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ORIGINAL ARTICLE

One-step reconstruction of the right inferior hepatic veins using auto-venous grafts in living donor liver transplantation

Toru Ikegami · Ken Shirabe · Shohei Yoshiya · Yuji Soejima · Tomoharu Yoshizumi · Hideaki Uchiyama · Takeo Toshima · Takashi Motomura · Yoshihiko Maehara

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Abstract
Purposes Reconstruction of the right inferior hepatic vein (RIHV) presents a major technical challenge in living donor liver transplantation (LDLT) using right lobe grafts.
Methods We studied 47 right lobe LDLT grafts with RIHV revascularization, comparing one-step reconstruction, performed post-May 2007 ($n = 16$), with direct anastomosis, performed pre-May 2007 ($n = 31$).
Results In the one-step reconstruction technique, the internal jugular vein ($n = 6$), explanted portal vein ($n = 5$), inferior vena cava ($n = 3$), and shunt vessels ($n = 2$) were used as venous patch grafts for unifying the right hepatic vein, RIHVs, and middle hepatic vein tributaries. By 6 months after LDLT, there was no case of occlusion of the reconstructed RIHVs in the one-step reconstruction group, but a cumulative occlusion rate of 18.2 % in the direct anastomosis group. One-step reconstruction required a longer cold ischemic time (182 ± 40 vs. 115 ± 63, $p < 0.001$) and these patients had higher alanine transaminase values (142 ± 79 vs. 96 ± 46 IU/L, $p = 0.024$) on postoperative day POD 7. However, the 6-month short-term graft survival rates were 100 % with one-step reconstruction and 83.9 % with direct anastomosis, respectively.
Conclusion One-step reconstruction of the RIHVs using auto-venous grafts is an easy and feasible technique promoting successful right lobe LDLT.

T. Ikegami (✉) · K. Shirabe · S. Yoshiya · Y. Soejima · T. Yoshizumi · H. Uchiyama · T. Toshima · T. Motomura · Y. Maehara
Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 812-8582 Fukuoka, Japan
e-mail: tikesurg@yahoo.co.jp

Keywords Living donor liver transplantation · Short hepatic vein · Right inferior hepatic vein · Right lobe · Venous reconstruction

Abbreviations

ALT	Alanine transaminase
AST	Aspartate aminotransferase
EPV	Explanted portal vein
GV	Graft volume
IJV	Internal jugular vein
IVC	Inferior vena cava
LDLT	Living donor liver transplantation
MELD	Model for end-stage liver disease
MHV	Middle hepatic vein
POD	Postoperative day
PT-INR	Prothrombin time international normalized ratio
RHV	Right hepatic vein
RIHV	Right inferior hepatic vein
SLV	Standard liver volume
V5	Segment 5 vein
V8	Segment 8 vein

Introduction

One of the major technical concerns in right lobe living donor liver transplantation (LDLT) is the complexity of the vessels, which need to be revascularized [1, 2]. Specifically, the venous systems in procured right lobe grafts may include several vessels such as the middle hepatic vein (MHV) tributaries and the right inferior hepatic veins (RIHVs). Revascularization of these outflow vessels is imperative for a fully functional right lobe graft, which affords vigorous portal inflows in a LDLT recipient with end-stage liver disease [3–5].

There are two options for reconstructing the MHV tributaries: one technique uses an extended right lobe graft, including the MHV trunk [5]; and the other uses interposition grafts [6]. We described previously how we used the explanted portal vein (EPV) for this purpose [7], but techniques for reconstructing the RIHV are not as well documented. Since 2007, we have been practicing the one-step reconstruction technique exclusively, unifying all the RIHVs with the interposed MHV tributaries and right hepatic vein (RHV) using auto-venous grafts [8]. In this technique, RIHVs are never connected to the inferior vena cava (IVC) separately, but are unified with other outflow vessels and connected with the IVC at the same time. Our technique differs from the conventional one, anastomosing graft RIHVs directly with the IVC.

This article reviews the outcomes of reconstructed RIHVs using the one-step reconstruction technique with an auto-venous graft in right lobe LDLT.

Materials and methods

Patients

Between July 1998 and October 2011, 125 LDLTs using right lobe grafts were performed at Kyushu University Hospital. Among these 125 recipients, 47 (37.6 %) required reconstruction of the RIHVs, whereas 78 (62.4 %) did not. Before May 2007, the RIHVs in the grafts were directly anastomosed ($n = 31$) to the IVC in situ; however, after May 2007, the RIHVs were connected to other outflow veins, including the RHV and the interposed graft from the MHV tributaries, using a patch-shaped venous graft ($n = 16$). All the LDLTs were performed after obtaining full informed patient consent and approval by the Liver Transplantation Committee of Kyushu University.

Graft selection

Grafts were selected as previously described [9]. Left lobe grafts were considered as the primary graft type if the desired GV/SLV was >35 %, whereas right lobe grafts were considered if the simulated GV/SLV of the left lobe graft was <35 % and the donor's remnant liver volume was >35 %. Before May 2007, the RIHVs or MHV tributaries were reconstructed if the estimated corresponding congested volume was >25 % or the deducted congested volume from the GV was <40 %. After May 2007, the indication for reconstruction of the RIHVs and MHV tributaries became more simplified: those with a congested volume >10 % of the GV or a size >5 mm were considered for reconstruction.

Donor surgery

In order to prevent biliary complications, donor hilar dissection was performed only at the corresponding first Glissonean branch [10] and donor parenchymal transection was performed using the Cavitron Ultrasonic Surgical Aspirator (CUSA™, Valleylab Inc., Boulder, CO). The significant RIHV and MHV tributaries were double-clamped with large clips and divided. After donor hepatectomy, the graft was perfused, weighed, and stored in University of Wisconsin solution (Viaspan™, DuPont Inc., Wilmington, DE).

Bench surgery and recipient surgery

In order to procure the IJV, a collar or oblique incision was made in the neck and the sternocleidomastoid muscle was retracted laterally. The overlying omohyoid muscle was divided to expose the IJV, which was isolated with vessel tape and dissected from the surrounding tissue, down to the level of its junction with the subclavian vein. To avoid injuring the branches of the facial nerve, cranical dissection was never performed above the level of the angle of the mandible. The procured IJV, usually 7–9 cm in length, was placed in heparinized saline for the bench surgery.

The hilar portal vein was procured from the explanted liver, as previously described [8]. If available, a shunt vessel of appropriate length and caliber was also procured. This type of suitable shunt vessel is usually available in patients with portal vein thrombosis [11], providing two auto-vein grafts for the bench surgery. We recently began reserving the IJV graft for interposing the MHV tributaries and other venous grafts for the patch graft to unify the orifices of the outflow veins. Next, the MHV tributary was anastomosed to the interposition graft using continuous 7-0 Prolene™ sutures (Ethicon Inc, Somerville, NJ), taking care to prevent stenosis. The venous orifices, including the RHV, RIHVs, and the interposed venous graft for the MHV tributaries, were connected together using continuous 6-0 Prolene™ sutures. To make the in situ anastomosis easier, a cuff might be attached around the RHV.

Recipient surgery

A right lobe graft with a large, unified venous orifice was implanted into the recipient, after dividing the bridge between the hepatic veins by creating a longitudinal incision in the anterior wall of the IVC [8]. The venous anastomoses were all performed using 5-0 continuous PDS-II™ sutures (Ethicone Inc, Somerville, NJ). After reconstruction of the portal vein with continuous 6-0 PDS-II™ sutures, the liver graft was reperfused. Arterial reconstruction was then performed under microscopy with interrupted 8-0 Prolene™ sutures.

Evaluation of the patency of the grafts

Follow-up computed tomography (CT) scans with intravenous contrast were taken 1 week, 1 month, 3 months, 6 months, and yearly after the LDLT. CT scans after abnormal liver function test results were also performed as necessary. Non-visualized RIHVs or parenchyma that was poorly enhanced by intravenous contrast were judged to be occlusions.

Statistical analysis

Values are expressed as mean ± SD. Variables were analyzed using the χ^2 tests for categorical values or the Mann–Whitney's test for continuous variables. Cumulative survival analyses were calculated using the Kaplan–Meier method. A P value < 0.05 was considered significant.

Results

Donor and recipient data

Forty-seven patients underwent reconstruction of the RIHVs during right lobe LDLT. All of the right lobe grafts were modified right lobe grafts that did not include the main middle hepatic vein (Table 1). The donors comprised 20 men and 27 women, with a mean age of 37.9 ± 10.8 years. The mean operation time was 448 ± 53 min and the mean blood loss was 421 ± 194 ml. The mean graft volume was 571 ± 60 ml and the mean graft volume (GV)/standard liver volume (SLV) was 46.9 ± 4.9 %. The grafts with one-step reconstruction of the RIHVs ($n = 16$) had less GV/SLV than those ($n = 31$) with direct anastomosis (43.6 ± 3.8 vs. 48.7 ± 6.9, $p = 0.010$).

