+) HCV(+)##	HIV(+) HCV(−)	HIV(−) HCV(+)	HIV(−) HCV(−)
11/21 (52.4)	3/3 (100)	10/17 (58.8)	1/2 (50.0)

#HBsAb, hepatitis B viral surface antibody; HBcAb, hepatitis B viral core antibody.
##HIV, human immunodeficiency virus; HCV, hepatitis C virus.

[Makris et al., 1990; Troisi et al., 1993], and GBV-C [Hanley et al., 1998; Toyoda et al., 1998] has been reported in many studies. The status of serological markers on HBV infection has also been reported [Kumar et al., 1993; Goedert et al., 2002]. However, occult HBV infection in this population has not been examined. Because a large number of patients with hemophilia have HIV infection and changes in immune status in these patients can occur partly due to the disease itself and partly to the effect of HAART therapy, clarification of the status of occult HBV infection in these patients is important because of the potential for reactivation of occult HBV in association with changes in immune status.

HBV DNA was detected in serum in around one-half of the patients. The rate of detection was similar to that of HBV DNA detected in serum of the Japanese patients without hemophilia who have chronic HCV infection [Fukuda et al., 1999]. Neither the severity of hemophilia nor coinfection with HIV and HCV indicated the potential for occult HBV infection. In a previous study, Nunez et al. [2002] found no HIV-infected patients (most were intravenous drug users) in whom occult HBV infection could be confirmed. In contrast, we confirmed occult HBV infection in 11 HIV-infected patients. Only a high HBc antibody titer, which has already been reported to be an indicator of occult HBV infection [Nirei et al., 2000], and HCV genotype 1 (1a or 1b) in patients coinfected with HCV may indicate the high risk of occult HBV coinfection.

On the basis of our results, occult HBV infection appears to have no significant clinical impact when the infection is evaluated by the HBV detection for the C-region. On the contrary, occult HBV may increase serum ALT levels, which indicates severe liver damage, in patients with HCV infection when HBV DNA is positive by PCR for the S-region. Further study will be required to clarify the difference in clinical significance of HBV occult infection between PCR positive for the C-region and that positive for the S-region.

The HBV genotype detected in the Japanese patients with hemophilia was exclusively genotype C, which is the most common genotype in Japan. This shows the distinct characteristics of occult HBV infection in hemophilia patients in Japan, which are different from those of other transfusion-transmissible viruses in this population. The genotypes of viruses such as HCV or GBV-C in the Japanese hemophilia patients are foreign and not domestic genotypes [Kinoshita et al., 1993; Toyoda et al., 1998]. This is because, in this population, transmission of these viruses has been by imported blood products, as well as HIV transmission in this population [Tsuchie et al., 1985]. In contrast, only the domestic HBV genotype was found in the Japanese hemophilia patients. This, together with the lack of difference in the prevalence of occult HBV infection between hemophilia patients with HCV and HCV-infected patients who have not undergone repeated transfusions in Japan, suggests a route of transmission of this virus, different from that in cases of HIV, HCV, and GBV-C infection. The lack of correlation in the rate of coinfection between HIV or HCV and HBV also supports this suggestion. Screening for HBV in blood donors using HBs antigen as a marker started in 1973 in Japan. Some patients have a history of blood transfusion, which may have caused the occult HBV infection. Nosocomial infection in relation to the injection of blood products through the repeated use of needles, syringes, or other medical instruments, which could have occurred under medical conditions in Japan prior to the 1970s, might have also played a role.

In summary, among 43 Japanese patients with hemophilia, occult HBV infection was observed in about one-half of patients without detectable HBs antigen, a prevalence similar to that of the Japanese patients with chronic HCV infection. Occult HBV infection did not have significant clinical implications as a whole, although patients in whom HBV was detected with S-region-specific PCR showed higher ALT levels. The HBV genotype was exclusively a domestic type, suggesting a different route of transmission of HBV from that of HCV or HIV in this population. Further studies are required to determine occult HBV infection in the Japanese patients with hemophilia.

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Hepatic Arterial Anatomy for Right Liver Procurement From Living Donors

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Living donor liver transplantation (LDLT) using right liver grafts is now widely performed. Anatomic classifications of the hepatic artery for right liver procurement, however, are limited. In this study, celiac and mesenteric angiograms of 223 consecutive living donors in a single institution were evaluated. Details of the arterial anastomosis and results were reviewed in 72 patients who underwent primary LDLT using right liver grafts. There was a 6% incidence of hepatic arterial bifurcations that might provide multiple orifices in a right liver graft. Only one right liver graft (1%) had multiple arterial orifices. Single arterial anastomosis without interposition was possible in all patients with right liver grafts and none of them were complicated with hepatic arterial thrombosis. Single arterial anastomosis, therefore, has a high probability of success in right liver graft implantation. (*Liver Transpl 2004;10:129–133.*)

Living donor liver transplantation (LDLT) is a preferable treatment for adults with end-stage liver disease due to the limited number of available cadaveric donors.[1] Fundamental to the application of this technique is an understanding of hepatic vascular anatomy.[2] Michels first reported 10 basic types of hepatic arterial supply.[3] Since then, common and rare hepatic artery variants have been reported. Most of these studies, however, focused only on replaced or accessory arterial branches that are helpful for whole-liver harvesting and transplantation. Without information regarding bifurcation of the right hepatic artery (RHA), the classification is of little help for right liver harvesting.

Recently, Marcos et al proposed the use of interposition arterial grafts in right liver graft because double hepatic arteries were common in their series.[7] Their report conflicted with our experience because, in our series, no patients underwent double hepatic artery reconstruction in right liver LDLT. To clarify this inconsistency, we evaluated celiac and mesenteric angiograms of 223 consecutive living donors in a single institution. The aim of the study was to determine a useful anatomic classification of the hepatic arteries for LDLT using right liver grafts.

Materials and Methods

Donors

From January 1996 until May 2003, 223 consecutive living donors underwent hepatectomy at the University of Tokyo Hospital. They comprised 126 men and 97 women with a median age of 34 years (range, 18–63 years). Details regarding selection criteria and evaluation are described elsewhere.[8] Only one case was rejected due to arterial anatomy.[9] All of the donors were related to the recipients. The relation of the donors to the patients was 84 parents, 65 children, 37 siblings, 22 spouses, 9 nephews, and 4 uncles and two cousins. The type of graft was determined by volumetric analysis and not by vascular anatomy. The graft estimate was determined by computed tomography (CT). A graft-volume-to-recipient-standard-liver-volume ratio[10] of 40% was the lower limit. Candidates in whom the right liver comprised more than 70% of the whole liver were rejected as prospective donors. The most common procedure was left liver with or without caudate lobe resection (n = 85), followed by right liver resection (n = 72), left lateral segmentectomy (n = 51), and right lateral resection of right lateral sector (n = 15). All donors provided written informed consent.

Angiography of celiac and mesenteric arteries was performed in each donor to evaluate the anatomy of the donor's hepatic artery. First, the anatomy was reviewed according to Michels's classification.[3] Thereafter, the anatomy was classified from the point of view of whether single or multiple anastomoses were needed in LDLT using the right liver. In

Abbreviations: A6, accessory branch from segment VI; CT, computed tomography; Ce, celiac axis; GDA, gastroduodenal artery; LDLT, living donor liver transplantation; LHA, left hepatic artery; LGA, left gastric artery; MHA, middle hepatic artery; PSPDA, superior pancreaticoduodenal artery; RHA, right hepatic artery; RL, lateral branch of right hepatic artery; RPM, paramedian branch of right hepatic artery; SA, splenic artery; SMA, superior mesenteric artery.

