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Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation?

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Abstract: As treatments for acute cellular rejection (ACR) and recurrent hepatitis caused by hepatitis C virus (HCV) are dramatically different, making a precise diagnosis is considered to be essential in patients after liver transplantation. Therefore, we investigated whether immunohistochemical detection of FOXp3, a marker for regulatory T cells (CD4+ CD25+), could be used to differentiate between recurrent hepatitis C and ACR. From a group of 103 cases of living-donor liver transplantation (LDLT), 48 samples were taken via liver biopsy from 20 patients with HCV infection. An initial diagnosis was made based on hematoxylin and eosin staining, which was scored with the hepatitis activity index (HAI) grading, whereas ARC was scored with the rejection activity index (RAI). The FOXp3 immunohistochemical staining on serial specimens was retrospectively analyzed, scoring from 0 to III. The time after LDLT was a median of 270 (range: 14–2000) days, whereas the median number of biopsies per patient was 3 (range: 1–8). The HAI was significantly different between 0 vs. I, and II vs. III, in terms of the FOXp3 score. On the other hand, a significant difference in the RAI was only found between 0 vs. I. In conclusion, FOXp3 may represent a surrogate marker for recurrent HCV infection after LDLT.

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Key words: FOXp3; recurrent hepatitis C; acute cellular rejection; living-donor liver transplantation; CD25

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Recurrent hepatitis C virus (HCV) infection after liver transplantation (LT) remains a therapeutic challenge, especially in a setting with living donors, where the possibility of retransplantation is limited. To date, the best treatment for chronic HCV infection is interferon (IFN) combined with ribavirin, with a 50% sustained virologic response rate (1). In fact, diagnosis of recurrent hepatitis C is sometimes difficult to make, because the presentation, in the histologic view, has many similarities to rejection (2).

Regulatory T cells (Tregs) are supposed to regulate an over-reactive autoimmune response, and were detected to be CD4+ CD25+. Tregs are engaged in the maintenance of self-tolerance by suppressing the activation and expansion of self-reactive lymphocytes (3–5). Loss of this suppressing function may lead to chronic inflammation and/or autoimmunity (6–12). Currently, the best indicator of Tregs function is thought to be the intracellular expression of forkhead box P3 (FOXp3), which is also crucial for Tregs development (13). One of our co-authors (H.M.) previously reported the usefulness of examining liver-targeted Tregs as indicators of chronic hepatitis B virus and HCV infection (14).

In the setting of LT, it was also reported that needle biopsy could provide a source for determining FOXp3 messenger RNA (mRNA) expression after LT (15). In addition, it was reported that FOXp3 mRNA in peripheral blood is a useful marker for acute cellular rejection (ACR), whereas CD4+ CD25+ numbers in peripheral blood may be a marker to predict recurrence of HCV after LT (16, 17).

However, to the best of our knowledge, studies have not previously examined the use of FOXp3 in

Characteristics of liver transplant patients with HCV

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis		FOXp3	IFN
				HAI	RAI		
1	58/M	1b	1800	5	4	0	+
			2000	5	3	1	+
2	57/F	1b	540	0	0	0	+
			720	3	0	2	+
3	53/F	1b	30	3	3	0	–
			180	6	6	1	–
			540	3	7	0	+
			1020	3	0	2	+
4	52/M	II	20	1	8	1	–
			35	6	4	2	–
5	63/F	1b	150	3	4	1	+
			270	4	0	2	+
			510	5	0	2	+
			630	0	0	1	– (SVR)
6	57/M	1b	14	1	4	0	–
			180	6	2	2	+
			540	6	0	0	+
7	55/F	1b	180	3	6	1	–
			194	7	5	3	–
			208	6	4	2	–
			360	7	3	1	+
			1080	7	3	1	+
8	64/F	1b	21	2	3	2	–
			74	2	3	2	–
			180	6	3	3	+
9	61/F	1b	30	3	0	3	–
			720	4	2	1	–
			780	4	2	1	–
10	62/M	II	60	6	4	3	+
11	67/M	1b	14	7	7	1	–
			400	7	3	2	+
			720	3	5	1	–
12	58/M	1b	90	2	1	0	–
			360	3	6	1	+
			720	2	4	2	+ (SVR)
13	51/F	1b	450	10	5	3	–
			900	10	6	3	–
			1380	10	5	3	+
			1835	10	4	3	+
14	59/M	1b	360	2	0	0	+ (SVR)
15	54/M	1b	390	0	0	0	– (SVR)
16	68/F	1b	150	3	3	1	–
			300	6	3	1	+
17	59/F	1b	180	4	0	0	–
			360	5	0	0	+
			480	2	1	0	+
18	65/F	1b	30	4	4	1	–
			120	9	6	3	+
			135	3	3	2	+

Table 1 continued

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis		FOXp3	IFN
				HAI	RAI		
19	59/M	1b	21	4	3	0	–
			41	1	3	0	–
20	65/M	1b	480	4	0	2	+
			570	3	0	1	+

HCV, hepatitis C virus; Pt no., patient number; LDLT, living-donor liver transplantation; HAI, hepatitis activity index; RAI, rejection activity index; FOXp3, marker for regulatory T cells; IFN, interferon; M, male; F, female; SVR, sustained virologic response.

Table 1

liver infiltrating lymphocytes to differentiate recurrent HCV infection from ACR.

Patients and methods

Patients

Of 103 cases of living-donor LT (LDLT), 29 patients (mean age: 57.8 ± 10.6, male:female ratio: 17:12) were positive for anti-HCV antibodies. Fifty-eight samples were taken via liver biopsy from 20 patients (Table 1). Liver biopsy tissue specimens were taken by a needle puncture for diagnostic purposes. HCV serotype was type I in 18 of those patients, whereas it was type II in 2 patients. In all patients, IFN therapy was eventually attempted. Immunosuppression was based on our protocol using cyclosporine as previously reported (1).

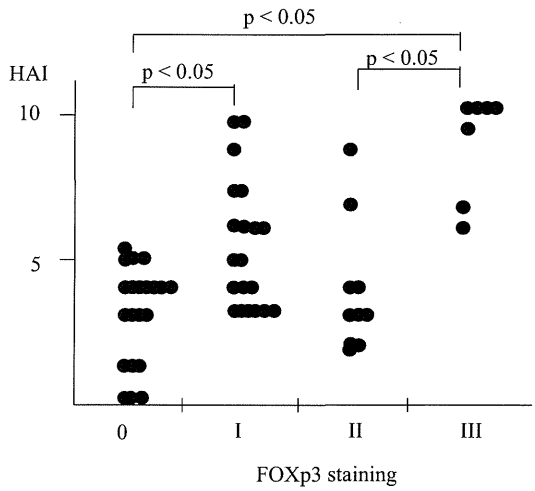

Fig. 1. The relationship between hepatitis activity index (HAI) grading and FOXp3 staining. Significant differences were seen between 0 and I, II and III, and 0 and III with regard to the FOXp3 staining.

Methods

All tissues were fixed in 10% neutral buffered formalin and were then embedded in paraffin, and 4-mm-thick serial sections were cut from each paraffin block. T cells were examined immunohistochemically using an anti-CD4 antibody (Novocastra, Newcastle, UK).

Initial diagnosis was made based on hematoxylin and eosin (H&E) staining, followed by FOXp3 immunohistochemical staining (eBioscience, San Diego, California, USA) on serial specimens. Among aggregated lymphocytes, the number of FOXp3-positive CD4+ lymphocytes was scored as 0 = none, I = 1–9 cells, II = 10–19 cells, and III = >20 cells, as in our previous report (14). The association of FOXp3 with hepatitis activity index (HAI) and/or rejection activity index (RAI) (median 3, range: 0–8) was investigated.

To classify the degree of hepatic inflammation (hepatic activity), we used the HAI score as described by Knodell et al. (18). Based on their criteria, the H&E-stained specimens of the non-cancerous liver tissues were examined and classified into 4 categories. ACR was scored based on the RAI according to the Banff schema (19, 20).

All data are expressed as the median values with ranges. The statistical analysis was performed using the Mann–Whitney *U*-test for continuous values, and the chi-squared test for categorical values. A significant difference was defined as a *P*-value of <0.05. The StatView 5.0 statistical software package (Abacus Concepts, Berkeley, California, USA) was used for all statistical analyses.