The recipients comprised 28 men and 19 women, with a mean age of 49.6 ± 8.4 years. The causes of liver disease were acute liver failure ($n = 2$), cholestatic liver diseases ($n = 9$), post-necrotic liver cirrhosis ($n = 35$), and others ($n = 1$). Twenty of these patients had hepatocellular carcinoma. The mean model for the end-stage liver disease score was 17.2 ± 4.2. The mean operative time was 939 ± 149 min, the mean blood loss during surgery was 6.7 ± 4.0 L, and the mean cold and warm ischemic times were 136 ± 51 and 51 ± 8 min, respectively. The grafts with one-step reconstruction of the RIHVs ($n = 16$) were subjected to longer cold ischemic time than those ($n = 31$) with direct anastomosis (182 ± 40 vs. 115 ± 63, $p < 0.001$).

Table 1 Patient characteristics

	One-step reconstruction ($n = 16$)	Direct anastomosis ($n = 31$)	p value
Donor			
Age (year)	37.6 ± 12.6	38.1 ± 11.7	0.892
Gender, male	6 (37.5)	14 (45.2)	0.614
Operative time (min)	448 ± 93	449 ± 49	0.923
Blood loss (ml)	363 ± 163	452 ± 386	0.385
Graft			
GV (g)	542 ± 54	586 ± 83	0.063
GV/SLV (%)	43.6 ± 3.8	48.7 ± 6.9	0.010
RIHV			
Size (mm)	12.7 ± 3.2	12.3 ± 4.9	0.781
Number >2	4 (25.0)	8 (26.7)	0.943
Recipient			
Age (year)	48.4 ± 12.6	50.2 ± 11.2	0.615
Gender, male	11 (68.7)	17 (54.8)	0.357
MELD score	18.2 ± 5.6	16.8 ± 5.7	0.456
Acute liver failure	1 (6.3)	1 (3.3)	0.916
Recipient surgery			
Operative time (min)	902 ± 171	997 ± 211	0.105
Blood loss (L)	4.7 ± 2.9	7.7 ± 4.6	0.035
Cold ischemic time (min)	182 ± 40	115 ± 63	<0.001
Warm ischemic time (min)	50 ± 13	52 ± 7	0.304

GV graft volume, *MELD* model for end-stage liver disease, *RIHV* right inferior hepatic vein, *SLV* standard liver volume

Venous grafts used for the one-step reconstruction technique

The venous grafts used for one-step reconstruction of the RIHVs and the MHV tributaries are summarized in Table 2. The auto-venous patch grafts for RIHVs included the EPV ($n = 4$), internal jugular vein (IJV, $n = 6$), shunt vessels ($n = 2$), IVC ($n = 3$) and saphenous vein ($n = 1$). The shunt vessels available for this purpose included the umbilical vein ($n = 1$) and ovarian vein ($n = 1$).

Figure 1 illustrates a one-step reconstruction of the RIHVs and MHV tributaries of a right lobe LDLT graft. Preoperative three-dimensional venous images obtained by thin-slice computed tomography (CT) showed that the graft had two RIHVs and two MHV tributaries. The dilated ovarian vein was procured and used for interposing the MHV tributaries and the IJV was opened and used for a patch graft to unify the venous orifices. The patency of the RIHVs and the MHV tributaries was confirmed on an enhanced CT scan performed 5 months after LDLT.

The IVC was used as an auto-venous patch graft in three patients. In two patients, the anterior wall of the recipient's hepatic IVC was procured under clamping of the supra- and infra-hepatic IVC and on veno-venous bypass. The

Table 2 The vascular grafts used for one-step reconstruction of the right inferior hepatic veins with or without middle hepatic vein tributaries

Patch graft for RIHV	Interposition graft for MHV tributaries	*N*	Comments
EPV	IJV	3	
	EPV	1	
IJV	IJV	2	
	EPV	3	
	Shunt vessels	1	Ovarian vein ($n = 1$)
Shunt vessels	EPV	2	Umbilical vein ($n = 1$)
			Ovarian vein ($n = 1$)
IVC	EPV	3	Anterior IVC wall ($n = 1$)
			Full IVC ($n = 2$)
Others	EPV	1	Saphenous vein

EPV explanted portal vein, *IJV* internal jugular vein, *IVC* inferior vena cava, *MHV* middle hepatic vein, *RIHV* right inferior hepatic vein

procured auto-IVC was sutured with the venous orifices of the liver graft on the back-table and the graft with completed venoplasty was implanted in situ. In one patient, the total hepatic IVC was procured from the recipient and sutured with the veins of the right lobe graft, followed by implantation.

Liver function tests after LDLT

The changes in liver function test results, including aspartate aminotransferase (AST), alanine transaminase (ALT), prothrombin time international normalized ratio (PT-INR) and total bilirubin, were compared between the 16 patients who underwent one-step reconstruction and the 31 patients who underwent direct anastomosis (Fig. 2). The AST, PT-INR, and total bilirubin values did not differ significantly at any time; however, the ALT values were increased significantly in the patients with one-step venous reconstruction (142 ± 79 vs. 96 ± 46 IU/L, $p = 0.024$) on postoperative day (POD) 7.

Patency of the reconstructed RIHVs

Follow-up CT scans showed no obstructed RIHVs in the one-step reconstruction group, but five in the direct anastomosis group. The mean time from LDLT to the occlusion was 21 ± 12 days (7, 9, 19, 22, 50 days). Daily ultrasound detected an occluded RIHV in three (60 %) of five patients. Because four patients with occlusions presented minor clinical signs including increased ascites, neither stenting nor revision was performed. Only one patient died, of drastic circulatory collapse 12 h after detection of the occluded RIHV, so active treatment could not be performed. The 6-month occlusion rate of RIHVs in the one-step reconstruction group and the direct anastomosis group was 0 versus 18.2 %, respectively (Fig. 3).

Graft survival

The 3- and 6-month graft survival rates for grafts with one-step reconstruction and those with direct reconstruction were 100 and 100 % vs. 87.1 and 83.9 %, respectively. Among the five patients with grafts associated with early mortality in the direct anastomosis group ($n = 31$), only one died with graft dysfunction caused by an obstructed RIHV. Other causes of mortality included occluded MHV tributaries ($n = 1$) and graft dysfunction due to a small graft size ($n = 1$), and sepsis ($n = 2$). These patients all had patent RIHVs.

Discussion

The optimal technique for creating hepatic venous outflow in right lobe LDLT remains elusive. Right lobe grafts, especially modified right lobes without the main MHV, frequently have multiple venous orifices to be reconstructed, including the main RHV, MHV tributaries, and the RIHVs [2–4]. Although several reports focus on the technical refinements devised to resolve the issues of the MHV tributaries, little attention has been paid to the reconstruction of the RIHVs; thus, direct anastomosis of the RIHV to the IVC has remained the standard procedure. However, direct anastomosis is difficult because the in situ anastomosis of the small RIHV is usually performed in a deep, narrow and often bloody surgical field [7]. Moreover, it requires adjustments to the exact length, size, and orientation of the vessels, considering the changes resulting from graft regeneration.

Since 2007, we have used a one-step reconstruction technique for such cases [8]. This technique involves joining all of the venous orifices together in and around a large square venous patch graft. The most useful feature of this one-step technique is the ease of the in situ veno-venous anastomosis, with no kinking or malalignment of the RIHVs [8]. The complex quilting creates a wide unified venous orifice that needs to be completed during the cold phase. In the present series, although the cold ischemic time was longer and the ALT was higher in the patients who received grafts with the one-step reconstruction technique than in those who underwent the direct anastomosis, there was no significant difference in short-term graft outcomes. The one-step reconstruction technique resulted in a 100 % patency rate of the revascularized