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Table 1. Anatomy Classification Stratified by Michels

Type	Description	Michels's Series (n = 200)	Present Series (n = 223)
1	Normal	55%	61%
2	Replaced LHA from LGA	10%	14%
3	Replaced RHA and MHA from SMA	11%	4%
4	Replaced LHA from LGA, and replaced RHA from SMA	1%	0
5	Accessory LHA	8%	12%
6	Accessory RHA	7%	3%
7	Accessory LHA and accessory RHA	1%	2%
8	Accessory LHA and replaced RHA, or replaced LHA and accessory RHA	2%	0
9	PHA from SMA	4.5%	6%
10	PHA from LGA	0.5%	0

Abbreviations: LGA, left gastric artery; LHA, left hepatic artery; MHA, middle hepatic artery; RHA, right hepatic artery; SMA, superior mesenteric artery.

this classification, the anatomy of the left hepatic artery (LHA) was not always considered.

Patients Receiving Right Liver Grafts

The actual arterial anastomosis was reviewed in 72 patients who underwent primary LDLT using right liver grafts. The patients were 53 males and 19 females with a mean age of 50 years. The indications for LDLT in these patients included hepatitis C virus with cirrhosis (n = 25), hepatitis B virus with cirrhosis (n = 13), primary biliary cirrhosis (n = 12), fulminant hepatic failure (n = 8), cryptogenic cirrhosis (n = 5), metabolic disorders (n = 3), biliary atreasia (n = 3), primary sclerosing cholangitis (n = 2), and autoimmune hepatitis (n = 1).

The surgical details of the recipients were described previously.[11] In brief, hepatic arterial reconstruction was performed under a surgical microscope by a microsurgeon (HA). The donor and recipient arterial branches were anastomosed in an end-to-end manner with interrupted sutures using 9-0 monofilament nylon. When the donor's arterial branch was long enough to turn over, the anterior suture was performed first. Otherwise, the posterior wall was sutured with an inside-outward procedure using double needles; thereafter, the anterior wall was sutured without turning the anastomotic site over.[12] After reconstruction, the intrahepatic arterial signals in each segment were examined using Doppler ultrasonography; the other branches were ligated after confirming pulsatile back-bleeding from the nonanastomosed cut stumps[13] (k). When these criteria were not satisfied, the remaining arteries were anastomosed to the recipient hepatic arteries.

Results

Angiographic Classification

The frequency of each type proposed by Michels is shown in Table 1. There was a similar distribution between types in Michels's series and ours. There were no Michels types 4, 8, and 10 in our series, however.

To predict the number of hepatic artery stumps in right liver LDLT, the anatomy was classified into four types (Fig. 1). The frequency of each type is shown in Table 2. Type I secures a single arterial orifice in the right liver graft. This type is divided into six subcategories. Type IA, normal anatomy in which RHA originates from the common hepatic artery and the middle hepatic artery (MHA) originates from the LHA; Type IB, same variation as Type IA, except that the MHA originated from the RHA; Type IC, replaced RHA from superior mesenteric artery (SMA); Type ID, replaced RHA and MHA from the SMA; Type IE, entire common hepatic artery from the SMA and the MHA from the LHA; Type IF, same as with Type IE except for the MHA originated from the RHA.

The hepatic arterial bifurcations that might provide multiple orifices in the right liver graft were divided into three types. A total of 14 donors (6%) were classified into these types. In Type II, the MHA originated from the paramedian (Type IIA) or lateral branch (Type IIB) of the RHA. In Type III, the right paramedian and lateral branch of the RHA had separated origins. This type was divided into two subtypes with a right lateral branch from the LHA (Type IIIA) or from the SMA (Type IIIB). In Type IV, there was an accessory branch from segment VI (A6). This type was divided into three subtypes according to the root of A6 as follows: Type IVA, from the hepatic artery proper; Type IVB, from the celiac trunk; and Type IVC from the superior pancreaticoduodenal artery. The relation between Michels's classification and ours is shown in Table 3.

Donors of Right Liver Resection

Among the 72 donors who underwent right liver resection, angiographic analysis predicted one Type IVA

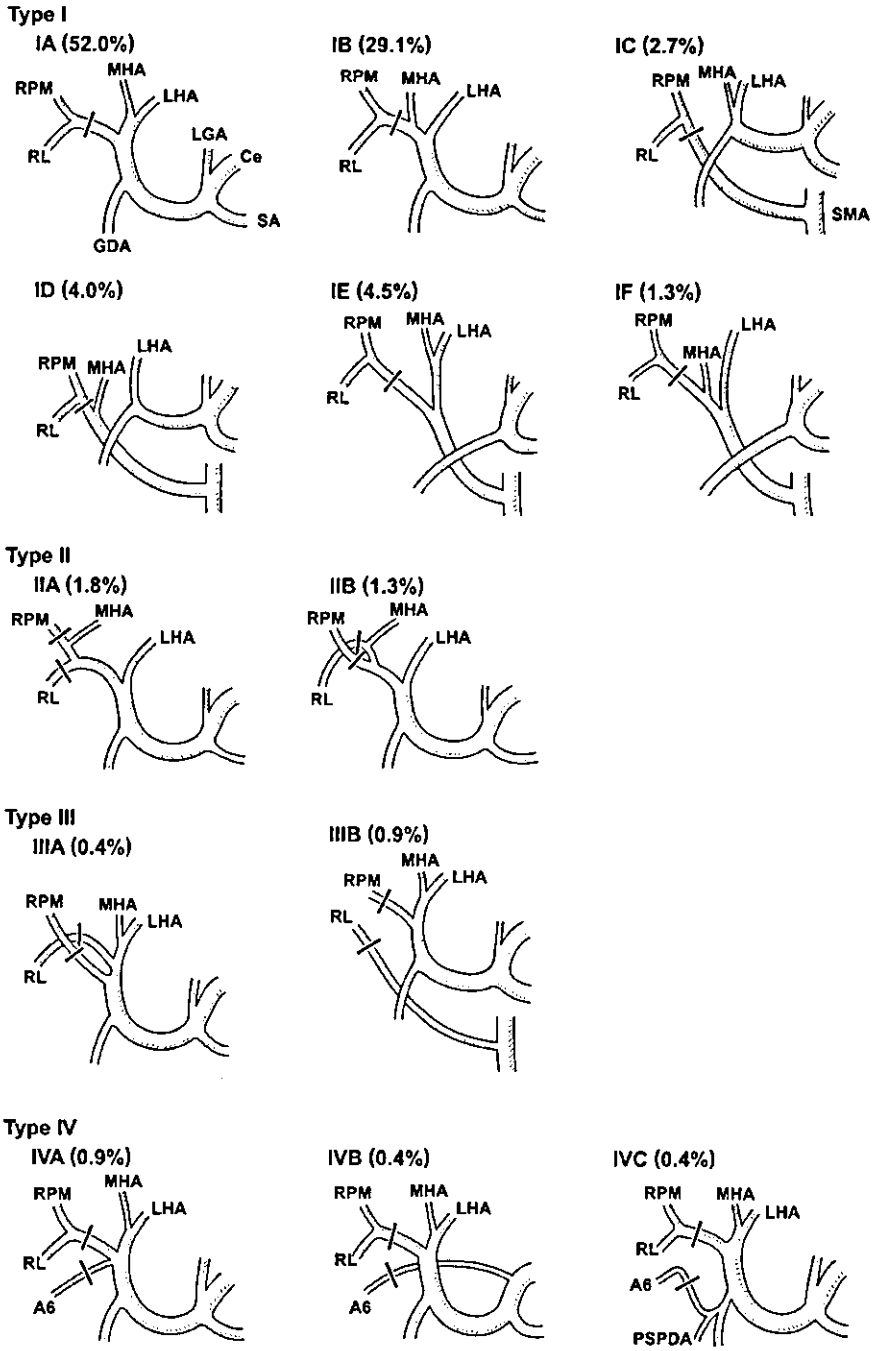

Figure 1. Arterial classification for right liver procurement.

donor who had multiple arterial orifices after right liver harvesting, confirmed during surgery. In this case, pulsatile backflow from A6 was observed after the graft RHA was anastomosed with the recipient's RHA. Therefore, the stump of graft A6 was ligated. As a result, single arterial anastomosis was possible in all right liver grafts in our series.