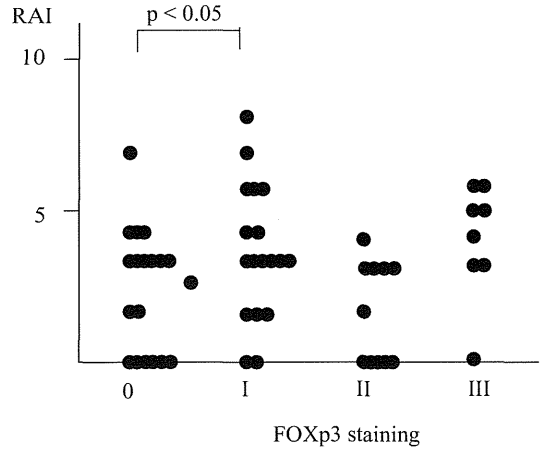

Fig. 2. The relationship between the rejection activity index (RAI) and FOXp3 staining. A significant difference was only seen between 0 and I in terms of FOXp3 staining with regard to the RAI.

Results

Table 1 showed characteristics of the patients and a summary of the histologic findings. The median days from the time of liver biopsy was 270 days (range: 14–2000 days) after LT. The median grade, based on the HAI, was 4 (0–10). The median degree of rejection, based on the RAI, was 3 (0–8). The difference in HAI was significant between 0 vs. I, as well as II vs. III, based on the number of FOXp3-positive cells (Fig. 1). On the other hand, a significant difference in the RAI was only seen for 0 vs. I (Fig. 2).

Figure 3 shows a representative liver biopsy specimen of a patient with recurrent HCV infection after LT. The patient was a 58-year-old woman who had undergone LT 4 years earlier. Her aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels were elevated at 107/80. H&E staining revealed an HAI grade of 10 and RAI of 5.

Figure 4 shows another representative liver biopsy specimen. This patient was a 62-year-old woman who underwent LT 4 months before the biopsy. Her AST/ALT levels were elevated at 68/65. H&E staining revealed an HAI grade of 9 and RAI of 6.

On the other hand, Figure 5 shows a representative liver biopsy specimen from a patient with ACR. The patient was 58-year-old woman who had undergone LT 4 years before the biopsy samples were taken. Her AST/ALT levels were elevated to 120/108. H&E staining revealed HAI grade of 3 and RAI of 6.

Discussion

This study examined the distribution and frequency of the appearance of Tregs in the liver after LT. After LT, in patients with hepatitis C, both ACR and recurrent HCV can lead to elevation of transaminases, in

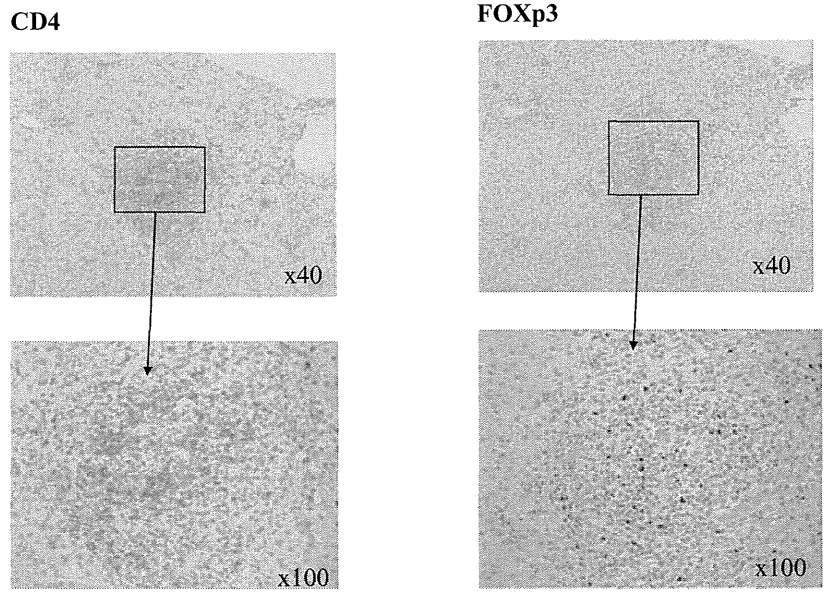

Fig. 3. Case 13. Representative findings in a liver with recurrent hepatitis C. Many liver infiltrating lymphocytes were positive for CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

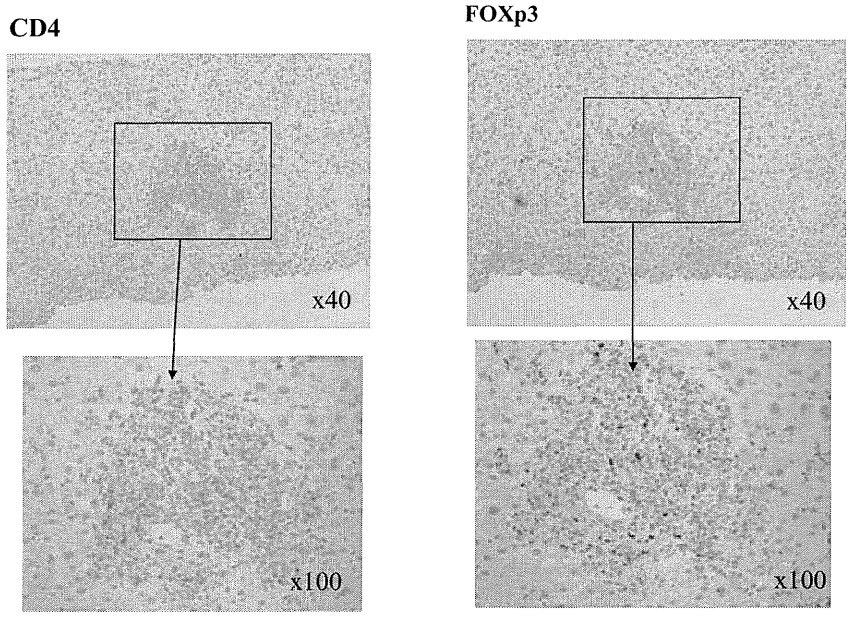

Fig. 4. Case 18. Representative findings in a liver with recurrent hepatitis C. Many liver infiltrating lymphocytes were positive for CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

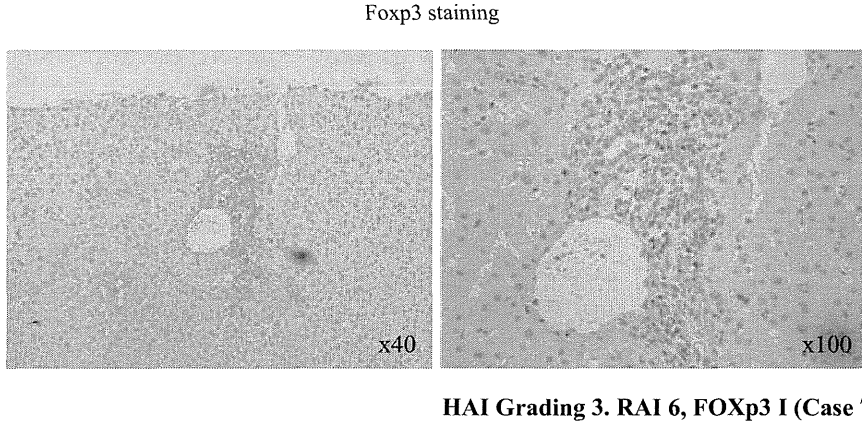

Fig. 5. Case 7. Representative findings in a liver with acute cellular rejection. Few liver infiltrating lymphocytes were positive for both CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.

addition to possible vascular abnormalities. Previously, Jain et al. (21) reported the significance of C4d expression in infiltrating lymphocytes, as CD4, CD8, and CD56 were similar in both ACR and recurrent HCV infection. However, accurately differentiating ACR from hepatitis C can sometimes be very difficult.

In previous reports regarding LT, the significance of Tregs in the grafted liver has been controversial. One report showed a relationship between ACR and an increase in Tregs. Intrahepatic detection of FOXp3 gene expression after LT can be accomplished using minimally invasive aspiration biopsy (15). With regard to recurrent hepatitis C, FOXp3 mRNA expression was used to differentiate between the two conditions. Based on needle biopsy, they reported that intrahepatic FOXp3 levels are associated with HCV re-infection and a history of acute rejection, and that the level increased within the first year after LT (15).

Generally speaking, Tregs are associated with graft tolerance in organ transplantation. It seems likely that FOXp3 mRNA expression is associated with graft acceptance (22). It was reported that CD4+ FOXp3 cells are present within grafts in a subset of tolerant patients after human LT (23). However, in the present study, no clear relationship was observed between

ACR and Tregs, except to find a statistical difference between 0 and I in FOXp3 staining. This relationship needs further investigation without the interference of HCV infection.