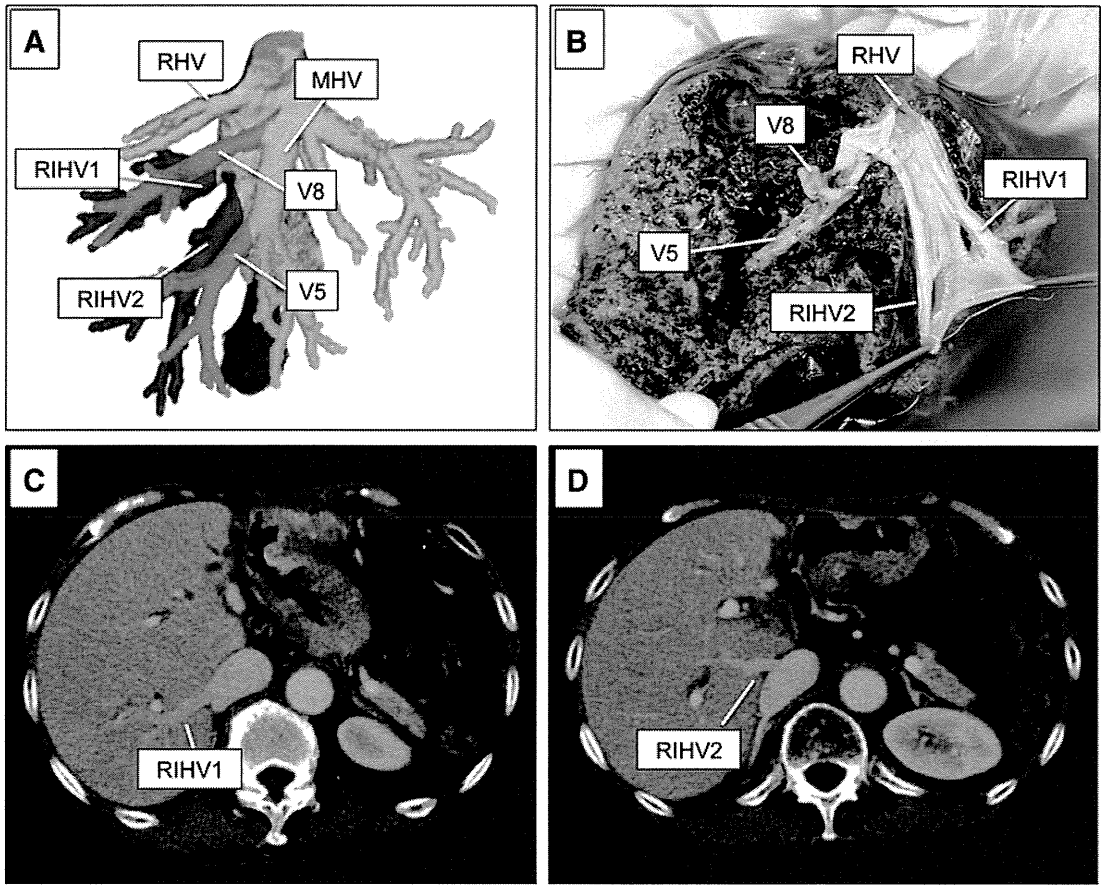

Fig. 1 Preoperative three-dimensional venous images showed two right inferior hepatic veins (*RIHVs*) and two middle hepatic vein (*MHV*) tributaries in the graft (**a**). The dilated ovarian vein was used for interposing the segment 5 vein (*V5*) and the segment 8 vein (*V8*), and the internal jugular vein (IJV) was used for a patch graft to unify the venous orifices (**b**). The patency of the RIHVs was confirmed by computed tomography (**c**, **d**). *PT-INR* prothrombin time international normalized ratio, *RHV* right hepatic vein

RIHVs and a 100 % short-term graft survival rate, which could be attributed to the ease of the one-step technique for creating a fine and wide venous anastomosis in a large surgical field.

Several other techniques for RIHV reconstruction without direct anastomosis have been reported. Sugawara et al. [6] proposed double IVC techniques, in which the venous orifices of a right lobe graft are anastomosed in a cryopreserved IVC procured from a deceased donor and then the newly created pouch-shaped cava is anastomosed side-to-side to the recipient's native IVC. Although in situ anastomosis is easy in the double IVC technique, the regenerating graft might compress the reservoir-like pouch, causing outflow insufficiency under the long slit-shaped anastomosis. Moreover, there seems to be no evidence of forming a reservoir. Yaprak et al. [12] recently described using a cryopreserved aortic patch for a similar purpose. The non-tubular shape of the aortic patch with its durable properties would be more appropriate; however, the availability of aortic grafts without atherosclerosis might be limited. Furthermore, we cannot exclude the possible transmission of uncommon pathogens when these cryopreserved vascular grafts are used [13]. Hwang et al. [14] recently reported how the funnel-shaped procurement of RIHVs with its accurate anastomosis to the recipient's IVC, under extensive IVC dissection, was the key for directly reconstructing the RIHVs, resulting in a RIHV stenosis rate as low as 2.9 %. Although the stenosis rate in their series is low, the difficulties of in situ anastomosis of the RIHVs in a restricted surgical field make us reluctant to use their techniques. We used EPV and shunt vessels as auto-venous grafts. Shunt vessels that can be used as venous grafts are usually limited to a large paraumbilical vein or large meso-systemic shunts including a dilated ovarian vein or a dilated inferior mesenteric vein [15, 16]. These veins have the properties of a straight shape and a large diameter without branches. Other shunt vessels, including the splenorenal shunt, gastrorenal shunt and gastroparaesophageal shunt are not suitable for venous grafts. EPV usually offers a larger caliber with a thick wall

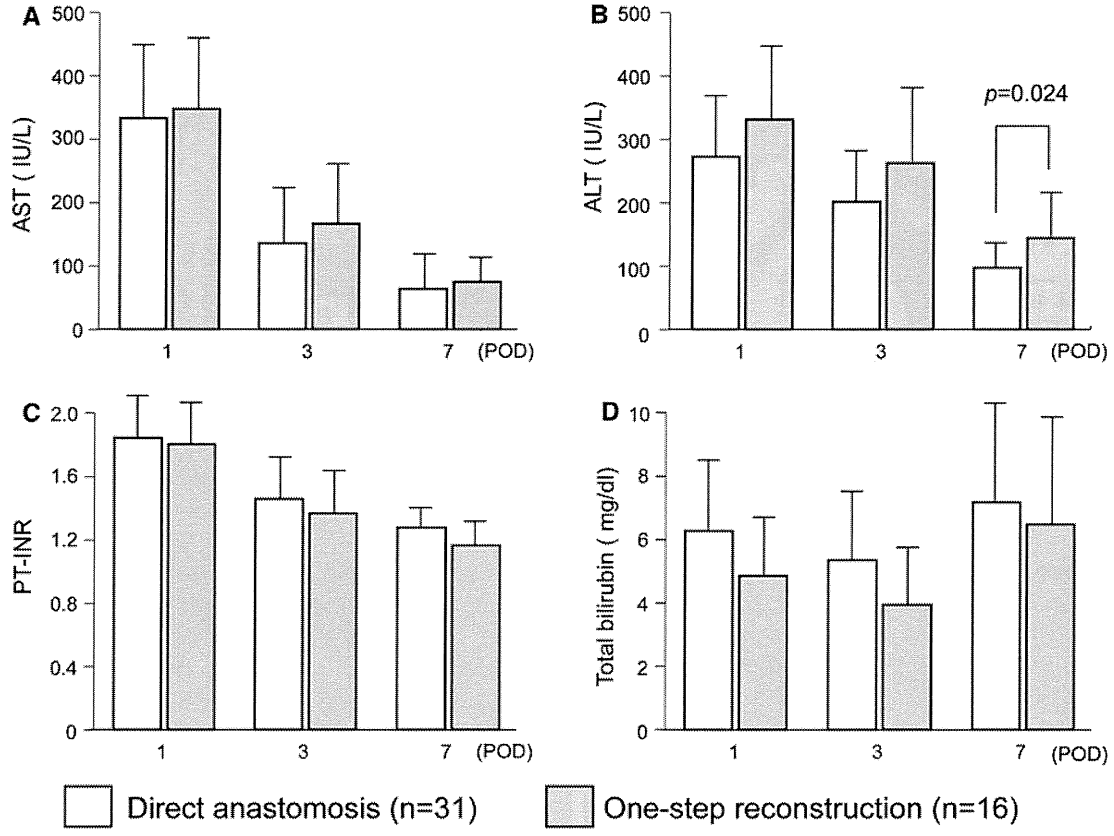

Fig. 2 Chronological changes in the liver function tests in the direct anastomosis group ($n = 31$) versus the one-step reconstruction group ($n = 16$). *ALT* alanine transaminase, *AST* aspartate aminotransferase, *POD* postoperative day, *PT-INR* prothrombin time international normalized ratio