Clinical Results of the Patients Receiving a Right Liver

The duration of the operation ranged between 650 and 1,890 minutes (median, 915 min). The time for arterial reconstruction was 28 to 92 minutes (median, 43 min). No patients were complicated with hepatic arterial throm-

Table 2. Anatomic Classification of Right Hepatic Artery

Type	Total Donors (n = 223)	Donors of Right Liver (n = 72)
One arterial orifice	209 (94%)	71 (99%)
IA	116	38
IB	65	26
IC	6	1
ID	9	0
IE	10	6
IF	3	0
Multiple arterial orifices	14 (6%)	1 (1%)
IIA	4	0
IIB	3	0
IIIA	1	0
IIIB	2	0
IVA	2	1
IVB	1	0
IVC	1	0

bosis. Portal vein thrombosis was observed in two patients and was repaired by reoperation. Acute rejection was confirmed in 22 patients (31%) with a mean time to rejection of 17 days. Bile-duct leakage and stenosis of the anastomosis, which necessitated surgical revision, occurred in four and six patients, respectively. Two patients died during hospitalization, one for bleeding from the ileum (99 days) and the other for refractory acute rejection (49 days). The hospital-stay duration after LDLT of the survivors ranged from 16 to 143 days (median, 40 days). Four patients experienced late death due to virus-associated hemophagocytic syndrome (n = 2; 146 days and 370 days), hepatocellular carcinoma recurrence (n = 1; 229 days), and cholestatic hepatitis C (n = 1; 351 days). The other patients achieved survival with a median follow-up of 14 months.

Discussion

In the present study, visceral angiography was performed in 223 consecutive donors, and the findings were classified to reflect the number of RHA orifices after right liver harvesting. The results of Michels's data and those in our series were similar, indicating that there is little difference in hepatic arterial anatomy between the Japanese and the population in the United States. The analyses revealed that while the frequency of multiple orifices was predicted to be 6% in the overall series, it was actually only 1% in the donors of right liver resection. The results contrasted with the results of left liver grafts. Sakamoto et al classified hepatic arterial variations in 101 left liver donors.[13] They reported a 53% incidence of multiple arterial stumps in left liver grafts. The high incidence could be due to the MHA, which often originated independently from the LHA.

Our results contrasted with the recent data by Marcos et al.[14] They analyzed the results of 95 consecutive LDLT using right liver grafts, and of these, 11 grafts (12%) had double arteries. The double arterial orifices were sutured with auto Y-shaped arterial grafts at the bench. It is difficult to explain the discrepancy in the difference of anatomic variation in the subjects, because the precise classification was not shown in the previous data[14] They commented that the multiple arterial orifices in some grafts resulted from distal arterial division to spare the main trunk, common bile duct, and MHA. Although the MHA bifurcated from the RHA with 40% possibility (Types IB, ID, IF, and II), the trunk of the RHA could be cut near its root with a short but sufficient margin for its closure.

There is often only a single and very short arterial stump in the graft. The short conduit can be overcome, however, using microsurgical techniques.[15] When the donor's arterial branch was long enough to be clamped and turned over, it could be anastomosed using threads with double needles. Actually, in 72 patients who underwent right liver graft, there was no arterial thrombosis. We preferred not to use the interposition technique proposed as a reversed extension graft.[14] Harvesting an arterial graft for interposition will subject either the donors or recipients to an additional incision or

Table 3. Relation Between Michels's Classification and Ours

Description of RHA	Michels's Classification	Present Classification
Normal	1 + 2 + 5	IA + IB + II + IIIA + IVA
Replaced RHA and replaced MHA from SMA	6 + 8a*	IC
Replaced RHA from SMA	3 + 8b*	ID
CHA from SMA	9	IE + IF
Accessory RHA	7 + 8b	IIIB + IVB + IVC

*The classification was modified as 8a, accessory LHA and replaced RHA; and 8b, replaced LHA and accessory RHA.
Abbreviations: CHA, common hepatic artery; LHA, left hepatic artery; MHA, middle hepatic artery; RHA, right hepatic artery; SMA, superior mesenteric artery.

more extensive dissection. Additionally, the technique takes longer, and the risk of thrombosis in the recipient is increased. We consider the indication of interposition graft in arterial reconstruction quite limited.

It remains a debate in LDLT whether all arterial stumps should be anastomosed. The previous report demonstrated that small arteries supplying the left liver could be ligated safely if pulsatile back-bleeding was observed after anastomosis of the main artery.[16] Marcos et al commented that no portion of the right liver was supplied by secondary arterial perfusion, which was different from segment IV, so that any tributaries should not be ligated[7] In one case of right liver graft with two arterial stumps, however, a simple method of only one anastomosis was sufficient if backflow from another tributary was confirmed. Our experience was limited; however, it might indicate that compensation of arterial perfusion exists in right liver grafts as well.

Less invasive examination is favorable for donors, and the effectiveness of CT angiography and gadolinium-enhanced magnetic resonance angiography was recently proposed for donor evaluation.[17,18] Although the sensitivities of CT angiography and magnetic resonance angiography for the depiction of hepatic arterial variants are reported in several articles, the variants in these studies are limited to those commonly reported.[19,20] Kopka et al described their experiences evaluating hepatic arterial variants in 60 patients using both magnetic resonance angiography and digital subtraction angiography.[20] Magnetic resonance angiography did not correctly depict the visceral anatomy in three cases. We believe that precise and definite assessment of arterial anatomy is necessary, and we will continue to perform digital subtraction angiography for anatomic evaluation until the accuracy of the less invasive examinations are at least as complete as conventional examination.[21]

In conclusion, multiple arterial tributaries in right liver graft procurement are rare. The anatomic characteristics of RHA allow simple and safe anastomosis in a high probability.

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Volume Regeneration After Right Liver Donation

Shojiro Hata, Yasuhiko Sugawara, Yoji Kishi, Takashi Niiya, Junichi Kaneko, Keiji Sano, Hiroshi Imamura, Norihiro Kokudo, and Masatoshi Makuuchi

After right hepatectomy with the middle hepatic vein trunk for a graft, the venous outflow in segment IV is disturbed. There are limited data, however, regarding the effect of middle hepatic vein deprivation on liver regeneration or functional recovery. Living donors who underwent right hepatectomy with preservation of the middle hepatic vein (Group A, n = 58) and those deprived of the middle hepatic vein (Group B, n = 13) were reviewed. When the donor was under 50 years old and the remnant left liver was estimated to be more than 35% of the whole liver, right liver graft harvesting with the middle hepatic vein trunk was considered. Volume regeneration of segments I–III, segment IV, and overall liver volume was assessed at the third postoperative month using computed tomography. The regeneration rate of segment IV was significantly impaired in Group B donors compared with that in Group A donors (125% vs. 45%, $P = 0.008$). In contrast, the regeneration rate of segments I –III was significantly higher than that in Group A (208% vs. 263%, $P = 0.004$). There was no significant difference in the regeneration rate of the whole left liver or functional recovery between groups. Multivariate analysis revealed that the resection type (group) was a significant predictive factor for the regeneration rate of segments I–III and segment IV. When deprived of the middle hepatic vein, liver regeneration of segment IV was impaired but was compensated for by the regeneration of segments I–III. In conclusion, extended right hepatectomy can be safely performed with careful preoperative consideration using these criteria. (*Liver Transpl 2004;10:65–70.*)

The shortage of cadaveric donors has led to an increase in the practice of living donor liver transplantation (LDLT).[1] A vital issue in LDLT is the preservation of a satisfactory blood supply and venous return in both the right and left livers to maximize donor safety and graft function. When splitting the liver along the main portal fissure to harvest a hemiliver graft, however, it is impossible to maintain complete venous outflow in both of the bisected livers, because the middle hepatic vein (MHV) can be preserved on only one side.