Sakamoto et al. (24) reported increased expression of FOXp3 mRNA immediately after LDLT, probably because of the activation of T cells, including Tregs and other T-cell subsets. In addition, it was reported that expression of FOXp3 mRNA on days 14, 21, and 28 after transplantation were lower in recipients with ACR within 60 days after LDLT. In our study, the median time since transplantation was 270 days. This is different from previous reports, which focused on short-term diagnosis using FOXp3 staining in the liver and peripheral blood. Usually, 6 months after LT, the level of immunosuppression is stabilized. HCV infection could occur during this period, and antiviral therapy is often initiated. In our study, most patients were undergoing or had already received antiviral therapy with IFN and ribavirin. Although we showed a relationship with FOXp3 expression, we were unable to clarify the function of Tregs in recurrent HCV infection after LT. Further investigation will be needed.

After effective IFN therapy, the number of infiltrating lymphocytes seemed to decrease, which made scoring FOXp3 staining difficult. It was unclear whether the character of the infiltrating lymphocytes changed over the course of treatment. In settings other than transplantation, the FOXp3 staining system may be used to differentiate hepatitis C from autoimmune-like disease or other causes of hepatitis.

In conclusion, FOXp3 staining in infiltrating lymphocytes in the liver may represent a surrogate marker for recurrent HCV infection after LDLT.

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Authors' contributions: S.E., T.K., and K.N carried out study conception and design. M.H. and A.S. provided acquisition of data. M.T., T.I., and H.M. performed analysis and interpretation of data. M.T. and S.E. were responsible for drafting of the manuscript. T.K., S.E., and K.N. performed critical revision.

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ORIGINAL ARTICLE

Use of stepwise versus straightforward clamping of biliary drainage tubes after living-donor liver transplantation: a prospective, randomized trial

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Abstract
Background/purpose There has been no report describing the optimal clamping method for biliary drainage tubes in living-donor liver transplantation (LDLT), although biliary splinting and drainage plays an important role in this procedure.
Methods When performing LDLT, we generally use a 2-mm drainage tube for the splint at the biliary anastomosis, and externalize it through the lower common bile duct. In the present study, when the serum levels of total bilirubin were lower than 5 mg/dl, and negativity for biliary complications and good passage of contrast media to the duodenum were confirmed, the drainage tubes were clamped. To determine the optimal clamping method, patients were randomly divided into two groups; those whose drainage tubes were subjected to stepwise clamping for 3, 6, 12, and 24 h per day ($n = 20$), and those whose drainage tubes were subjected to straightforward clamping ($n = 20$).
Results The results of liver function tests and rates of clamping failure were not different between the two groups after the different clamping methods were used.
Conclusions Straightforward clamping could be a simple and reasonable method to close a biliary drainage tube after LDLT.

Keywords Clamp · Liver transplantation · Biliary drainage · Tube

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Introduction

Biliary drainage and splinting plays an important role in living-donor liver transplantation (LDLT) because the rate of biliary complications is higher in LDLT than in deceased-donor whole LT [1, 2]. We generally use an external biliary splint and have previously reported the two-step method used for removal of the splint [3].

Anecdotally, a stepwise clamping method has sometimes been preferred to straightforward clamping to train the sphincter of Oddi in the papilla of Vater after decompression through the drainage tube following LT. The preference for the stepwise method is due to concerns that straightforward clamping may lead to dysfunction of the sphincter of Oddi after long-term decompression through the stent tube. However, it is not known whether stepwise clamping truly yields a better outcome, and there has been no report examining this matter in LT.

We investigated 40 LDLT patients who were randomly allocated to two groups in which different methods were used for clamping the biliary drainage tube.

Methods

Patients

Of 66 patients in whom we performed liver transplantations between May 2006 and October 2009, 65 were adult-to-adult LDLTs. Of these 65, 40 patients who underwent duct-to-duct biliary reconstruction with a tube splint at the anastomotic site and survived beyond 3 months were included in this study. This prospective randomized control study was conducted with the permission of the institutional ethics committee. Six ABO-incompatible patients

received a single dose of rituximab 1 week prior to the LDLT [4].

Biliary drainage tube placement

As reported previously, we used a polyvinyl chloride tube of 2-mm diameter, which was originally used for retrograde transhepatic biliary drainage, for our LDLT patients [3]. The tube was equipped with a malleable metallic dull-tipped splint at one end. Prior to the performance of duct-to-duct biliary anastomosis, the metallic splint of the tube was inserted from the lumen of the recipient's side of the hepatic duct and externalized through the common bile duct above the upper edge of the duodenum. Subsequently, duct-to-duct anastomosis was performed with interrupted sutures of 6-0 polydioxanone, and the tube was placed inside the graft intrahepatic bile duct for decompression and splinting. After the tube placement, the externalized site of the common bile duct was treated with purse-string sutures of 6-0 polydioxanone. Ductoplasty was performed in 4 patients with a right lobe graft; in 3 of these patients two tubes were placed, one in the anterior and one in the posterior branches of the bile duct. In the other patient with a right lobe graft, two tubes were placed when anterior and posterior branches of the bile ducts were too distant to perform ductoplasty.

Groups

When the serum levels of total bilirubin were lower than 5 mg/dl, and negativity for any biliary complications (leakage or severe stricture) and a good passage of contrast media to the duodenum were confirmed by fluoroscopic study, an attempt to clamp the biliary drainage tube was initiated 1 day after the fluoroscopic study. The following two methods were used for the clamping: for stepwise clamping ($n = 20$), the drain tube was clamped for 3 h on day 1, 6 h on day 2, 12 h on day 3, and 24 h per day thereafter. After each temporary clamping, the biliary drainage tube was opened and externally drained. For the straightforward clamping ($n = 20$), the drain tube was clamped and remained closed.

After the clamping, liver function tests (T. Bil: total bilirubin, ALT: alanine aminotransferase, ALP: alkaline phosphatase, GGT: gamma glutamyl transpeptidase) were performed on days 1 and 3. During the clamping period, the patients continued to eat hospital meals three times a day.

Statistics

All data were expressed as median values with ranges. Statistical analysis was performed using the Mann–Whitney *U*-test for continuous values. Statistical significance was defined as a p value of <0.05. The StatView 5.0 software program (Abacus Concepts, Berkeley, CA, USA) was used for all statistical analyses.

Table 1 Patient characteristics and liver function tests after the clamping

	Stepwise ($n = 20$)	Straightforward ($n = 20$)	
Age (years)	56 (31–67)	57 (33–68)	n.s.
Gender (M:F)	13:7	13:7	n.s.
Graft type (right-side graft:left-lobe graft)	10:10	10:10	n.s.
Bile ductoplasty	3	1	n.s.
Double tubes	3	0	n.s.
ABO-incompatible	1 (5%)	5 (20%)	n.s.
Starting day of the clamping	22 (12–54)	29 (9–59)	n.s.
T. Bil before clamping (mg/dL)	1.9 (0.6–5.6)	2.0 (0.6–11.1)	n.s.
After 1 day	1.9 (0.5–5.4)	1.8 (0.7–9.6)	n.s.
After 3 days	1.5 (0.5–4.6)	1.5 (0.4–7.2)	n.s.
ALT before clamping (IU/L)	73 (24–177)	89 (5–537)	n.s.
After 1 day	67 (21–178)	80 (7–567)	n.s.
After 3 days	60 (16–177)	81 (8–542)	n.s.
ALP before clamping (IU/L)	377 (115–1,744)	369 (176–1,100)	n.s.
After 1 day	382 (136–1,736)	377 (107–1,260)	n.s.
After 3 days	345 (138–1,698)	380 (169–1,410)	n.s.
GGT before clamping (IU/L)	94 (13–368)	100.5 (17–538)	n.s.
After 1 day	113 (17–358)	150 (16–549)	n.s.
After 3 days	94.5 (14–365)	100 (16–577)	n.s.

Numbers in parentheses are ranges, unless otherwise indicated. *n.s.* not significant, *T. Bil* total bilirubin, *ALT* alanine aminotransferase, *ALP* alkaline phosphatase, *GGT* gamma glutamyl transpeptidase

Results

Table 1 shows the characteristics of the patients in the study. There were no statistically significant differences in age, gender, graft type, the starting day of clamping after LDLT, or ABO incompatibility between the groups.

At the time of the clamping, there were also no significant differences between the groups in the serum levels of T. Bil, ALT, ALP, and GGT. After each type of clamping of the biliary drainage tube, there were no significant differences between the groups in the serum levels of total bilirubin, AST, ALP, or GGT on days 1 and 3. There was no clamping failure in either of the groups.