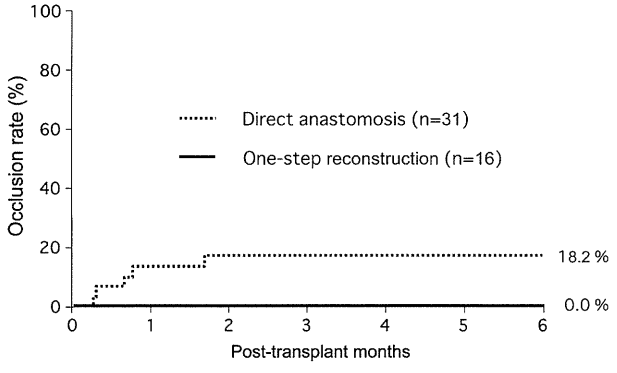

Fig. 3 Patency rate of the reconstructed right inferior hepatic veins (RIHVs) after direct anastomosis ($n = 31$) versus one-step reconstruction ($n = 16$)

and considerable length, making it a venous graft that is easy to handle [7]. However, it is not available in patients with portal vein thrombosis or hilar hepatocellular carcinomas. Recently, we used the IJV exclusively as a venous graft. The IJV has a large caliber of approximately 1 cm and sufficient length of up to 7–9 cm. In the field of liver transplantation, the IJV was first used as a jump portal venous graft in pediatric patients with extrahepatic portal venous obstruction [17]. Because the IJV usually has a healthy venous wall, it is suitable for fine anastomosis. The only technical concern is not to dissect into the cranial side over the mandible, to avoid facial nerve damage [18]. Therefore, we now prefer to use the IJV for reconstructing fine MHV tributaries. The current institutional guidelines for the selection of auto-venous grafts are summarized in Fig. 4. The most common combination is the IJV and EPV. If the EPV is not available, usable shunt vessels are sought and if neither the EPV nor shunt vessels are available, auto-IVC is used for reconstructing RIHVs.

One of the main limitations of this study is that the learning curve is unaccounted for. Knowledge gained not only in surgical techniques but also in post-transplant care could explain the better outcomes in the one-step reconstruction group. In fact, operative blood loss and acute rejection (data not shown) were significantly reduced in the one-step reconstruction group. The other limitation of this study is that no stenting was performed for the occluded vessels, although no occlusion was observed in the

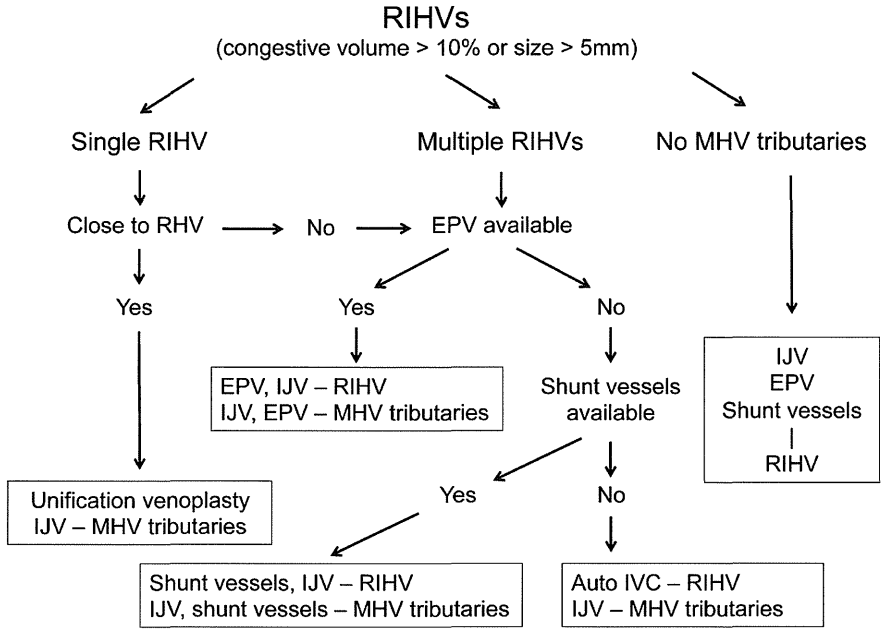

Fig. 4 Current institutional guidelines for reconstructing right inferior hepatic veins (*RIHVs*). *IVC* inferior vena cava, *IJV* internal jugular vein, *MHV* middle hepatic vein, *RHV* right hepatic vein

one-step reconstruction group. As Hwang et al. [14] reported aggressive stenting for reconstructed RIHVs should be performed by an experienced radiologist to optimize graft outflow.

In conclusion, we consider that one-step reconstruction of the accessory hepatic veins, including the RIHVs, using auto-venous grafts, including IJV, EPV or major shunt vessels, is feasible and effective in right lobe LDLT.

Conflict of interest No source of support was received for this work. No financial or other conflict of interest exists with the authors (Ikegami et al.).

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Hepatology Research

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Original Article

Impact of tumor size, number of tumors and neutrophil-to-lymphocyte ratio in liver transplantation for recurrent hepatocellular carcinoma

Tomoharu Yoshizumi, Toru Ikegami, Shohei Yoshiya, Takashi Motomura, Yohei Mano, Jun Muto, Tetsuo Ikeda, Yuji Soejima, Ken Shirabe and Yoshihiko Maehara

Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka, Japan

Aim: Hepatocellular carcinoma (HCC) is primarily treated with hepatic resection and/or locoregional therapy. When HCC recurs and further treatment is no longer possible owing to poor liver function, liver transplantation (LT) or living-donor LT (LDLT) is considered. The aim of this study was to clarify risk factors for tumor recurrence after LDLT in patients with recurrent HCC.

Methods: The study comprised 104 patients who had undergone LDLT because of end-stage liver disease with recurrent HCC. The recurrence-free survival rates after the LDLT were calculated. Risk factors for tumor recurrence were identified.

Results: The 1-, 3- and 5-year recurrence-free survival rates were 89.6%, 80.3% and 78.4%, respectively. By univariate analysis, the factors affecting recurrence-free survival were the sum of the largest tumor size and number of tumors of 8 or more ($P < 0.0001$), des-γ-carboxy prothrombin of more than 300 mAU/mL ($P = 0.0001$), and a neutrophil-to-lymphocyte ratio (NLR) of 4 or more ($P = 0.0002$), α-fetoprotein of more than 400 ng/mL ($P = 0.0001$) and bilobar tumor distribution ($P = 0.046$). A multivariate analysis identified independent risk factors for post-LDLT tumor recurrence including the sum of tumor size and number of tumors of 8 or more ($P = 0.0004$) and an NLR of 4 or more ($P = 0.01$). The 1- and 3- year recurrence-free survival rates in the recipients who had both risk factors were 30.0% and 15.0%, respectively.

Conclusion: LDLT should not be performed for patients who have both independent risk factors after any treatments for HCC.

Key words: hepatocellular carcinoma, living-donor liver transplantation, neutrophil-to-lymphocyte ratio, number of tumors, tumor size

INTRODUCTION

A SHORTAGE OF cadaveric organs for transplantation continues to impair our ability to provide liver transplantation (LT) despite progress in surgical techniques and immunosuppression.[1,2] Currently, there is no consensus on how to manage patients with hepatocellular carcinoma (HCC) while awaiting LT. Guidelines published in the UK state that locoregional therapy, such as transarterial chemoembolization (TACE), radiofrequency ablation (RFA), ethanol injection therapy and microwave coagulation therapy (MCT), should be considered for all listed patients with HCC.[3] In Asian countries, religious, cultural and political ideologies have created significant obstacles to the transplantation of organs from cadavers. As a result, HCC is primarily treated with hepatic resection and/or locoregional therapy.[4,5] However, when HCC recurs and further treatment is no longer possible owing to poor liver function, LT is considered.[4] Organ shortages have forced patients with recurrent HCC to endure long waiting periods that are associated with tumor development. Thus, living-donor LT (LDLT) is a potential choice for treating recurrent HCC patients after the use of other

Correspondence: Dr Tomoharu Yoshizumi, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Fukuoka 812-8582, Japan.
Email: yosizumi@surg2.med.kyushu-u.ac.jp
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treatments.[4] Since the 1994 report demonstrating successful LDLT, living donors have been increasingly used because of the disparity between demand and supply, even in Western countries.[2,6] Moreover, a blood relationship between the donor and the recipient in LDLT may give the recipient a chance to receive a transplant even during the suboptimal conditions of HCC.[7–9]

Thus, it is important to focus on factors that affect tumor recurrence after LDLT in patients with recurrent HCC.