An extended right liver graft,[2] which includes the MHV trunk, was devised by the Hong Kong group. This method is beneficial with regard to venous drainage of the graft. On the donor side, however, the venous outflow disturbances in segment IV are a concern, and they might disrupt the function of the relevant hepatic region.[3] Consequently, this type of graft is less commonly used than a right liver graft without the MHV trunk.[4]

In our institution, we adopted right hepatectomy with or without MHV as the donor procedures for LDLT in selected donor-recipient combinations. The aim of the present study is to clarify whether deprivation of the MHV truly causes adverse effects in donors, including disturbances in liver regeneration of segment IV or functional recovery.

Materials and Methods

Subjects

From March 2000 through March 2003, 138 consecutive living donors underwent hepatectomy at the University of Tokyo Hospital. Of these, 71 donors with right hepatectomy were investigated. Details regarding selection criteria and evaluation are described elsewhere.[5] All of the donors were related to the recipients. The relationships of the donors to the patients were 29 children, 20 siblings, 10 spouses, eight parents, and four nephews. Preoperative liver biopsy was indicated when the body mass index was over 25, and candidates with more than 30% steatosis on biopsy were rejected as donors.[6] All donors and patients provided written informed consent.

Right liver volume was preoperatively estimated using computed tomography (CT) as described previously.[7] Candidates in whom the right liver comprised more than 70% of the whole liver were rejected as prospective donors. The esti-

Abbreviations: CT, computed tomography; LDLT, living donor liver transplantation; MHV, middle hepatic vein.

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mated ratio of graft volume to recipient standard liver volume was 40%, which was the lower limit for right liver transplantation. The number and diameter of thick MHV tributaries draining the right paramedian sector were evaluated on CT. When the donor was under 50 years old and the remnant left liver was estimated to be more than 35% of the whole liver, extended right liver graft harvesting was considered. Otherwise, right liver graft harvesting without the MHV trunk was indicated.

The donors were divided into two groups. Group A (control group) included 58 donors who underwent resection of the right liver (segments IV–VIII). In this group, the MHV trunk was preserved in the remnant donor liver, and venous drainage of the right paramedian sector was thoroughly maintained after hepatectomy. Group B consisted of 13 donors with right liver resection involving the MHV. In this group, venous drainage of segment IV was interrupted after hepatectomy. Group A was comprised of 34 men and 24 women with a median age of 38 years (range, 36–61 yr), and Group B was comprised of two men and 11 women with a median age of 37 years (24–54 yr). Postoperative CT with contrast enhancement was routinely conducted 3 months after hepatectomy for evaluation of postoperative liver volume regeneration.

Surgical Technique and Postoperative Care

The surgical techniques of donor hepatectomy were described previously.[9] Briefly, a J-shaped incision was made to enter the abdominal cavity. Hepatectomy started with a careful hilar dissection. Intraoperative ultrasound was then performed to confirm the hepatic vein anatomy and to verify the transection plane. For right liver harvesting without the MHV trunk, the transection line was set at a plane to the right of the MHV. In this type of hepatectomy, MHV tributaries, if present and greater than 5 mm in diameter, were isolated and preserved. In contrast, for right liver harvesting with the MHV trunk, the transection line was set at a plane to the left of the MHV. Attention was paid to preserve a hepatic vein branch draining segment IV.

Parenchymal transection was performed using a combination of the clamp fracture technique and a Cavitron Ultrasonic Surgical Aspirator (SNOP 5000; Aloka Co., Tokyo, Japan). All sizable vascular and biliary structures were divided between ligatures. During transection, the inflow was intermittently occluded by Pringle's maneuver and sometimes selectively to the right portal vein and the paramedian branch of the right hepatic artery.[10] After the transection, the portal flow to segment IV was confirmed by Doppler ultrasound.

Postoperatively, all donors were observed in the intensive care unit for one night. Total bilirubin level, aspartate aminotransferase level, and prothrombin time were measured every day after the operation for 1 week and every other day for the next week.

Volume Regeneration Rate

The term "volume regeneration rate" is defined as "increasing percentage per 3 months," as defined previously[11] Accordingly, the volume regeneration rate of segments I–III and segment IV during the initial 3 postoperative months was calculated using the following formulas:

$$RR_{I-III} = (V2_{I-III} - V1_{I-III}) / V1_{I-III} \times 100\ (\%)$$
$$RR_{IV} = (V2_{IV} - V1_{IV}) / V1_{IV} \times 100\ (\%)$$
$$RR_{I-IV} = (V2_{I-IV} - V1_{I-IV}) / V1_{I-IV} \times 100\ (\%)$$

Abbreviations are as follows: RR_n, volume regeneration rate (%) of segment(s) *n* during the first three postoperative months; $V1_n$, volume (ml) of the segment(s) *n* on preoperative CT; $V2_n$, volume (ml) of the segment(s) *n* on CT at the third postoperative month.

The ratio of the remnant liver volume at the third postoperative month to the preoperative whole liver volume (RV), which is another index of liver mass restoration, was also calculated in both groups using the following formula:

$$RV = V2_{I-IV} / V1_{I-VIII} \times 100\ (\%)$$

Statistical Analysis

The clinical parameters were defined as follows: resection type (group), donor age, volume of blood loss during the operation, total ischemia time during hepatectomy, preoperatively estimated volume ratio to whole liver, and volume of the segment. These variables, except for resection type, were compared between groups using the Student *t* test. Multiple regression analysis was then performed to identify predictive factors independently associated with the regeneration rate. The clinical parameters were used as independent factors.

Intergroup comparison of intraoperative data was performed using the Student *t* test. Postoperative alanine aminotransferase level, total bilirubin level, and prothrombin time of the groups were compared using a two-way repeated measures analysis of variance. Differences were considered significant at a *P* value of less than 0.05. Values of measured variables were expressed as median and range or mean ± standard deviation.

Results

Operation

The median volume of blood loss was 420 ml (range, 110–1,537 ml), which was replaced by 320 ml (range, 0–1,200 ml) of each donor's own fresh frozen plasma or whole blood. The operation lasted 505 minutes (range, 355–1,495 min). The arterial blood supply was maintained, and venous congestion was not apparent on the remnant right liver surface at the time of hepatectomy. Intraoperative ultrasound, however, revealed hepatofugal portal flow to segment IV in 10 of 13 Group B donors (Fig. 1). In these cases, liver surface discoloration in a part of segment IV was observed after five minutes of clamping of the middle hepatic artery. There was no significant difference between the groups in any of the intraoperative parameters (Table 1).

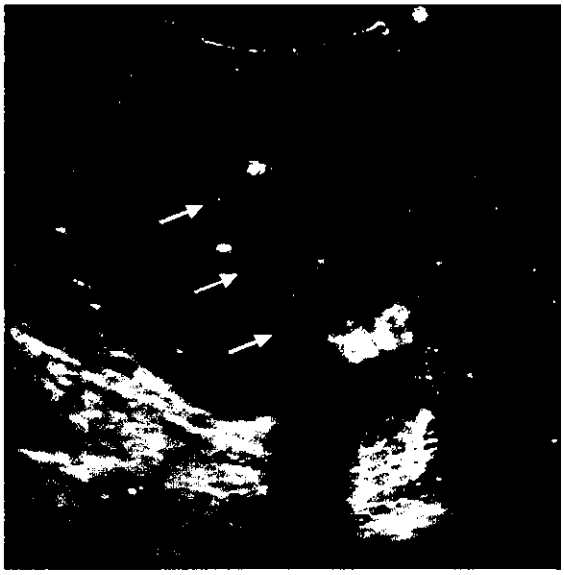

Figure 1. Intraoperative ultrasound after liver transection in a Group B donor. Note the hepatofugal flow to segment IV (arrows).