Discussion

In the present study, we demonstrated that there were no differences in the patient outcomes after using the stepwise versus the straightforward clamping method for the biliary drainage tube after LDLT.

Biliary splinting plays an important role in LDLT, as the rate of biliary complications is higher in LDLT than in deceased-donor whole LT [1, 2]. We generally use a 2-mm tube for stenting at the biliary anastomosis, externalize it through the lower common bile duct, and fistulize it using the duodenal serosa [3]. The safety of the two-step procedure for removal of the splint tube was reported previously by our group [3]. In order to clarify the effects of the stepwise clamping method, we performed the present prospective study.

In our patients, there were no differences between the groups in the distribution of graft type, i.e., right lobe grafts, right posterior grafts, and left lobe grafts. After the clamping, we observed no differences between the outcomes in the patients treated using the two different clamping methods. In addition, in our subgroup analysis of graft type within each group, there were no significant differences in any of the parameters. Moreover, ABO-incompatible patients did not show any additional response after clamping of the biliary drainage tube, regardless of the clamping method used.

In one patient, we started to clamp the tube when the level of total serum bilirubin was still more than 5 mg/dl because of a lack of any biliary complications at 2 months after LDLT. However, there was no increase in any of the examined parameters in this patient in the straightforward clamping group.

Studies on the duration of clamping procedures have been performed only in the area of total knee arthroplasty [5–8]. In one of these studies, a reduction of blood loss was confirmed when 1-h clamping was applied as compared to a 4-h clamping method [5]. However, there has been no previous report describing the clamping method or duration of use for a biliary drainage system; therefore, even specialists in this field sometimes adopt the conventional stepwise method after LDLT.

In conclusion, we performed a randomized control study to examine differences arising due to the use of different clamping methods. Our results indicate that the straightforward clamping method could be a simple and reasonable method to successfully close biliary drainage tubes after LDLT.

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HOW TO DO IT

Prevention of gastric stasis by omentum patching after living donor left hepatectomy

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Abstract Among 137 living liver donors who underwent partial hepatectomy between August 1997 and November 2010, 58 donated the left lobe of their liver, with or without the caudate lobe. Gastric stasis developed after surgery in 4 (7 %) of these 58 donors (Fig. 1); possibly because of dislocation of the stomach after hepatectomy and adhesion between the stomach and the cut surface of the liver. This complication is specific to left hepatectomy [1] and although not life-threatening, it is symptomatic and requires endoscopic or surgical intervention. We describe our surgical technic designed to prevent this complication.

Keywords Liver transplantation · Living donor · Omentum

Surgical procedures (Fig. 2)

After left hepatectomy, there is a large cavity between the stomach and the cut surface of the liver (Fig. 2a). A closed suction drain is generally placed along the cut surface via the dorsal route of the hepatoduodenal ligament. Our method involves stretching the omentum fully (Fig. 2b) into this space, covering the hepatoduodenal ligament and the cut surface of the liver (Fig. 2c), ensuring that the stomach and transverse colon are left in their natural positions. We simply leave the omentum in place without suturing (Fig. 2d). Patients with gastric stasis vomit frequently because their stomach is enlarged, as can be seen on abdominal X-ray and/or computed tomography images (Fig. 1). Computed tomography is performed routinely 1 month after surgery, mainly to check the regeneration of the liver.

We performed omental patching in the most recent 45 of the 58 donors who underwent left partial hepatectomy. The incidence of gastric stasis decreased significantly from 23 % (3/13) in the first 13 patients to 2 % (1/45) in the last 45 ($P < 0.05$; Fisher's test). Computed tomography after surgery confirmed that the omentum was still in place between the stomach and the liver (Fig. 3a), preventing adhesion between them in all except one patient, in whom gastric stasis was possibly caused by dislocation of the omentum. All 3 of the former 13 patients with gastric stasis after surgery without omentum patching were observed to have tight adhesion between the stomach and the cut surface of the liver (Fig. 3b).

Gastric stasis is not life-threatening, but it impairs the quality of life of living liver donors. In left hepatectomy, the stomach is twisted and falls into the space after the liver lobe is removed. This leads to adhesion between the stomach and the cut surface of the liver. None of the 62 patients who underwent right hepatectomy during the same period in this series suffered any gastric stasis. Although all four of our patients who suffered gastric stasis are now doing well, three required endoscopic repair, and one required surgical adhesiolysis. There are few studies on the prevention of gastric stasis after left hepatectomy. Yoshida et al. [2] proposed a procedure for fixing the greater omentum to the peritoneum to prevent the stomach from falling into the space after hepatectomy. We devised omentum patching because it is simple and requires no artificial materials. A sodium hyaluronate and carboxymethylcellulose membrane was recently introduced as an

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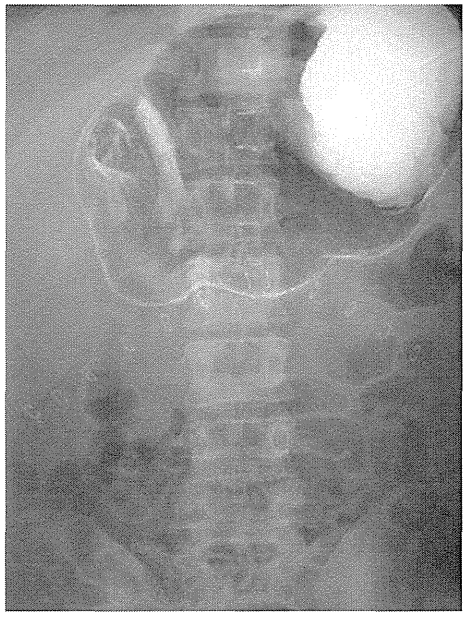

Fig. 1 Gastric stasis after living donor left hepatectomy. Fluorescent imaging study shows an enlarged stomach with no passage of radiofluorescence through the pylorus

effective material to prevent bowel obstructions being caused by adhesions [3, 4], but it is not clear whether it can be used to prevent adhesions between the cut surface of the liver and the stomach. Besides the omentum, another intra-abdominal material that could possibly be used is intestine, but this might lead to bowel obstruction due to adhesion. We simply left the omentum without any plasty in the space between the stomach and the cut surface of the liver, and without sutures. Even though it was not fixed, computed tomography confirmed that the omentum remained in place between the stomach and the liver in most of the patients. The omentum is used widely to prevent or treat various morbidities, including anastomotic leakage of the colon [5], perforation of a duodenal ulcer [6], hepatic hydatid cyst [7], and in some thoracic surgery [8]. It is generally used with some kind of plasty, but we simply placed it over the area without any plasty or sutures, and thus named the procedure as "omentum patching". This procedure cannot be applied if the omentum is too small to cover the cut surface of the liver, or if there are intra-abdominal adhesions involving the omentum from prior laparotomy. In our series, omentum patching was carried out easily in all patients, except for one who had previously undergone colectomy. We believe that the vast majority of living liver donors are candidates for omentum patching at the time of hepatectomy because they are healthy volunteers. This procedure is also useful for patients undergoing left hepatectomy for neoplasms, but it is more applicable in living donor hepatectomy, in which any complications, even minor ones, should be avoided.

One possible disadvantage of this procedure is that it may leave the person susceptible to severe peritonitis if intra-abdominal inflammation, such as appendicitis, occurs after surgery, because the general functions of the omentum include migration, covering, adhesion, and mending the absorption against peritoneal injury or infection. None of our patients have experienced any such adverse events