The neutrophil-to-lymphocyte ratio (NLR) has recently emerged as a useful prognostic factor for the recurrence of several malignancies. An NLR of 5 or more was reported to be a marker of survival in colorectal cancer patients.[10] Halazun *et al.* reported that an NLR of five or more was an independent predictor of the recurrence and poor overall survival in patients with colorectal liver metastases.[11] Recently, it was demonstrated that a preoperative NLR of 5 or more was an adverse predictor of recurrence-free survival for patients undergoing hepatic resection for HCC.[12] Furthermore, an elevated NLR significantly increased the risk of HCC recurrence after LT[13] or LDLT.[14]

Mazzaferro *et al.* recently proposed the "up-to-seven criteria", with 7 being the result of the sum of the largest tumor size (in cm) and number of tumors, to predict patient survival after LT, based on a large sample size.[15] We have reported the outcome of LDLT for otherwise unresectable and/or untreatable HCC patients[7,16] and proposed two risk factors for recurrence-free survival: a tumor size greater than 5 cm and des-γ-carboxy prothrombin (DCP) levels greater than 300 mAU/mL (Kyushu University [KU] criteria).[7] Furthermore, we previously reported a series of 68 cases of LDLT for patients who had received pretransplant treatment for HCC.[4] DCP above 300 mAU/mL was shown to be an independent risk factor for tumor recurrence after LDLT in the published work. Since this report, LDLT has become a more common treatment for such patients, thus generating a larger cohort for study.

Therefore, the aim of the present study was to clarify the risk factors of tumor recurrence after LDLT in patients with recurrent HCC.

METHODS

Recipients

ONE HUNDRED AND sixty-seven recipients underwent LDLT because of end-stage liver disease with HCC at Kyushu University Hospital between April 1999 and August 2012. In this study, 104 adult patients (41 female and 63 male) were enrolled who had undergone LDLT because of end-stage liver disease with recurrent HCC after treatment. The pretransplant treatments for HCC, such as RFA, TACE, MCT and/or hepatic resection, were dependent upon the recipient's liver function and tumor status. Graft types included left lobe with caudate lobe graft ($n = 63$), right lobe graft without the middle hepatic vein ($n = 37$) and posterior segment graft ($n = 4$). The etiology of liver cirrhosis included hepatitis C ($n = 75$), hepatitis B ($n = 20$), cryptogenic disease ($n = 4$), alcohol abuse ($n = 3$) and primary biliary cirrhosis ($n = 2$) (Table 1). Our selection criteria to perform LDLT for HCC patients were as follows: (i) no modality except LDLT available to cure the patients with HCC; (ii) no extrahepatic metastasis; and (iii) no major vascular infiltration.[4,7] There were no restrictions on the tumor size, number of tumors or pretransplant treatment. Since defining the KU criteria, we have not performed LDLT for HCC patients with a tumor size greater than 5 cm and DCP levels greater than 300 mAU/mL.

Pretransplant imaging was used to estimate the maximum tumor size, number of tumors and up-to-seven criteria. α-Fetoprotein (AFP), DCP and NLR were measured before the LDLT. The histological grades obtained from the explanted livers were used for tumor differentiation.

Donor and graft selection

Donors were selected from among the candidates who hoped to be living donors.[1,8] Donors were required to be within the third degree of consanguinity with recipients or spouses, and to be between 20 and 65 years of age. For a donor who was not within the third degree of consanguinity, individual approval was obtained from the Ethics Committee of Kyushu University Hospital. Good Samaritan donations were not used.

Eligible donors proceeded to the imaging studies, including chest and abdominal X-rays and 3-mm-slice computed tomography (CT) scans for graft volumetric analysis. 3-D CT was introduced for volumetric analysis and delineation of vascular anatomy. The standard liver weight (SLW) of recipients was calculated according to the formula of Urata *et al.*[17] Graft weight (GW) was predicted by CT volumetric analysis. Decisions regarding the graft type for recipients were based on the preoperatively predicted GW to SLW (GW : SLW) ratio. The left lobe with caudate lobe graft was used when the preoperatively predicted GW : SLW ratio was more than 35%. A posterior segment graft was used when the donor's vascular variation was suitable to take the posterior segment.

Table 1 Characteristics of recipients and donors

Variables	*n*
Recipient	
Sex (male/female)	63/41
Age (years, range)	58.0 (41–72)
Etiology	
HCV	75
HBV	20
Cryptogenic	4
Alcohol	3
PBC	2
MELD score (range)	11.5 (4–31)
Diabetes mellitus (yes/no)	31/73
Splenectomy (yes/no)	60/44
CNI (TAC/CyA/None)	44/57/3
Donor	
Sex (male/female)	75/29
Age (years, range)	34.3 (20–63)
Graft (left/right/posterior)	63/37/4
GW : SLW ratio (%, range)	41.0 (23.6–67.6)
Tumor	
Maximum size (cm, range)	2.4 (0–7.0)
n (range)	17 (0–400)
Milan criteria (yes/no)	52/52
NLR (range)	3.1 (0.44–20.2)
AFP (ng/mL, range)	1516 (1–43 000)
DCP (mAU/mL, range)	349 (3–5934)
Duration between first Tx and LDLT (days, median, range)	1198 (61–4272)
Duration between last Tx and LDLT (days, median, range)	349 (30–2140)
Times of treatment (range)	3 (1–11)
Microvascular invasion (yes/no)	39/65
Pathological differentiation (well/moderate/poor)	7/63/34

AFP, α-fetoprotein; CNI, calcineurin inhibitor; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus; Tx, pretransplant treatment.

Postoperative management

The graft retrieval technique, recipient surgery and perioperative management of the recipients, including immunosuppression regimens, have been described elsewhere.[9,18] Immunosuppression was initiated using a protocol based on either tacrolimus (Prograf; Astellas Pharma, Tokyo, Japan) or cyclosporin A (Neoral; Novartis Pharma, Tokyo, Japan) with steroid and/or mycophenolate mofetil (MMF; Chugai Pharmaceutical, Tokyo, Japan). Tacrolimus was used in 44 recipients and cyclosporin in 57 recipients. Three recipients did not receive calcineurin inhibitor owing to postoperative poor disease course. A target trough of tacrolimus was set at 10 ng/mL for 3 months after LDLT, followed by 5–10 ng/mL thereafter. A target trough level of cyclosporin A was set at 250 ng/mL for 3 months after LDLT, followed by 150–200 ng/mL thereafter. Methylprednisolone was initiated on the day of LDLT, tapered and converted to prednisolone 7 days after LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDLT. MMF was used in 91 recipients and was started at 1000 mg/day on the day after LDLT, tapered and discontinued until 6 months after LDLT. A trough level was not measured for MMF.

All patients had monthly follow ups, and the median follow-up period was 1738 days, with 723 days and 2891 days as the 25th and 75th percentiles, respectively.

Post-LDLT tumor recurrence and risk factors

Hepatocellular carcinoma recurrence after the LDLT was set as the primary end-point of this study. All patients underwent abdominal CT scan every 3 months, and chest CT scan and bone scintigraphy every 6 months within 5 years after LDLT. Tumor recurrence was defined as when any imaging studies revealed the recurrence of HCC. Recurrence-free survival was defined as the time period between LDLT and tumor recurrence.

Univariate and multivariate analyses were performed to identify the factors associated with recurrence-free survival after the LDLT.

Statistical analysis

Recurrence-free survival rates were calculated by the Kaplan–Meier product-limited method. Data were expressed as means.

Cox regression analysis was applied to the multivariate analyses. Variables that were used for the analysis included recipient age, donor age, Model for End-Stage Liver Disease score, presence of hepatitis C virus, presence of diabetes mellitus, recipient sex, donor sex, GW : SLW ratio, the sum of the largest tumor size (in cm) and the number of tumors, pretransplant NLR, pretransplant AFP, pretransplant DCP, graft type, splenectomy, duration between first treatment for HCC and the LDLT, duration of last treatment for HCC and the LDLT, times of pretransplant treatment and type of calcineurin inhibitor. All statistical analyses were performed using JMP ver. 9.0 software (SAS, Cary, NC, USA). $P < 0.05$ was considered significant.

Approval of institutional review board

The Institutional Review Board of Kyushu University Hospital approved this study protocol (no. 23–58).

RESULTS

THE CHARACTERISTICS OF the recipients and donors from this study are shown in Table 1. Fifty-two of 104 patients (50.0%) exceeded the Milan criteria. Patients previously underwent at least one of the following treatments for primary or recurrent HCC: TACE ($n = 85$), RFA ($n = 54$), ethanol injection therapy ($n = 30$), MCT ($n = 17$), hepatic resection ($n = 11$) and hepatic arterial infusion chemotherapy ($n = 7$). Median times of treatment were 3.0 (1–11 times), median duration from first treatment to LDLT was 1199 days (61–4272 days) and median duration from last treatment to LDLT was 348 days (30–2140 days).