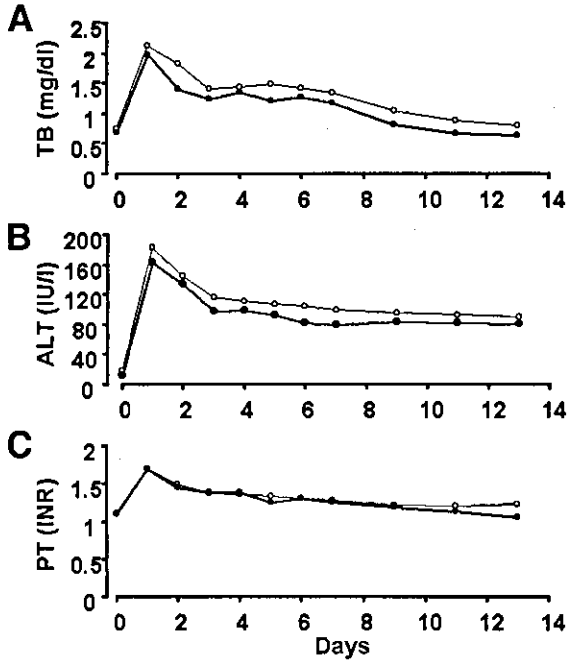

Figure 2. Changes in total bilirubin (A), alanine aminotransferase levels (B), and prothrombin time (C) for 2 weeks after transplantation. Group A is represented by open circles with a thin line (-○-). Group B is represented by closed circles with a thick line (-●-). Abbreviations: TB, total bilirubin; ALT, alanine aminotransferase; PT, prothrombin time.

Postoperative Course and Complications

All donors survived the operation. Postoperative bile leakage occurred in seven donors in Group A and in one donor in Group B. Of these, four donors in Group A required reoperation for repair. Bile leakage was seen from the stump of the right bile duct branch in three and dissection plane of the liver was seen in one, which was closed meticulously. Another donor in Group A was complicated with abscess formation in the dissection plane of the liver and underwent reoperation for drainage.

Laboratory Data

In both groups, total bilirubin level, alanine aminotransferase level, and prothrombin time peaked on the first postoperative day and gradually decreased thereafter (Fig. 2). There was no significant difference between the groups in any of these parameters.

Liver Volumetric Regeneration

The volumetric data are summarized in Table 2 and the volume regeneration rate of each sector is illustrated in Figure 3. There was a significant difference in the ratio of remnant liver volume between the groups. In Group B, RR_{IV} (45 ± 33%) was lower than RR_{I-III} (263 ± 48%, Fig. 4). In Group A, the regeneration rate was more proportional. RR_{IV} in Group B was significantly lower than that in Group A ($P = 0.008$), whereas RR_{I-III} in Group B was significantly higher than that in Group A ($P = 0.004$). There was no significant differ-

Table 1. Intraoperative Data

	Group A (n = 58)	Group B (n = 13)	*P* Value
Duration (min)	533 ± 159 (505-1495)	491 ± 75 (395-650)	0.36
Blood Loss (ml)	449 ± 230 (250-1537)	563 ± 268 (165-1125)	0.12
Autologous Blood transfusion (ml)	358 ± 307 (0-1200)	215 ± 289 (0-600)	0.42
Ischemic Time (min)	53 ± 17 (45-89)	59 ± 18 (40-95)	0.23

NOTE: Numbers in parentheses indicate range.

Table 2. Volumetric Data

	Group A (n = 58)	Group B (n = 13)	*P* Value
$V1_{I-IV}/V1_{I-VIII}$ (%)	34 ± 2 (30-39)	37 ± 2 (35-41)	0.04
$V1_{I-III}$ (ml)	228 ± 55 (131-381)	200 ± 38 (141-263)	0.17
$V1_{IV}$ (ml)	136 ± 38 (85-205)	134 ± 37 (83-194)	0.91
$V2_{I-III}$ (ml)	506 ± 124 (348-849)	557 ± 157 (423-935)	0.32
$V2_{IV}$ (ml)	300 ± 105 (150-659)	194 ± 72 (118-343)	0.008
RR_{I-III} (%)	208 ± 32 (149-280)	263 ± 48 (205-337)	0.004
RR_{IV} (%)	125 ± 62 (50-307)	45 ± 33 (9-101)	0.008
RR_{I-IV} (%)	125 ± 38 (72-218)	124 ± 37 (70-180)	0.98
RV (%)	75 ± 10 (56-98)	80 ± 12 (63-98)	0.19

Abbreviations: $V1_n$, volume of the segment(s) *n* on preoperative CT (ml); $V2_n$, volume of the segment(s) *n* on CT at the third postoperative month (ml); RR_n, volume regeneration rate of segment(s) *n* during the first three postoperative months (%); RV, ratio of the remnant liver volume at the third postoperative months to the preoperative whole liver volume given as $V2_{I-IV}/V1_{I-VIII} \times 100$ (%).
NOTE: Numbers in parentheses indicate range.

ence between the groups in RR_{I-IV} or RV ($P = 0.19$ or $P = 0.98$, respectfully).

The results of multiple regression analysis are shown in Table 3. The resection type was the sole significant predictive factor for the regeneration rate of segments I–III and segment IV. In contrast, the preoperative volume percentage rate to the left liver (segments I–IV), but not the graft type, affected the regeneration rate of the remnant liver.

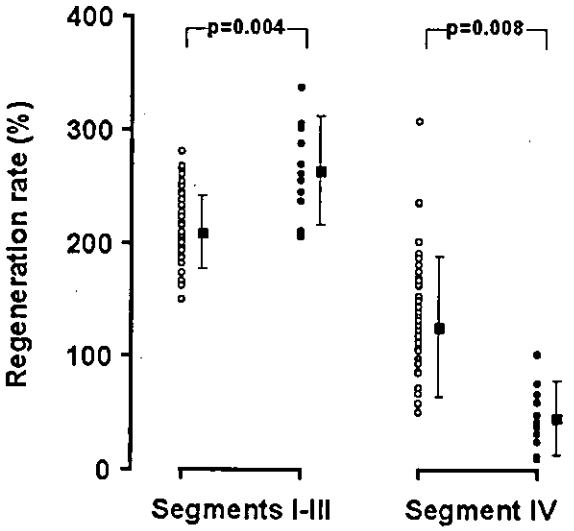

Figure 3. Volume regeneration rate of segments I–III and IV in each group. Group A is represented by open circles. Group B is represented by closed circles. Closed squares and vertical lines indicate the average levels ± standard deviation.