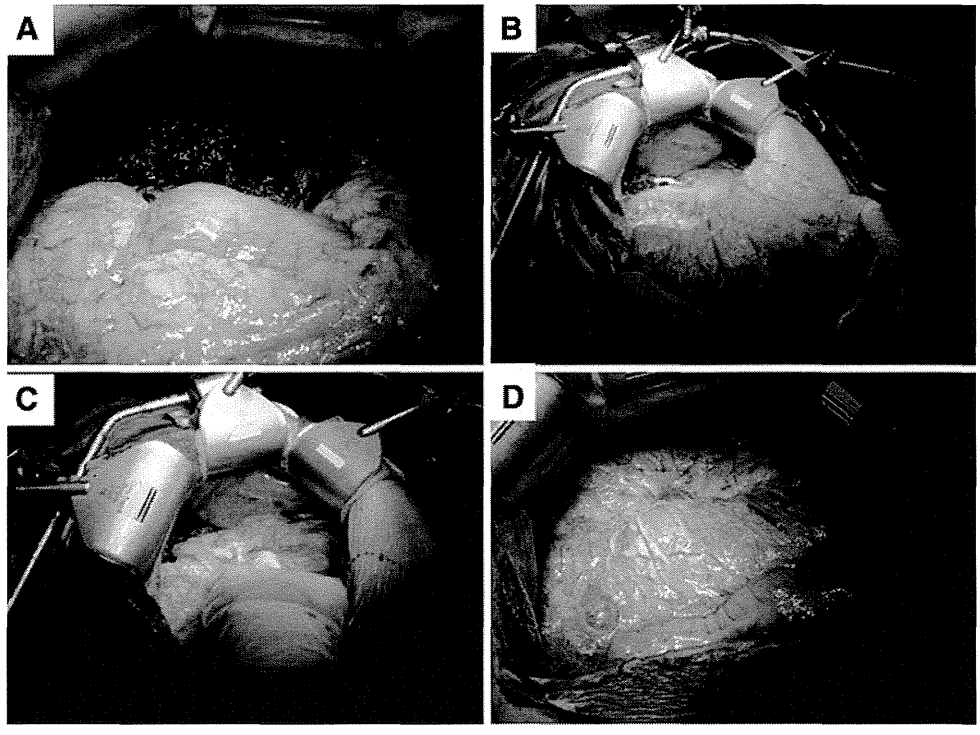

Fig. 2 Surgical procedure for omentum patching. There is a large cavity between the stomach and the cut surface of the liver (**a**). The omentum is fully stretched (**b**) and placed over the hepatoduodenal ligament and the cut surface of the liver (**c**). The omentum is left in place without sutures (**d**)

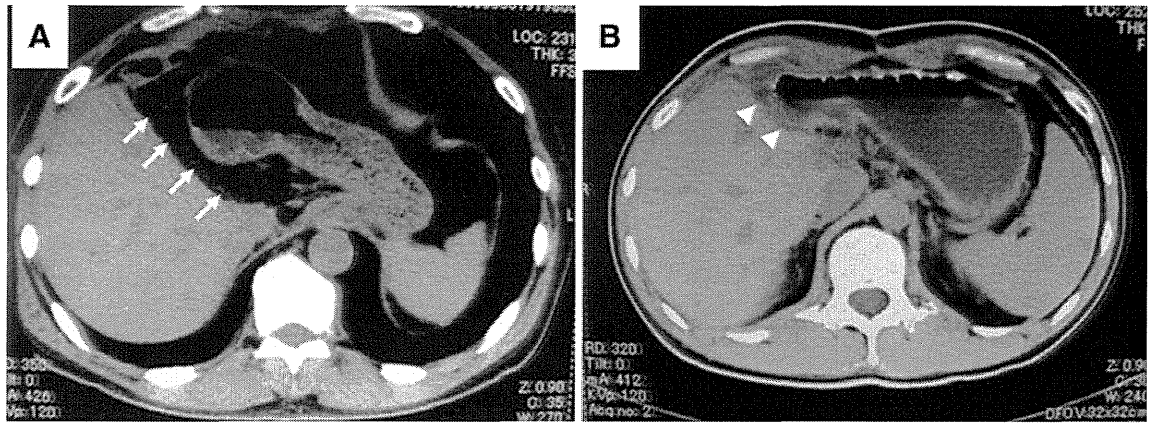

Fig. 3 Computed tomography scan after surgery with (**a**) and without (**b**) omentum patching. After omental patching, the omentum remains in place between the stomach and the cut surface of the liver (*arrows*, **a**), whereas without omental patching, there are tight adhesions in a person suffering from gastric stasis (*arrowheads*, **b**)

within a median follow-up period of 16 months (range 1–42 months). Another possible cause of adhesion between the stomach and the cut surface of the liver is bile leakage. Thus, it is essential to cut the bile duct at an adequate point [9]. There were no cases of bile leakage causing tight adhesion in our series, as we cut the bile duct at the optimal cutting point during donor surgery using C-arm cholangiography [10].

In conclusion, although a randomized study should be done, the findings of this series demonstrate that omentum patching prevents gastric stasis after living donor left hepatectomy.

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ELSEVIER

Standardized Less Invasive Living Donor Hemihepatectomy Using the Hybrid Method Through a Short Upper Midline Incision

A. Soyama, M. Takatsuki, M. Hidaka, I. Muraoka, T. Tanaka, I. Yamaguchi, A. Kinoshita, T. Hara, and S. Eguchi

ABSTRACT

Background. Recently, applications of less invasive liver surgery in living donor hepatectomy (LDH) have been reported. The objective of this study was to evaluate the safety and efficacy of a hybrid method with a midline incision for LDH.

Methods. Hemihepatectomy using the hybrid method was performed in the fifteen most recent among 150 living donors who underwent surgery between 1997 and August 2011. Six donors underwent right hemihepatectomy and 9 underwent left hemihepatectomy. An 8-cm subxiphoid midline incision was created for hand assistance during liver mobilization and graft extraction. After sufficient mobilization of the liver, the hand-assist/extraction incision was extended to 12 cm for the right hemihepatectomy and 10 cm for a left hemihepatectomy. Encircling the hepatic veins and hilar dissection were performed under direct vision. Parenchymal transection was performed with the liver hanging maneuver. Bile duct division was performed after visualizing the planned transection point by encircling the bile duct using a radiopaque marker filament under real-time C-arm cholangiography.

Results. All procedures were completed without any extra subcostal incision. All grafts were safely extracted through the 10–12-cm upper midline incision without mechanical injury. No donors required an allogeneic transfusion; all of them have returned to their preoperative activity levels.

Conclusion. LDH by the hybrid method with a short upper midline incision is a safe procedure.

DONOR safety is of the utmost importance for living donor liver transplantation (LDLT). Recently, the application of less invasive liver surgery has been reported during living donor hepatectomy (LDH).[1,2] We have adopted laparoscopy-assisted donor hepatectomy through a short upper midline incision with hilar dissection and parenchymal transection under direct vision as a new LDH method. For this procedure, we have applied useful techniques that we established for the conventional open LDH. Herein we have described the procedure for laparoscopic-assisted donor hepatectomy at our institute, providing an evaluation of its safety and efficacy using a short midline incision for LDH.

PATIENTS AND METHODS

Between 1997 and August 2011, we performed 150 LDLT, including the most recent 15 donors who underwent a laparoscopy-assisted donor hepatectomy, which consisted of a 2-phase laparoscopic procedure and an open procedure, the hybrid technique.[3] Six donors underwent a right and 9 underwent a left hemihepatectomy using the laparascopy-assisted hybrid procedure.

Right Hemihepatectomy

The donor was placed in the supine position with abducted arms. An 8-cm subxiphoid midline incision was created for hand assistance during liver mobilization and graft extraction, using a GelPort handport device (Applied Medical, Rancho Santa Margarita, Calif, United States). Pneumoperitoneum (CO_2 at 8 mm Hg)

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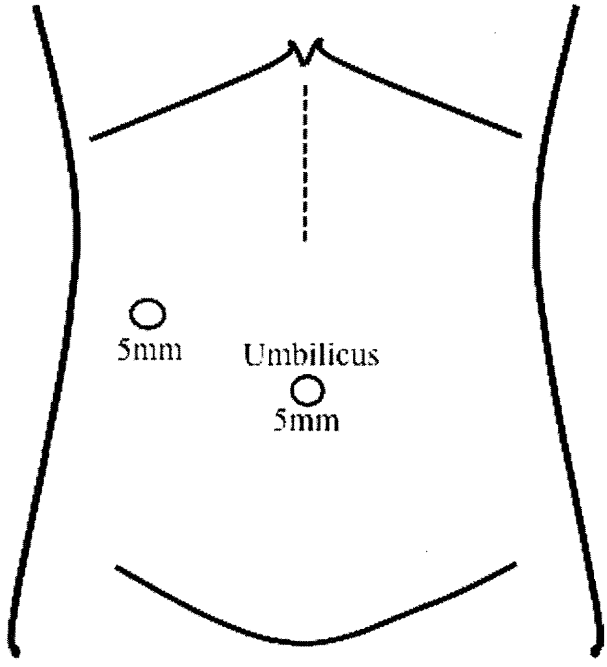

Fig 1. Trocar placement for laparoscopy-assisted donor hemihepatectomy. A 5-mm camera port was placed at the umbilicus. An 8-cm upper midline incision was made for setting the Gel Port handport device for hand-assisted mobilization of the liver.

was established through a 5-mm umbilical camera port. Once the liver was visualized, we placed an additional 5-mm port at the right flank or through the GelPort (Fig 1). The first assistant, who stood on the left side of the donor, manipulated the liver for the mobilization with hand-assistance through the hand port. Through the right flank port, the surgeon, who stood on the right side of the donor, used hook-type electrocautery to divide the ligaments and perform the dissection to sufficiently mobilize the liver until reaching the lateral wall of the inferior vena cava. At this point, we removed the hand port and the other ports. The midline incision was then extended to 12 cm for the subsequent open procedure. To provide sufficient exposure, the short incision was retracted with an Omni-Tract (Omni-Tract Surgical, St. Paul, Minn, United States). After exposing the inferior vena cava, we divided the short hepatic and encircling veins under direct vision.