Receiver–operator curve (ROC) analysis for tumor recurrence after LDLT was used to detect the cut-off line of the sum of the largest tumor size (in cm) and number of tumors, and NLR. The area under the ROC (AUROC) of the sum of the largest tumor size (in cm) and number of tumors was 0.833. A cut-off value of the sum was set as 8.0, because ROC analysis revealed that a cut-off value of 8, which had 84.2% of the sensitivity and 80.0% of the specificity, was the most suitable value. Similarly, the AUROC of NLR was 0.700 and a cut-off value of NLR of 4 was set using the analysis.

The 1-, 3- and 5-year recurrence-free survival rates in enrolled recipients were 89.6%, 80.3% and 78.4%, respectively. Among the 104 recipients, 19 patients developed tumor recurrence after LDLT. A univariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more, had an NLR of 4 or more, AFP levels of more than 400 ng/mL, DCP levels of more than 300 mAU/mL and bilobar tumor distribution were risk factors for tumor recurrence after LDLT ($P < 0.0001$, $P = 0.0002$, $P < 0.0001$, $P < 0.0001$, and $P = 0.046$, respectively) (Table 2). Although the nodule size and number of nodules were risk factors of tumor recurrence by the univariate analysis, these factors statistically interfered with the sum of the largest tumor size (in cm) and number of tumors for performing multivariate analysis. The AUROC of the number of nodules was 0.790 and that of the largest nodule size was 0.753. Both data were less than that of the sum of the largest tumor size and number of tumors (0.833). Thus, we selected the sum of the largest tumor size and number of tumors for multivariate analysis. Multivariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more and an NLR of 4 or more were independent risk factors for tumor recurrence after LDLT in this study ($P = 0.0004$ and $P = 0.011$, respectively) (Table 3).

Table 4 shows the correlation between explant pathology and each risk factor. The frequency of microvascular invasion and poorly differentiated tumors increased among patients who had both independent risk factors of tumor recurrence.

The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an NLR of 4 or more were 100%, 81.8% and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0%, and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained (Fig. 1). The differences among the four groups were significantly different ($P < 0.0001$).

DISCUSSION

THIS IS THE largest study to investigate LDLT with recurrent HCC.[4] It is crucial to clarify when patients with poor liver function and HCC should be listed as candidates for LDLT. We chose recurrence-free survival rate as the end-point in this study because preliminary analysis revealed that 27 deaths occurred in the enrolled recipients, of which 14 causes of death were not tumor-related.

To date, several studies have attempted to extend the Milan criteria to encompass HCC patients with potentially curable tumors.[7,14,19–22] The up-to-seven criteria may predict patient survival even after LDLT.[4,14] The ROC analysis for tumor recurrence after LDLT revealed that the sensitivity of the cut-off value of 7 was 89.4% and the specificity was 71.7%. It meant that a cut-off value of 7 was less suitable than that of 8 in this study. Although we previously proposed that the number of tumors did not affect tumor recurrence after LDLT,[4,7,16] the results obtained from the present study suggest that the number of tumors as well as largest tumor size should be taken into consideration to select HCC patients for LDLT.

The precise mechanism of how NLR affects tumor recurrence is still unclear. Infiltration of pro-inflammatory macrophages, cytokines and chemokines in the tumor microenvironment can boost tumor

Table 2 Risk factors for tumor recurrence: univariate analysis

Variables	*n*	Recurrence-free survival (%)			*P*
		1 year	3 years	5 years	
Recipient variables					
Sex					
Male	63	84.5	82.7	79.5	0.81
Female	41	97.4	75.7	75.7	
Age (years)					
>60	46	88.1	82.3	82.3	0.67
≤60	58	90.8	79.1	76.1	
Etiology					
HCV	75	88.8	79.6	77.2	0.64
Others	29	91.4	82.0	82.0	
Pretransplant MELD					
<15	84	91.2	80.1	78.0	0.99
≥15	20	82.1	82.1	82.1	
Diabetes mellitus					
Yes	31	89.1	84.4	78.8	0.75
No	73	89.7	78.5	78.5	
NLR					
≥4	21	72.7	55.9	41.9	0.0002
<4	83	93.5	86.2	86.2	
Splenectomy					
Yes	60	90.9	79.9	79.9	0.82
No	44	87.8	80.2	77.4	
Calcineurin inhibitor					
TAC	44	90.0	80.9	80.9	0.78
CyA	57	89.4	80.1	77.3	
Donor variables					
Sex					
Male	75	92.7	82.9	80.4	0.34
Female	29	82.1	74.1	74.1	
Donor age (years)					
>40	25	95.2	89.6	89.6	0.19
≤40	79	88.0	77.6	75.3	
Graft type					
Others	67	90.2	75.4	72.5	0.13
Right	37	88.6	88.6	88.6	
GW : SLW ratio					
<35	24	86.1	76.0	76.0	0.62
≥35	80	90.5	81.5	79.1	
Tumor variables					
Nodule size (cm)					
≥5	6	50.0	33.3	33.3	0.0004
<5	98	92.2	83.5	81.4	
No. of nodules					
≥5	34	75.2	58.0	58.0	0.0002
<5	70	96.8	91.6	88.7	
Nodule size + number					
≥8.0	33	67.9	46.4	46.4	<0.0001
<8.0	71	100	96.5	93.8	

Table 2 *Continued*

Variables	*n*	Recurrence-free survival (%)			*P*
		1 year	3 years	5 years	
DCP (mAU/mL)†					
>300	19	51.6	38.7	38.7	<0.0001
≤300	84	97.3	89.5	87.1	
AFP (ng/mL)					
>400	22	75.8	53.1	44.3	<0.0001
≤400	82	93.3	87.5	87.5	
Tumor distribution					
Bilobar	65	85.3	74.7	72.1	0.046
Unilobar	39	97.0	90.4	90.4	
Duration between the first treatment and the LDLT					
<1 year	21	80.0	68.7	68.7	0.20
≥1 year	83	92.1	83.3	80.7	
Duration between the last treatment and the LDLT					
<1 year	72	86.5	76.5	76.5	0.26
≥1 year	32	96.6	89.1	82.3	
Times of treatment					
≥4	36	85.0	67.9	67.9	0.06
<4	68	91.9	86.7	83.9	

†Data of one case was lacking because of warfarin intake.
AFP, α-fetoprotein; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HCV, hepatitis C virus; KU, Kyushu University; LDLT, living-donor liver transplantation; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus.

growth, invasion and metastases.[23,24] Recently, Motomura *et al.* reported that interleukin (IL)-17-producing T cells are thought to release CXC chemokines that recruit neutrophils, leading to elevated NLR, and promote the differentiation of tissue macrophages in peritumoral regions into tumor-associated macrophages (TAM).[14] Both IL-17-producing T cells and TAM may accelerate tumor progression and antitumor T-cell exhaustion. As shown in Table 4, pathological examination revealed poorly differentiated HCC and microvascular invasion in the explanted liver in seven of eight recipients who had both independent risk factors of tumor recurrence. The use of routine biopsy to identify tumor grading has been abandoned owing to concerns of tumor seeding, leading to an extensive search for suitable surrogate markers to predict tumor differentiation or vascular invasion. Halazun *et al.* showed that elevated NLR correlated with microvascular invasion and poorly differentiated tumors.[13] The results from our study are consistent with this previous report. The interpretation

Table 3 Risk factors for tumor recurrence: multivariate analysis

Variables	Odds ratio	95% CI	*P*
Nodule size + number ≥8.0	15.2	3.34–68.9	0.0004
NLR ≥4	4.02	1.38–11.6	0.011
DCP >300 mAU/mL	3.09	0.87–11.0	0.082
AFP >400 ng/mL	1.23	0.37–4.08	0.73
Bilobar distribution	1.12	0.24–5.21	0.88

AFP, α-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; NLR, neutrophil-to-lymphocyte ratio.

Table 4 Correlation between explant pathology and risk factors

Variables	No risk factor ($n = 58$)	NLR ≥4 ($n = 13$)	Tumor size and number of tumors ≥8 ($n = 25$)	Both risk factors ($n = 8$)	*P*
Microvascular invasion	12 (20.7%)	4 (30.8%)	16 (64.0%)	7 (87.5%)	<0.0001
Poorly differentiated tumor	12 (20.7%)	3 (23.1%)	12 (48.0%)	7 (87.5%)	0.0005

NLR, neutrophil-lymphocyte ratio.