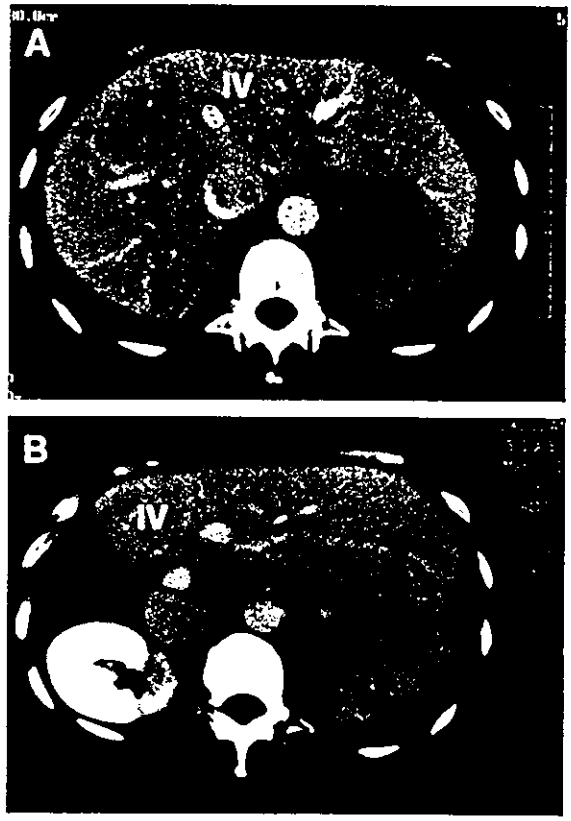

Figure 4. Computed tomography images of a donor in Group B taken preoperatively (A) and at the third postoperative month (B). The amount of parenchyma of segments I–III was more significant than that of segment IV. The broken line was drawn between segments I–III and IV. The regeneration rate of segments I–III and IV in the donor were 205% and 9%, respectively.

Table 3. Predictive Factors for Volume Regression Expressed as *P* Value

	Group	Age	Blood Loss	Ischemic Time	Preoperative Volume Ratio to Whole*	Preoperative Volume*
RR_{I-III}	0.0007	0.66	0.15	0.58	0.21	0.32
RR_{IV}	0.007	0.93	0.44	0.92	0.54	0.61
RR_{I-IV}	0.17	0.95	0.60	0.47	0.001	0.11

NOTE: Numbers indicate *P* values.
*Corresponded to that of the dependent factor.

Discussion

The present study demonstrated effects of outflow deprivation on liver regeneration. The regeneration rate of segments I–III and that of segment IV after right hepatectomy was proportional when the blood circulation was maintained. In contrast, segment IV without the MHV had impaired volume regeneration compared with cases in which the MHV was preserved. Conversely, in such cases, segments I–III underwent accelerated volume regeneration, probably due to a compensatory mechanism. The results are consistent with those of a recent report,[11,12] on the volumetric changes of the right liver after left or right liver donation.

Deprivation of the MHV tributaries induces hepatofugal portal flow of a part of segment IV.[13] Poor portal blood supply leads to unsatisfactory regeneration of segment IV, because portal blood is the most important nutritional supply for the liver parenchyma, and suspension of partial portal blood inflow results in impaired regeneration of the corresponding hepatic area.[14] Cheng et al.[15] reported that in LDLT using the extended right lobe graft (segments II, III, and a part of segment IV), a part of segment IV decreased in volume when the MHV tributaries were not reconstructed. This observation can be explained by the hepatofugal flow of the portal branch of segment IV induced by deprivation of the MHV tributaries. A previous report[16] revealed that in LDLT, venous flow of the ligated MHV tributaries drained into the right hepatic vein by way of the venous collaterals that rapidly develop approximately 1 week after transplantation, which was confirmed by Doppler ultrasonography. Liver regeneration generally begins during the first 3 to 5 days after hepatectomy.[17]

Volume regeneration of segment IV without MHV drainage was not uniform among the individuals, ranging from 9 to 101%. The left medial vein draining the left part of the medial segment is close to the confluence of the middle and left hepatic veins.[18] This tributary flows into the left hepatic vein in the majority of cases, but sometimes it flows into the MHV. The variation in volume enlargement of segment IV might reflect an anatomic difference in left medial vein bifurcation. Thus, detailed recognition of the venous territory pattern on preoperative CT and ultrasonography in individual donors is essential.

As for whole remnant liver regeneration, the ratio of the preoperative left liver to the whole liver was a significant predictor. The results indicate that smaller livers will regenerate more quickly, which is consistent with previous data that regeneration of the partial liver converges to the standard liver volume.[19] In addition, partial venous disruption did not lead to overall retardation of mass restoration with the balance between impaired and accelerated regeneration of respective segments. Additionally, postoperative liver functional recovery was comparable between groups. These results suggest that extended right hepatectomy can be safely performed using our criteria. The procedure may be more frequently adopted, because it was not as risky for donors as previously estimated and could prevent a complex reconstruction strategy in MHV reconstruction in recipients. A previous report[20] suggested that a residual liver volume of 30% of the total volume is the lower limit. We believe, however, that a larger safety margin should be added to the limitation. We made a limitation of age less than 50 years for the donor for extended right hepatectomy. Previous studies reported that liver grafts from older donors had an inferior ability to regenerate.[21,22] The present multivariate analysis, however, failed to support the theoretical background of the age limitation. Nonetheless, without more data we will continue to employ the present criteria for donor selection for extended right hepatectomy.

Although the multivariate analysis revealed that the total blood-loss volume was not a significant predictor for liver regeneration, minimizing blood loss is clearly important for donor safety. Severe bleeding is associ-

ated with decreased hepatic blood flow and ischemic injury.[20] Although the upper limitation on ischemic duration should be discussed, previous data[10] indicated a beneficial effect of Pringle's maneuver on graft outcome. As the application of Pringle's maneuver requires no specific skills, surgeons should not hesitate to apply this technique to donor hepatectomy.

In summary, the present data indicated that right hepatectomy with MHV resection was associated with latent impairment in postoperative liver regeneration of segment IV. However, we could perform extended right hepatectomy with low postoperative morbidity when the donor was under 50 years of age, and the remnant left liver was estimated to be more than 35% of the whole liver. For donor safety, careful preoperative consideration should be given on a case-by-case basis to the extent of right liver harvesting.

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Refinement of Venous Reconstruction Using Cryopreserved Veins in Right Liver Grafts

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Short and direct vein anastomosis is generally performed in living donor liver transplantation using a right liver graft. The graft will regenerate, however, and might thus compress the anastomosis. We formulated a strategy for outflow reconstruction in right liver graft. When reconstruction of multiple short hepatic veins was necessary, a cryopreserved inferior vena cava graft was anastomosed with the hepatic veins of the graft in a basin. When there were no major short hepatic veins in the graft, a rectangular-shaped vein graft was used to make a single orifice using the middle and right hepatic veins in the graft. When there were no tributaries of the middle hepatic vein to be reconstructed, a diamond-shaped vein patch was anastomosed on the anterior wall of the right hepatic vein orifice of the graft. These techniques were satisfactorily applied in 40 patients with no torsion or tension at the anastomotic site of the hepatic venous reconstruction or other complications in outflow. The present strategy seemed to be technically feasible for outflow reconstruction in a right liver graft. (*Liver Transpl 2004;10: 541–547.*)

Living donor liver transplantation (LDLT) is now widely performed to compensate for the critical cadaveric organ shortage in adult patients.[1] The significant increase might be due to the introduction of right liver graft for adult patients.[2]

An extended right liver graft (ERLG)[3] which includes the trunk of the middle hepatic vein (MHV), was devised by Fan and colleagues. Although the extent of the donor hepatectomy might be increased, this method is beneficial with regard to venous drainage of the graft because the MHV is a major draining vein of the right paramedian sector, and its role in the left paramedian sector is limited.[4] A right liver graft without the MHV trunk (RLG) is now more commonly used. This type of graft, however, can cause severe congestion of the right paramedian sector (segments V and VIII). MHV drainage into the recipient's venous system can be reconstructed using vein grafts[5] to provide a functioning liver mass comparable to an extended right liver.[6]

Vein reconstruction in a right liver graft is technically challenging.[7] The different strategy may be devised according to the existence of thick MHV tributaries or inferior right hepatic vein (IRHV). Additionally, the average right liver graft is only half size for the recipient, and regenerates in all directions after LDLT. As a result, the graft might compress the venous anastomotic site. In the present manuscript, we formulated a strategy for vein reconstruction tolerable to the compression.