After encircling the right hepatic vein, a Penrose drain was passed around the hepatic vein for the liver-hanging maneuver. As a result of the sufficient mobilization of the right lobe and using the liver-hanging maneuver, the transection line came just beneath the upper midline incision (Fig 2). A parenchymal transection was performed using the CUSA (Integra Life Sciences, Plainsboro, NJ, USA) and Tissue Link dissecting sealers (Salient Surgical Technologies, Portsmouth, NH, United States), which is the so-called two-surgeon technique.[4] We performed bile duct division after visualizing the planned transection point by encircling the bile duct using a radiopaque marker filament under real-time C-arm cholangiography.[5] The resected right lobe was retrieved through a short upper midline incision.

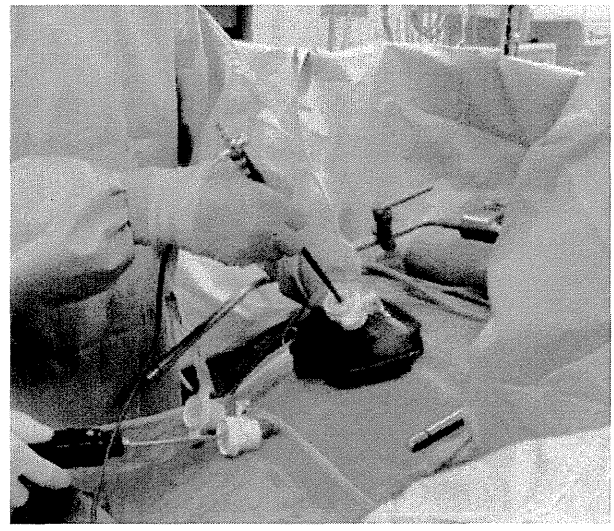

Fig 3. For dissection of the left triangular ligament and left side of the coronal ligament, a 5-mm trocar is placed through the GelPort hand port device.

Left Hemihepatectomy

The donor position and settings of the laparoscopic procedure were the same as the right hemihepatectomy. In the same manner as during the right hemihepatectomy, the right lobe of the liver was sufficiently mobilized during the left hemihepatectomy. Otherwise, the transection line along the Cantlie line was not safely positioned under direct vision through the short upper midline incision. To dissect the left triangular ligament and the left side of the coronal ligament, a 5-mm port was placed through the GelPort (Fig 3).

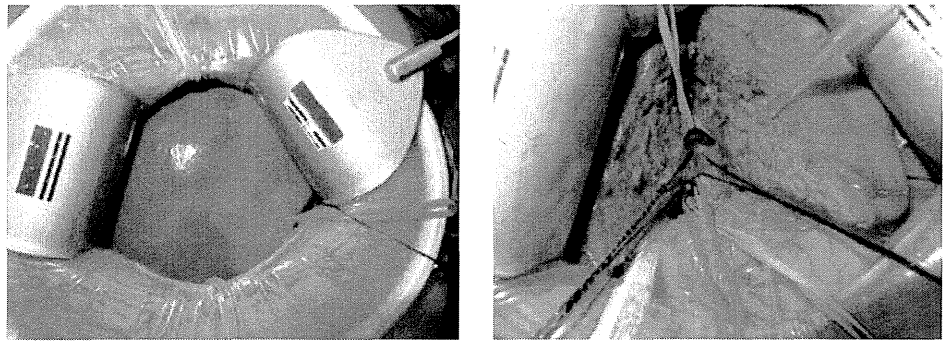

Fig 2. The appearance of the demarcation line after the clamping of the right hepatic artery and right branch of the portal vein. After the mobilization of the right liver, the transection line comes just beneath the short upper midline incision after performing the liver-hanging maneuver.

Intraoperative and postoperative outcomes

The median length of the operation and blood loss were 456 minutes (range, 328–581) and 520 g (range, 230–1000), respectively. The donors were transferred to the surgical intensive care unit for an overnight stay. On postoperative day 1, they were transferred to the nursing ward with surgical site pain controlled by parenteral analgesics. Donor recovery was uneventful except for 1 donor who required a relaparotomy to remove a portal venous thrombus. Serum chemistry findings were similar to those of our open donors (data not shown). All donors fully recovered, returning to their previous activities. On follow-up as outpatients, wound healing was favorable in all donors (Fig 4).

DISCUSSION

Herein we have described 15 donor laparoscopic-assisted hemihepatectomies performed through a short upper midline incision. Koffron et al reported the first laparoscopic, hand-assisted living donor right hepatic lobectomy using an upper midline incision.[1] In laparoscopy-assisted LDH at our institute, besides the procedures that Koffron et al reported, we have introduced other techniques that we had already established for open procedures.

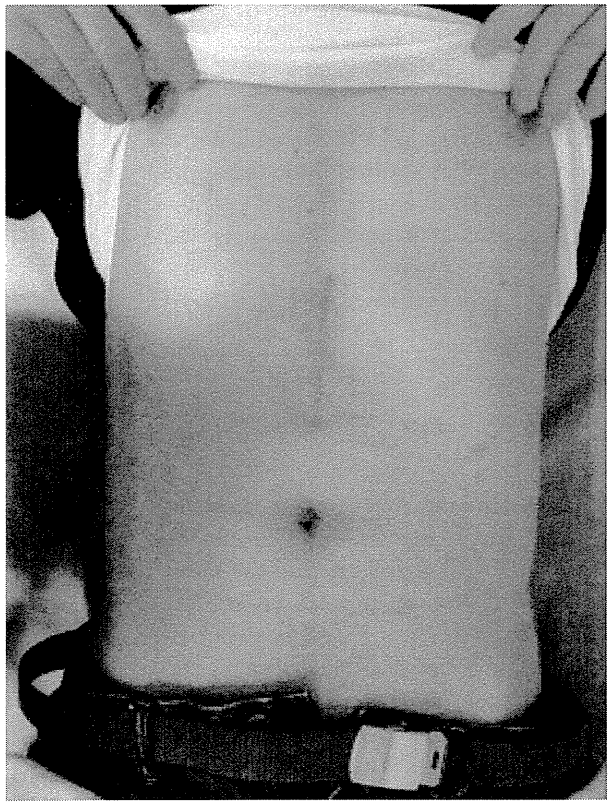

Fig 4. The wound left by the upper midline 10-cm incision 1 month after the left hemihepatectomy. The wound from the umbilical port was nearly invisible.

We consider dividing the short hepatic veins and the subsequent encircling of the right hepatic vein or the common trunk of the middle hepatic vein and left hepatic vein can be more securely performed under direct vision compared with a laparoscopic procedure. Minimizing blood loss is the most important element of laparoscopic liver surgery. Because the retrohepatic vena cava and the hepatic veins can be controlled for urgent extensive bleeding, dissecting those vessels under direct vision seems to be a reasonable approach. Once the right lobe is mobilized, the liver can be rotated to the left of the midline for retraction; therefore, the surgeon can easily approach the inferior vena cava and the right hepatic vein even through the mini-laparotomy with a short upper midline incision. The 2-surgeon technique that is performed during parenchymal tranresection can also be conducted during open procedures using the liver-hanging maneuver, which brings the transection line to just beneath the upper midline incision while pulling up the liver.[4] As a result, parenchymal transection can be completed through a 10–12-cm upper midline incision without stress to the surgeons.

Visualizing the planned transection point by encircling the bile duct using a radiopaque marker filament contributed to avoiding biliary complications when we divided the bile duct in the smaller working space compared with the open procedure.[5] Depending on the type of graft, we used different lengths of incisions: 12 cm for safe retrieval of a right and 10 cm for a left lobe graft.

In conclusion, LDH by the hybrid method via an upper midline incision was a safe procedure using the combination of hand-assistance and techniques that have been established for open donor surgery.