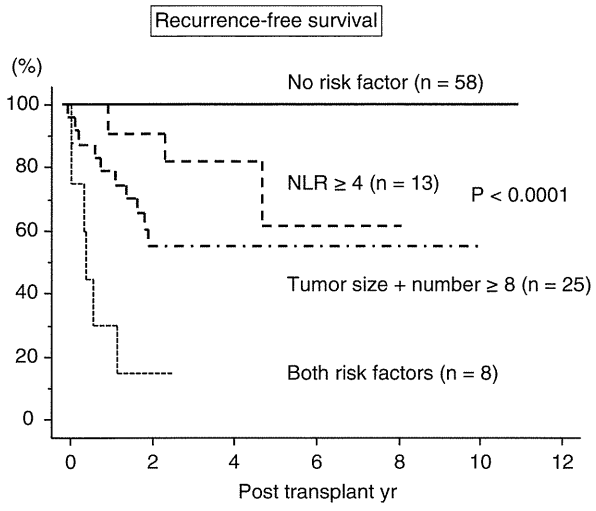

Figure 1 Recurrence-free recipient survival after living-donor liver transplantations for hepatocellular carcinoma. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an neutrophil-to-lymphocyte ratio (NLR) of 4 or more were 100%, 81.8%, and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0% and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained. The differences among the four groups were significantly different ($P < 0.0001$). yr, years.

of NLR in patients with end-stage liver disease, often complicated with hypersplenism and pancytopenia, seems to require caution. Furthermore, patients with end-stage liver disease often develop specific bacterial peritonitis or other bacterial infections because of impaired immune system. There may be limitation for the evaluation of NLR in such patients.

Seventy-eight of 104 patients underwent pretransplant treatment more than twice in this study. Moreover, the times of pretransplant treatment, the interval between the first treatment and LDLT, and the interval between the last pretransplant treatment and LDLT did not affect the outcome of LDLT. Next, we focused on how to predict patients with a high risk of tumor recurrence after LDLT. For the univariate and multivariate analysis, we chose variables that had been obtained before transplantation. The 5-year recurrence-free survival rate after the LDLT was 100% for recipients who did not have both risk factors of tumor recurrence. Therefore, according to our results, HCC can be treated with any treatment modality whenever the patient's liver function is tolerable to such treatments. However, patients who have the sum of the largest tumor size (in cm) and the number of tumors of 8 or more and have an NLR of 4 or more should be excluded from LDLT. Further study is needed on whether LDLT can be performed for patients who have a single independent risk factor or not, because the 5-year recurrence-free survival rate for patients who had the sum of the largest tumor size (in cm) and the number of tumors of 8 or more was 55.4%, and for patients who had an NLR of 4 or more was 61.4%. A recent report recommended giving psychosocial considerations careful attention for both donor and recipient in LDLT.[25]

In conclusion, the type or duration of treatment for HCC did not affect the outcome of LDLT, but LDLT should not be performed for patients who have the sum of the largest tumor size (in cm) and number of tumors of 8 or more and with an NLR of 4 or more after any treatments for HCC to prevent tumor recurrence.

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Original Article

Early extensive viremia, but not rs8099917 genotype, is the only predictor for cholestatic hepatitis C after living-donor liver transplantation

Toru Ikegami,[1] Ken Shirabe,[1] Takasuke Fukuhara,[1] Norihiro Furusyo,[2] Kazuhiro Kotoh,[3] Masaki Kato,[3] Shinji Shimoda,[4] Shinichi Aishima,[5] Yuji Soejima,[1] Tomoharu Yoshizumi[1] and Yoshihiko Maehara[1]

[1]*Department of Surgery and Science,* [2]*General Internal Medicine,* [3]*Medicine and Bioregulatory Science,* [4]*Medicine and Biosystemic Science, and* [5]*Anatomic Pathology, Graduate School of Medical Science, Kyushu University, Fukuoka, Japan*

Aim: Cholestatic hepatitis C is one of the most serious but still unaddressed disorders after liver transplantation.

Methods: In this study, we analyzed 49 patients who underwent living-donor liver transplantation (LDLT) to treat hepatitis C virus (HCV) infection.

Results: Five patients developed cholestatic hepatitis C, with total bilirubin of 15.2 ± 3.1 mg/dL at diagnosis 6.2 ± 1.0 weeks after LDLT. Univariate analysis showed that larger graft to standard liver volume ratio, higher HCV RNA titer at 2 weeks, earlier peak HCV RNA titer and cytomegalovirus infection were the significant risk factors. The development of cholestatic hepatitis C was not significantly associated with interleukin-28B genotype (rs8099917); four out of five affected patients had the T/T genotype. Multivariate analysis showed that higher HCV RNA titer at 2 weeks was the only significant factor ($P = 0.026$) for the development of cholestatic hepatitis C. Receiver–operator curve analysis showed that that HCV RNA titer of more than 7.2 $\log_{10}$IU/mL was the optimal cut-off for characterizing cholestatic hepatitis C. All of the patients were serum HCV RNA negative after treatment with pegylated interferon and ribavirin and all the patients are alive.

Conclusion: Early extensive viremia, but not the rs8099917 genotype, was the only predictor for cholestatic hepatitis C after LDLT.

Key words: cholestatic hepatitis, hepatitis C, interleukin 28B, liver transplantation, living donor, splenectomy

INTRODUCTION

ALTHOUGH END-STAGE LIVER disease secondary to hepatitis C virus (HCV) is the leading indication for liver transplantation (LT), re-infection of HCV is a widespread, unaddressed and serious event.[1] It has been reported that approximately one-quarter of patients develops cirrhosis within 10 years after LT for HCV; therefore, graft outcomes after LT for HCV are inferior to those for other indications.[2]

Nevertheless, recurrent hepatitis C after LT is represented by a spectrum of disorders, including mild to severe inflammation with various degrees of fibrosis progression over several years.[1,2] Of note, HCV re-infection can result in very aggressive hepatitis in a small number of patients, and is usually characterized by rapid progression of cholestasis with fibrosis resulting in graft failure and death.[3,4] This outcome has been termed post-transplant cholestatic hepatitis C and its risk factors include higher donor age, HCV genotype 1, extremely high viral titers and bolus steroid administration for acute rejection.[3,4] More recently, two reports

Correspondence: Dr Toru Ikegami, Department of Surgery and Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan. Email: tikesurg@surg2.med.kyushu-u.ac.jp
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have shown that single nuclear polymorphism (SNP) in the interleukin (IL)-28B gene was a significant risk factor for the disease process.[5,6] To date, however, the pathogenesis of recurrent cholestatic hepatitis C after LT has not been elucidated.

Therefore, in the current study, we examined the clinical characteristics of patients who developed this rare type of recurrent cholestatic hepatitis C after living-donor liver transplantation (LDLT). We investigated whether its pathogenesis could be attributed to viral factors, host factors, including IL-28B genotypes or graft-related factors.

METHODS

Patients

LIVING-DONOR LIVER TRANSPLANTATION was performed in 54 patients positive for the HCV antibody at Kyushu University Hospital between February 2007 and July 2012. All procedures were approved by the Ethics and Indications Committee of Kyushu University. Forty-nine patients who were HCV RNA positive before LDLT were included in the current study. The mean follow-up time was 2.8 ± 1.1 years.

Transplantation and postoperative care

The surgical procedures for both the donors and the recipients are described in more detail elsewhere.[7,8] The graft type, either left or right lobe, was determined based on the need for a graft volume (GV) of more than 35% of the recipient's standard liver volume (SLV).[7] Splenectomy was performed for 47 (95.9%) recipients to prevent pancytopenia caused by interferon (IFN) therapy.[9] A biliary stent over the biliary anastomosis was placed during the surgery and was kept in place for 3–4 months after LDLT to prevent early stricture.[10]

The immunosuppression regimen consisted of tacrolimus or cyclosporin with mycophenolate mofetil and steroids as previously reported.[8] The immunosuppression level was maintained at a standard level to prevent acute rejection; unfortunately, this hinders the diagnosis and treatment of hepatitis C after LDLT. The tacrolimus level was maintained at 10–14 ng/mL for 1 month after LDLT and was then decreased to 7–10 ng/mL over the next few months. The cyclosporin level was maintained at 150–250 ng/mL for 1 month after LDLT and then decreased to 100–150 ng/mL over the next few months. Mycophenolate mofetil at the dose of 2 g/day, was then tapered down to 1 g daily over 1–3 months and tapered off at 6 months. All the patients received steroids during the study period. Methylprednisolone (1 g) was given after reperfusion, and titrated from 200 mg/day to 20 mg/day in a week, then switched to oral prednisolone, and tapered off by 6 months. The immunosuppression protocol for blood type-incompatible LDLT consisted of pretransplant rituximab and plasma exchanges with tacrolimus or cyclosporin and mycophenolate mofetil and steroids, as previously described.[11]

Antiviral treatment

Interferon was indicated for recurrent hepatitis C associated with serum HCV RNA positivity, abnormal liver function tests and histological evidence of recurrent hepatitis C. Preemptive antiviral treatment was not performed.