Material and Methods

Patients

From January 2002 to April 2003, 62 adult patients underwent LDLT at our hospital. Of these, 40 patients (31 men, 9 women) received a right liver graft and were the subjects of the present study. The age ranged from 20 to 66 years (median age = 52 years). The indications for LDLT in these patients included hepatitis C virus–cirrhosis (n=12), hepatitis B virus–cirrhosis (n=8), primary biliary cirrhosis (n=6), cryptogenic cirrhosis (n=5), fulminant hepatic failure (n=4), biliary atresia (n=2), Wilson's disease (n=1), citrullinemia (n=1), and primary sclerosing cholangitis (n=1).

Donors

The donors were 20 men and 20 women. The age ranged from 20 to 61 years (median age = 36 years). Their relation to the patients included children (n=20), siblings (n=10),

Abbreviations: AST, aspartate aminotransferase; CT, computed tomography; ERLG, extended right liver graft; IRHV, inferior right hepatic vein; IVC, inferior vena cava; LDLT, living donor liver transplantation; LHV, left hepatic vein; MHV, middle hepatic vein; MRHV, middle right hepatic vein; RLG, right liver graft without the MHV trunk; RHV, right hepatic vein; SHV, short hepatic vein; VC, vena cava.

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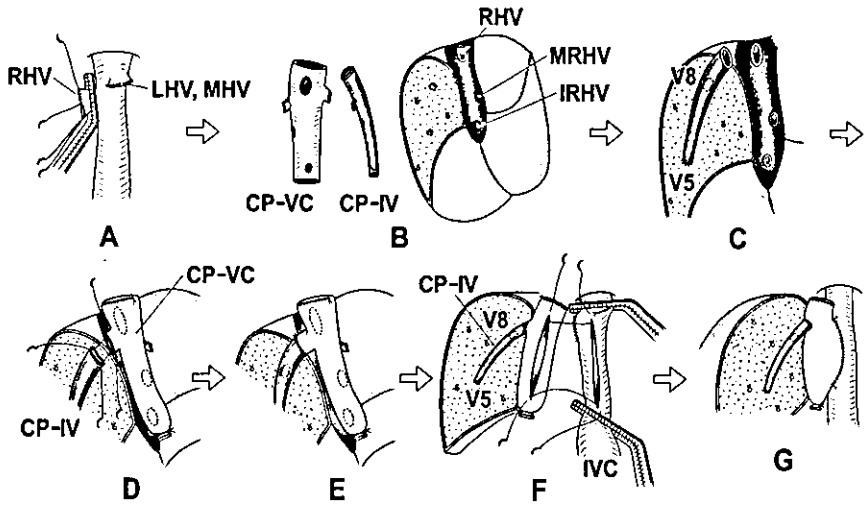

Figure 1. Schematic view of double vena cava technique. (A) All hepatic vein trunks of the recipient (LHV, MHV, RHV) were sutured at their roots. (B) Three side holes were created in the wall of the cryopreserved vena cava graft (CP-VC) for anastomosis with the right hepatic vein and the short hepatic veins (IRHV or MRHV) of the graft. (C, D) Another cryopreserved vein graft (CP-IV) can be used for middle hepatic vein reconstruction. (E) The stump of venous branch was anastomosed with jumping vein graft for the middle hepatic vein reconstruction. (F, G) Side-to-side anastomosis between the recipient inferior vena cava and CP-VC with continuous sutures was performed.

spouses (n=5), parent (n=4), and nephew (n=1). Right liver volume was preoperatively estimated by computed tomography (CT). Candidates in whom the right liver comprised more than 70% of the whole liver were rejected as prospective donors. An estimated graft volume to recipient standard liver volume[8] ratio of 40% was the lower limit for right liver transplantation.

The number and diameter of thick MHV tributaries draining the right paramedian sector were evaluated on CT. The tributaries were classified as V8, which drained the cranial part of the portal trunk of the right paramedian sector, and V5, which drained the corresponding caudal part. When the donor was under 50 years of age and the remnant left liver was estimated to be more than 35% of the whole liver, extended right liver graft (ERLG) harvesting was considered. Otherwise, a right liver graft without the middle hepatic vein trunk (RLG) graft harvesting was indicated. Details regarding the selection criteria and evaluation are described elsewhere.[9]

Homologous Vein Graft Preparation

Vein grafts were provided by the University of Tokyo Tissue Bank. The preservation and thawing methods were described previously.[10] In brief, the vein grafts were obtained from cadavers or nonheart beating donors within 24 hours after cardiac arrest after obtaining informed consent. The specimens were packed in a sterile bag and frozen slowly in a programmable freezer at a rate of 1°C/min to -40°C. They can be semipermanently stored in liquid nitrogen until use.

Surgical Procedure

The right liver was harvested as described previously.[9] In a basin, the graft was flushed with 1 liter of University of Wisconsin solution through a cannula inserted into the right portal vein. When there were major short hepatic veins, including inferior or middle right hepatic veins in the graft, the double vena cava (VC) technique was applied. The method was refined from our previous method.[11] A cryopreserved VC graft was prepared for venoplasty in a basin (Fig. 1). A side hole was made in the wall of the VC, which was anastomosed with the hepatic veins in the graft. When direct anastomosis was difficult for a distance between the orifice of the middle hepatic vein (MHV) tributaries and VC graft, an iliac branch of the VC vein graft was used for the interposition. If the iliac branch was not available, another cryopreserved vein graft was used for interposition. With this technique, there was no need to preserve any hepatic vein trunks of the recipient, which were sutured at their roots. Then, the inferior vena cava (IVC) of the recipient was partially clamped and incised longitudinally approximately 5 cm. The VC graft was similarly incised longitudinally, and then anastomosed side-to-side with the IVC of the recipient.

When there were no major short hepatic veins in the graft or a VC graft was not available, a rectangular-shaped patch method was applied (Fig. 2). The orifices of right hepatic vein (RHV), MHV, or MHV tributaries received venoplasty with a cryopreserved vein graft or recipient left portal vein. They were cut in a rectangular shape and placed on the orifices of the MHV and RHV of the graft. The vein grafts were sutured to the right side of the MHV orifice and the left side of the RHV orifice in a basin. When the distance between the orifice of V8/V5 and that of RHV was too great in RLG, and not appropriate for this technique, another vein graft was substituted as an MHV. Then, a rectangular-shaped vein patch was placed between the right side wall of the interposition graft