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Original article

Intraoperative portal venous pressure and long-term outcome after curative resection for hepatocellular carcinoma

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Background: **Outcomes of liver resection for hepatocellular carcinoma (HCC) have improved owing to better surgical techniques and patient selection. Portal hypertension may influence outcome but the preoperative definition and role of portal hypertension are far from clear. The aim of this study was to elucidate the influence of portal venous pressure (PVP) measured directly during surgery on outcomes of liver resection in patients with HCC.**

Methods: **Patients who had resection of HCC between 1997 and 2009, and who underwent direct measurement of PVP immediately after laparotomy were enrolled. These patients were divided into groups with high (at least 20 cmH_2O) and low (less than 20 cmH_2O) PVP. The influence of PVP on overall and recurrence-free survival was analysed and prognostic factors were identified.**

Results: **A total of 177 patients were enrolled, 129 in the low-PVP group and 48 in the high-PVP group. The 5-year overall survival rate (63·7 *versus* 31 per cent; $P < 0·001$) and recurrence-free survival rate (52·5 *versus* 12 per cent; $P < 0·001$) were significantly higher in patients with low PVP. In multivariable analysis, two or more tumours, tumour diameter at least 5 cm, high PVP, grade B liver damage and Hepatic Activity Index (HAI) grade 7 or more were significant predictors of poorer survival after liver resection. Two or more tumours, tumour diameter at least 5 cm and HAI grade 7 or more were significant predictors of poorer recurrence-free survival.**

Conclusion: **High PVP was associated with poor long-term outcome after liver resection for HCC.**

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide[1]. Outcomes of liver resection for HCC have improved greatly in recent years because of improved surgical techniques and better perioperative management[2,3]. Adequate estimation of preoperative liver function and tailoring the extent of hepatectomy based on liver function have reduced postoperative mortality and morbidity rates[2–4].

The degree of portal hypertension probably reflects the severity of fibrosis in patients with liver cirrhosis. Patients with cirrhosis often have portal hypertension before surgery, and currently are not candidates for liver resection, especially major hepatectomy, according to North American and European guidelines[5,6]. Several reports have shown an association between portal hypertension estimated before surgery and the prognosis of HCC[7,8]. Major hepatic resection increases portal venous pressure (PVP) in cirrhotic and non-cirrhotic livers. This increase in PVP after hepatectomy, however, does not seem to have a direct effect on early postoperative morbidity and mortality[9]. On the other hand, directly measured high PVP during hepatectomy has been associated with postoperative complications in patients with cirrhosis and HCC[10].

The aim of the present study was to clarify whether PVP reflects the prognosis of patients with HCC after hepatic resection, and to identify factors affecting recurrence and survival.

Methods

All patients with HCC who underwent curative hepatic resection between January 1997 and December 2009 in the

Department of Surgery, Nagasaki University Hospital, and in whom PVP was measured, were eligible for the study. Curative resection was defined as any operation in which all tumours were resected macroscopically.

Hepatic resection was performed based on preoperative tumour staging and liver function tests. The selection for minor resection (partial hepatectomy or segmentectomy) or major resection (here defined as bisegmentectomy and lobectomy) was based on the location and diameter of HCC and liver function tests[9]. Tumour staging included preoperative ultrasonography, multidetector computed tomography (CT) and magnetic resonance imaging (MRI) in all patients. Preoperative liver function was assessed by liver function tests, indocyanine green retention rate at 15 min (ICG-R15), liver scintigraphy represented by the liver to liver plus heart ratio at 15 min (LHL15) after ^{99m}Tc-labelled galactosyl sialyl albumin loading, and Child–Pugh classification.

Patient data collected before surgery included age, sex, virus status, platelet count, prothrombin time, albumin, total bilirubin, alanine aminotransferase (ALT), Child–Pugh grade, liver damage defined by the Liver Cancer Study Group of Japan[11], ICG-R15 and LHL15.

Intraoperative PVP measurement was performed as described previously[9,10]. Briefly, a catheter was inserted into a jejunal mesenteric vein around 100–120 cm from Treitz's ligament before liver mobilization and resection. PVP was then measured using a water pressure gauge with saline. Patients with a history of upper abdominal surgery and mesenteric membrane adhesions were excluded because intubation could not be done easily after laparotomy. A high PVP was defined as a pressure of at least 20 cmH_2O[9,10]. Pressure over 15 mmHg was considered an indicator to avoid small-for-size graft syndrome after liver transplantation. Generally, a PVP of 15 mmHg was taken to be equal to 20 cmH_2O (conversion factor 1·36)[12]. Patients were divided into groups with high (at least 20·0 cmH_2O) and low (below 20·0 cmH_2O) PVP at the time of surgery. Liver dysfunction was defined by hyperbilirubinaemia, severe ascites, lower prothrombin time and raised sustained levels in liver function tests after hepatectomy.

Postoperative follow-up included measurement of serum α-fetoprotein (AFP) and serum protein induced by vitamin K absence II (PIVKAII) levels, and ultrasonography, CT or MRI every 2 or 3 months. If indicated, chest CT or bone scintigraphy were performed. If tumour recurrence was found, the optimal treatment (transarterial chemoembolization for intrahepatic multiple recurrence, radiofrequency ablation for single small recurrence, repeat hepatectomy for single intrahepatic recurrence) was selected for patients with preserved liver function.

Statistical analysis

Preoperative clinical data in the high- and low-PVP groups were compared, including age, sex, virus status, Child–Pugh classification, liver damage, ICG-R15, LHL15, platelet count, prothrombin time, serum albumin, total bilirubin, ALT, AFP and PIVKAII, and pathological data, including number and diameter of tumours, vascular invasion, liver inflammation and fibrosis graded using the Hepatic Activity Index (HAI)[13]. Clinical and pathological factors related to the presence of high PVP were compared by means of the Mann–Whitney U test and χ^2 test. Survival was analysed from the day of surgery to most recent follow-up. Survival and recurrence-free survival rates were determined by the Kaplan–Meier method and compared using the log rank test. To identify prognostic factors for survival and recurrence, 14 clinical and pathological variables were included in univariable and multivariable analyses using the Cox proportional hazard model. $P < 0·050$ was considered statistically significant. Statistical analyses were done using SPSS® version 18.0 (SPSS, Tokyo, Japan).

Results

A total of 177 patients were included in the analysis, with a median age of 65 (range 20–81) years; 83·1 per cent were men (Table 1). Forty-seven patients (26·6 per cent) were seropositive for hepatitis B antigen (HBs-Ag), three (1·7 per cent) were seropositive for HBs-Ag and hepatitis C antibody (HCV-Ab), 84 (47·5 per cent) were seropositive for HCV-Ab, and 43 (24·3 per cent) were seronegative for both HBs-Ag and HCV-Ab.

Forty-eight patients had high PVP and the remaining 129 had low PVP. Patients with high PVP had a lower platelet count, a lower prothrombin time, lower albumin level, higher ALT concentration, higher Child–Pugh grade, higher grade of liver damage, higher ICG-R15, lower LHL15 and higher AFP level; solitary tumours were less common in this group, resulting in fewer major hepatectomies, and a higher HAI grade and stage (Table 1). Eighteen (38 per cent) of 48 patients in the high-PVP group had a platelet count of less than $10 \times 10^4/mm^3$ compared with 13 (10·1 per cent) of 129 in the low-PVP group.

Twenty patients (42 per cent) in the high-PVP group developed complications after hepatectomy, including ascites in eight (17 per cent), pleural effusion in eight (17 per cent) and infectious disease in eight (17 per cent). Fifty-four patients (41·9 per cent) with low PVP developed complications, with ascites in 18 (14·0 per cent), pleural effusion in 23 (17·8 per cent) and infectious disease in nine

Table 1 Clinical characteristics of patients with high or low portal venous pressure undergoing hepatectomy for hepatocellular carcinoma