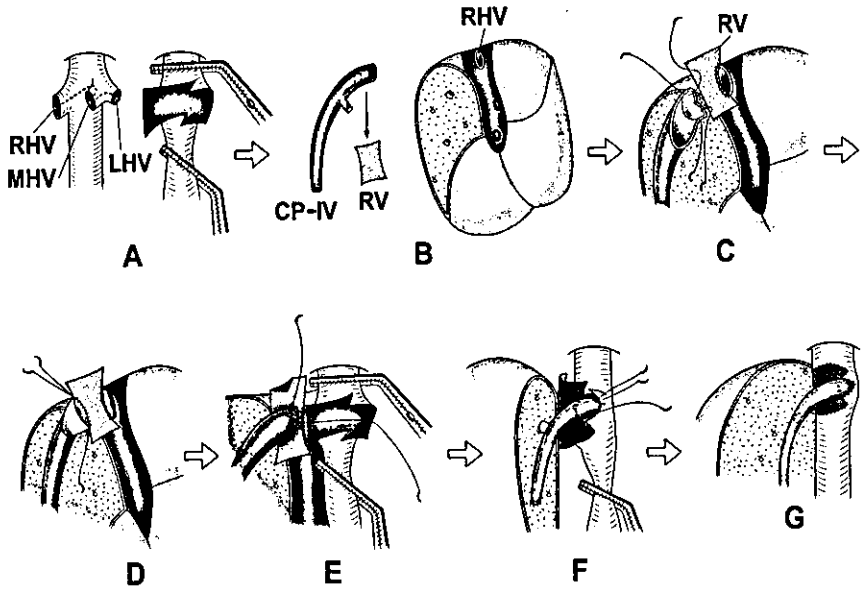

Figure 2. Schematic view of rectangular shaped patch method. (A) All hepatic vein trunks of the recipient (LHV, MHV, RHV) were cut and one wide single orifice was made. (B) The cryopreserved vein graft (CP-IV) was used for interposition graft for MHV reconstruction. A proximal part of the CP-IV was cut and used for patching between the orifice of graft RHV and the interposition graft (RV). (C, D) The RV patch was anastomosed with the left side of the RHV orifice of the graft. The posterior wall of the CP-IV was cut longitudinally, which was anastomosed with another edge of the RV patch. (E) The right side of the RHV orifice of the graft was anastomosed with the edge of the common hepatic vein of the recipient. (F,G) The anterior wall of the CP-V, RV patch and the edge of common hepatic vein of the recipient were sutured together to make a reservoir of outflow between the liver graft and recipient vena cava.

and the left side of the RHV orifice. In the recipient, a wide orifice was created by dividing three hepatic veins. The recipient IVC was cross-clamped above and beneath the roots of the hepatic veins. Anastomosis was started between the right edge of the recipient's common hepatic vein and the right side of the graft RHV orifice. Next, the left edge of the recipient's common hepatic vein and the left side of the graft MHV orifice were put together. Then, the caudal edges of the graft veins and recipient venous wall were sutured and the cranial edges were closed.

When the graft had no major MHV tributaries to be preserved, a diamond-shaped patch method was applied (Fig. 4). The method was refined from our previous method.[12] The anterior wall of the RHV orifice of the graft was cut short to widen the orifice while in the basin. An iliac vein graft or left portal branch of the recipient was anastomosed to the anterior

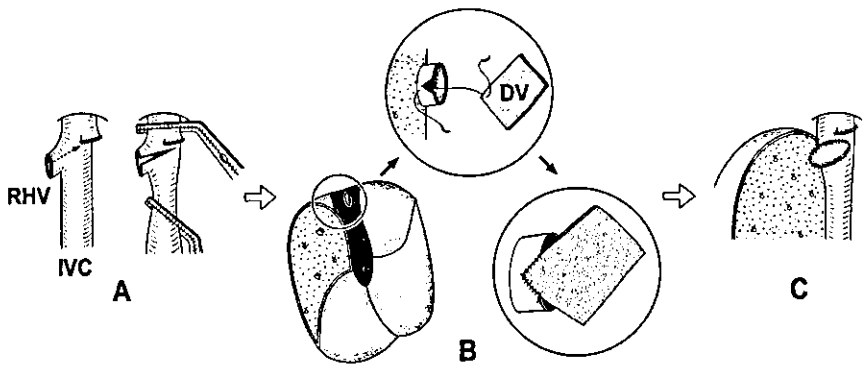

Figure 3. Schematic view of diamond-shaped patch method. (A) The anterior wall of the recipient right hepatic vein (RHV) was cut approximately 2 cm under cross-clamping of inferior vena cava (IVC). (B) The anterior wall of the RHV orifice of the graft was shortly (5 mm) cut for widening the area of orifice. The diamond shaped venous patch (DV) was anastomosed to the orifice of the RHV. (C) End-to-end anastomosis was done between the recipient and graft RHV with continuous sutures.

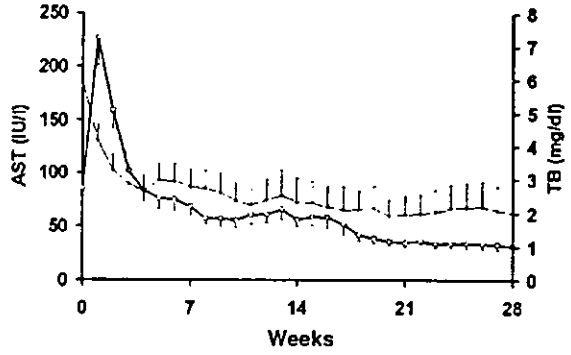

Figure 4. Changes in serum aspartate aminotransferase (AST, open circle with thick line) and total bilirubin level (TB, closed circle with thin line) for three weeks after the operation (n=40). Error bars represent standard error of the means.

wall of the graft RHV orifice. In this technique, the left and middle hepatic veins of the recipient were closed with a running suture. Under cross-clamping of the IVC, the anterior wall of the RHV and the IVC was incised. At first, the posterior wall of the RHV orifice of the graft and that of the recipient RHV were sutured together. Then, the diamond-shaped vein patch and anterior wall of the graft RHV orifice were anastomosed.

Postoperative Evaluation

Vascular flow in the graft or interposition vein patency was checked by Doppler ultrasound every day until the 14th postoperative day and once a week thereafter until hospital discharge. Enhanced CT was performed 1 and 3 months after LDLT to check for vein graft patency. Aspartate aminotransferase and total bilirubin levels were measured every day after LDLT for 4 weeks.

Results

Clinical Outcome of Donors

The graft types harvested consisted of 32 RLGs and 8 ERLGs. The weight of the graft ranged from 411–917 g (median, 607 g) and corresponded to 48–67% (54%) of donors' standard liver volume. Blood loss ranged from 160–1125 g (460 g), which was replaced by 0–1200 ml (310 ml) of the donors' own fresh frozen plasma or whole blood. The operation lasted 355–665 min (458 min). Bile juice leakage from the dissection plane of the liver (n=1) or stump of the bile duct (n=1) necessitated surgical repair. The median hospital stay was 16 days. All of the donors returned to their normal daily lives.

Venous Reconstruction and Patency

The number of grafts using each technique and the number of vein grafts are shown in Tables 1 and 2. A total of 19 of 32 RLG grafts received reconstruction of MHV tributaries. Reconstructed MHV tributaries consisted of both V8 and V5 (n=15), V8 (n=3) and V5 (n=1). No MHV tributaries were reconstructed in 13 grafts because of a negligible area of congestion in 11 and a lack of dominant tributaries in 2.[13] Upon reconstruction of the inflow, good hepatic venous drainage was confirmed by Doppler ultrasound.

The time for outflow reconstruction in each technique is shown in Table 3. The liver graft cold preservation time varied, ranging from 12–142 minutes (median 62 minutes). The median time for the venous reconstruction in the recipient (after the graft was taken off ice) was 27 minutes. Doppler ultrasound and CT examination revealed that all the vein grafts were patent for at least 3 months after LDLT.

Laboratory Data, Morbidity, and Mortality

The graft corresponded to 33–71% (median, 51%) of the standard liver volume of the recipients. The blood loss ranged from 30–961 g per body weight (kg, median, 920 g/kg). The operation lasted 735–1345 min (920 min). Postoperative complications included acute rejection in 11, and bile juice leakage from the anastomosis, which necessitated surgical revision in 2.

Table 1. Detail of MHV Reconstruction

Technique	Graft Type RLG:ERLG	Reconstruction V5	V8	SHV
Double VC (n = 16)	13:3	8/13	9/13	16/16
Using rectangular shaped vein patch (n = 14)	9:5	8/9	9/9	10/14
Using diamond shaped vein patch (n = 10)	10:0	0/10	0/10	5/10

Abbreviations: ERLG, Right liver graft which includes the trunk of the middle hepatic vein; RLG, Right liver graft without the middle hepatic vein trunk; SHV, Short hepatic vein; V5, V8, tributaries of middle hepatic vein; VC, vena cava.