	High PVP (≥ 20 cmH_2O) ($n = 48$)	Low PVP (< 20 cmH_2O) ($n = 129$)	P†
Age (years)*	63 (43–78)	66 (20–81)	0·162‡
Sex ratio (M : F)	40 : 8	107 : 22	0·856
Aetiology			0·347
Hepatitis B	14 (29)	33 (25·6)	
Hepatitis C	26 (54)	58 (45·0)	
Hepatitis B + C	1 (2)	2 (1·6)	
Hepatitis-negative	7 (15)	36 (27·9)	
Platelet count (× $10^4/mm^3$)*	11·8 (4·1–35·6)	15·9 (2·6–47·0)	0·001‡
Prothrombin time (%)*	84 (63–105)	91 (54–122)	0·002‡
Albumin (g/dl)*	3·8 (2·5–4·7)	4·0 (2·8–4·8)	0·001‡
Total bilirubin (mg/dl)*	0·9 (0·4–4·8)	0·7 (0·3–2·4)	0·060‡
ALT (units)*	55·5 (18–190)	34·5 (7–222)	0·002‡
Child–Pugh grade			0·004
A	38 (79)	122 (94·6)	
B	10 (21)	7 (5·4)	
Liver damage grade			0·001
A	30 (63)	112 (86·8)	
B	18 (37)	17 (13·2)	
ICG-R15 (%)*	18 (3–39)	11 (1–40)	0·004‡
LHL15*	0·89 (0·77–0·96)	0·93 (0·61–0·97)	0·001‡
AFP (ng/ml)*	47·5 (4·2–454 300)	13·1 (1·2–151 367)	0·030‡
PIVKAII (mAU/ml)*	73 (21–10 173)	133 (2–60 380)	0·522‡
Tumour diameter (cm)*	2·9 (1·0–13·0)	4·0 (0·5–17·0)	0·080‡
Solitary tumour	29 (60)	101 (78·3)	0·016
Type of hepatectomy			0·001
Minor	41 (85)	76 (58·9)	
Major	7 (15)	53 (31·1)	
Vascular invasion	10 (21)	40 (31·0)	0·207
HAI*			
Grade	9·1 (3–13)	4·8 (1–13)	0·001‡
Stage	3·8 (2–4)	2·1 (0–4)	0·001‡

Values in parentheses are percentages unless indicated otherwise; *values are median (range). PVP, portal venous pressure; ALT, alanine aminotransferase; ICG-R15, indocyanine green retention rate at 15 min; LHL15, liver to liver plus heart uptake ratio at 15 min; AFP, α-fetoprotein; PIVKAII, protein induced by vitamin K absence II; AU, arbitrary units; HAI, Hepatic Activity Index. †χ^2 test, except ‡Mann–Whitney U test.

(7·0 per cent). There were no differences in postoperative incidence of pleural effusion, ascites and infections between groups. However, patients with a high PVP significantly more often had liver dysfunction (7 *versus* 2 patients; $P < 0·001$).

Overall and recurrence-free survival

Median follow-up of all patients was 39·2 (range 1·1–207) months. Five patients died from liver failure and sepsis with multiple organ failure after hepatectomy. Recurrence developed after resection in 37 patients (77 per cent) in the high-PVP group and in 93 (72·1 per cent) in the low-PVP group. One-, 3- and 5-year overall survival rates in the low-PVP group were 92·0, 78·2 and 63·7 per cent respectively. This was significantly better than corresponding rates of 73, 49 and 31 per cent in the high-PVP group ($P < 0·001$) (*Fig. 1*). One-, 3- and 5-year recurrence-free survival rates in the low-PVP group were 73·9, 61·0 and 52·5 per cent respectively, again better than those in the high-PVP group: 48, 27 and 12 per cent ($P < 0·001$) (*Fig. 2*).

Prognostic factors for overall and recurrence-free survival

Univariable analysis identified seven significant predictors of poor overall survival: presence of multiple tumours, tumour diameter at least 5 cm, high PVP, liver damage grade B, HAI grade 7 or more, AFP 100 ng/ml or greater and vascular invasion (*Table 2*). A multivariable analysis based on the significant variables in univariable analysis revealed the presence of multiple tumours, tumour diameter at least 5 cm, high PVP, liver damage and HAI grade 7 or more as independent prognostic indicators for survival.

Table 3 shows the results of univariable analysis to identify factors related to recurrence. Poor prognostic factors were the presence of multiple tumours, tumour diameter

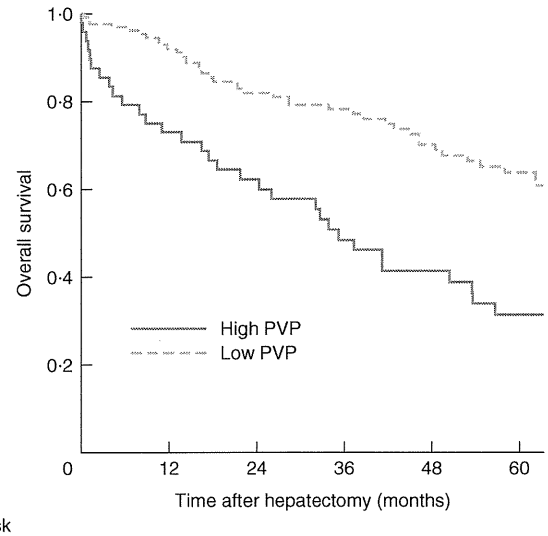

Fig. 1 Comparison of overall survival of patients with hepatocellular carcinoma and high or low portal venous pressure (PVP) after hepatectomy. $P < 0{\cdot}001$ (log rank test)

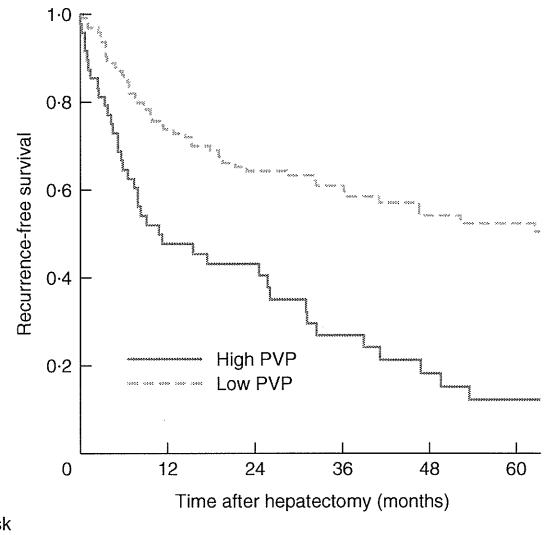

Fig. 2 Comparison of recurrence-free survival of patients with hepatocellular carcinoma and high or low portal venous pressure (PVP) after hepatectomy. $P < 0{\cdot}001$ (log rank test)

at least 5 cm, HAI grade 7 or more, high PVP, vascular invasion and AFP level 100 ng/ml or greater. The presence of multiple tumours, tumour diameter at least 5 cm and an HAI grade 7 or greater were identified as significant independent prognostic indicators for recurrence in the multivariable analysis.

Discussion

Portal hypertension is considered to be a contraindication to liver resection according the guidelines of the European Association for the Study of the Liver/American Association for the Study of Liver Diseases[5,6]. These guidelines indicate that treatment of HCC for such patients

Table 2 Results of univariable and multivariable Cox proportional hazards analyses of prognostic factors for overall survival after hepatectomy

	Univariable analysis		Multivariable analysis	
	Hazard ratio	*P*	Hazard ratio	*P*
≥ 2 tumours	3·15 (2·02, 4·90)	< 0·001	2·52 (1·58, 4·02)	< 0·001
Tumour diameter ≥ 5 cm	1·67 (1·09, 2·54)	0·018	2·22 (1·41, 3·50)	0·001
PVP ≥ 20 cmH_2O	2·44 (1·60, 3·60)	< 0·001	1·74 (1·24, 3·03)	0·004
Liver damage grade B	1·91 (1·19, 3·07)	0·007	1·74 (1·07, 2·82)	0·026
HAI grade ≥ 7	2·14 (1·42, 3·25)	< 0·001	1·65 (1·04, 2·63)	0·034
AFP ≥ 100 ng/ml	1·69 (1·11, 2·57)	0·013	1·24 (0·80, 1·93)	0·354
Vascular invasion	1·68 (1·08, 2·61)	0·020	1·21 (0·71, 2·03)	0·521
Platelet count ≤ 10 × 10^4/mm^3	0·99 (0·60, 1·63)	0·985		
ICG-R15 ≥ 15%	1·21 (0·81, 1·80)	0·354		
LHL15 ≤ 0·9	0·60 (0·34, 1·07)	0·079		
PIVKAII ≥ 100 mAU/ml	0·85 (0·53, 1·37)	0·515		
Child–Pugh grade B	1·49 (0·83, 2·70)	0·177		
Partial hepatectomy	1·17 (0·77, 1·76)	0·465		
HAI stage 4	1·31 (0·84, 2·03)	0·237		

Values in parentheses are 95 per cent confidence intervals. PVP, portal venous pressure; HAI, Hepatic Activity Index; AFP, α-fetoprotein; ICG-R15, indocyanine green retention rate at 15 min; LHL15, liver to liver plus heart uptake ratio at 15 min; PIVKAII, protein induced by vitamin K absence II; AU, arbitrary units.