Antiviral treatment consisted of pegylated (PEG) IFN-α-2b with ribavirin (Pegintron with Rebetol; Merck, Whitehouse Station, NJ, USA) or PEG IFN-α-2a with ribavirin (Pegasys with Copegus; Chugai Pharmaceutical, Tokyo, Japan) was used for antiviral treatment. Although PEG IFN-α-2b was primarily used for post-transplant induction of antiviral treatment, PEG IFN-α-2a could also be used for refractory or severe cases. The type of PEG IFN drug, regarding conversion between the products, was determined for individual cases. PEG IFN-α-2b and ribavirin were started at doses of 0.5–1.0 mcg/kg per week and 200–400 mg/day, respectively. The doses were escalated in a stepwise manner, in accordance with the individual's tolerability, to 1.5 mcg/kg per week and 800 mg/day, respectively. PEG IFN-α-2a and ribavirin were started at doses of 90–120 mcg/week and 200–400 mg/day, respectively, to 180 mcg/week and 800 mg/day respectively. The recommended duration of treatment was 48 weeks after achieving viral response (VR), defined as undetectable serum HCV RNA.

Measurement of the serum HCV RNA titer

The serum HCV RNA titer was determined by a real-time HCV assay (AccuGene HCV; Abbott Molecular, Des Plaines, IL, USA). The lower and higher limits of quantification for this assay are 1.08 log IU/mL and 8.0 log IU/mL, respectively. The serum HCV RNA titer was measured before LDLT, 2 weeks after LDLT and monthly thereafter.

IL-28B genotyping assay

DNA from the donors and the recipients was extracted from a biopsy or explanted liver tissue obtained during LDLT, and genotyping was performed using TaqMan

GTX press Master Mix (Life Technologies, Tokyo, Japan), in accordance with the manufacturer's instructions. The Custom TaqMan SNP Genotyping Assay (Life Technologies) was used to identify IL-28B genetic polymorphisms. We used rs8099917 as the representative SNP for IL-28B because of its higher sensitivity and specificity for IFN sensitivity in Asian individuals.[12] The T/T genotype of rs8099917 was defined as the major allele, while the T/G and G/G genotypes were regarded as the minor alleles.

Diagnosis of cholestatic hepatitis

Cholestatic hepatitis C was defined according to the factors as proposed by Wiesner *et al.*[13] with minor modifications: (i) total bilirubin of more than 6 mg/dl; (ii) elevated biliary enzymes with alkaline phosphatase (ALP) and/or γ-glutamyltransferase (GGT) of more than 5 times the upper limit of normal; (iii) very high serum HCV RNA titer of more than 6 log IU/mL; (iv) histological findings that include predominant ballooning of hepatocytes in the perivenular zone and limited inflammation; (v) occurring between 1 and 6 months after LT; and (vi) absence of surgical complications at the time of diagnosing cholestatic hepatitis C.

Percutaneous liver biopsy was obtained and evaluated for patients with abnormal liver function tests suggestive of recurrent hepatitis C or acute rejection. Biopsies were also obtained every year in accordance with the established protocol.

Statistical analysis

Values are expressed as the mean ± standard deviation. Variables were analyzed using the χ^2-test for categorical values or the Mann–Whitney *U*-test for continuous variables. Multivariate analyses were performed using the logistic regression model and odds ratios were calculated. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of patients with cholestatic hepatitis C

FIVE PATIENTS DEVELOPED cholestatic hepatitis C after LDLT (Table 1). The mean ages of the donors and the recipients were 58.2 ± 7.7 years and 29.2 ± 10.0 years, respectively. The mean GV/SLV ratio was 45.0 ± 7.3%. Donor age was less than 40 years old in all of the cases except for case 5. GV/SLV was more than 35% in all of the cases, except in case 3. Splenectomy was performed in all five cases.

Hepatitis C virus genotype was type 1b, except in case 4 (2a) and the mean HCV RNA titer before LDLT was 5.2 ± 0.7 $\log_{10}$IU/mL. The HCV RNA titer was more than 5 $\log_{10}$IU/mL in all the cases except case 5. The IL-28B (rs8099917) genotype was T/T in both the donors and recipients except in case 2, where the donor and recipient both had the T/G genotype.

The mean values of liver function parameters were 15.2 ± 3.1 mg/dL for total bilirubin, 357 ± 79 IU/L for aspartate aminotransferase (AST) and 859 ± 497 IU/L for GGT. The peak HCV RNA titer was 7.9 ± 0.1 $\log_{10}$IU/mL and more than 7.7 $\log_{10}$IU/mL in all five patients at diagnosis of cholestatic hepatitis C, 6.2 ± 1.0 weeks after LDLT. Although cases 1 and 5 had biliary anastomotic stenosis after LDLT, this complication occurred after treatment for cholestatic hepatitis C.

All of the five patients were treated with PEG IFN with ribavirin after histological confirmation of cholestatic hepatitis C. PEG IFN-α-2b was used in two patients and PEG IFN-α-2b was used in three patients. VR was observed in all of the patients. Among the patients who received IFN ($n = 41$) after LDLT, the total dosage of IFN was larger in patients with ($n = 5$) cholestatic hepatitis C (10.5 ± 3.0 vs 6.0 ± 4.6 mg, $P = 0.040$), compared with those without ($n = 36$). However, the total dosage of ribavirin (24.6 ± 26.1 vs 24.4 ± 20.7 g, $P = 0.981$) and the treatment period (90.0 ± 44.7 vs 62.2 ± 38.8 g, $P = 0.147$) was not different between the groups. Discontinued antiviral treatment was observed in no case in the patients with cholestatic hepatitis ($n = 5$) and 10 cases (27.8%) in the patients without ($n = 36$) due to intolerance and adverse reactions. Dose modification of IFN during the treatment course was observed in three patients (60%) and 18 patients (50.0%), respectively.

Risk factors for cholestatic hepatitis C

We next determined possible risk factors for cholestatic hepatitis C after LDLT. In univariate analyses, larger GV/SLV (45.0 ± 7.3% vs 39.2 ± 5.9%, $P = 0.049$), higher HCV RNA titer at 2 weeks after LDLT (7.7 ± 0.4 vs 5.8 ± 1.3 $\log_{10}$IU/mL, $P = 0.002$), earlier period for having peak HCV RNA titer (3.7 ± 2.3 vs 9.4 ± 5.6 weeks, $P = 0.031$) and cytomegalovirus infection (80.0% vs 27.2%, $P = 0.017$) were significantly associated with cholestatic hepatitis C after LDLT. By contrast, donor and recipient age, cold and warm ischemic time, HCV genotype, and donor and recipient IL-28B genotype were not associated with the occurrence of cholestatic hepatitis C (Table 2).

Table 1 Clinical characteristics of the five cases of cholestatic hepatitis C

Case	1	2	3	4	5
Recipient age, sex	54, F	62, F	52, M	53, F	70, F
MELD score	16	18	8	18	12
Hepatocellular carcinoma	Yes	Yes	Yes	No	Yes
Splenectomy	Yes	Yes	Yes	Yes	Yes
Donor age, sex	21, F	36, M	20, M	23, M	43, F
Immunosuppression regimen	FK-based	CyA-based	CyA-based	CyA-based	CyA-based
ABO incompatible	No	Yes	Yes	No	No
Graft type	Left	Left	Left	Right	Right
GV (g)	460	440	510	598	502
GV/SLV (%)	39.9	44.0	37.0	55.4	48.9
HCV genotype	1b	1b	1b	2a	1b
HCV RNA titer (log_{10}IU/mL)	5.7	5.7	5.3	5.5	3.9
Recipient IL-28B genotype	T/T	T/G	T/T	T/T	T/T
Donor IL-28B genotype	T/T	T/G	T/T	T/T	T/T
Peak liver function tests					
Total bilirubin (mg)	17.4	13.6	19.1	16.7	9.0
AST (IU/L)	354	382	486	163	399
GGT (IU/L)	519	1939	415	1023	401
HCV RNA (log_{10}IU/mL)	7.7	7.7	8.0	8.0	7.7
Weeks after LDLT	4	8	6	6	7
Histological findings					
Hepatocyte ballooning	++	++	++	+++	++
Cholestasis	+	–	–	–	–
Perivenulitis	+++	+	++	+	–
Portal infiltration	+	+	–	–	+
Ductular reaction	+	+	+	–	+
Interferon treatment					
Type and dose (μg/week)	α-2b (50)	α-2a (180)	α-2b (90)	α-2a (180)	α-2a (180)
Ribavirin dose (mg/day)	400	0	400	200	200
Response (weeks)	VR (130)	VR (17)	VR (15)	VR (49)	VR (23)
On treatment (weeks)	Yes (170)	Yes (74)	Yes (70)	Yes (69)	Yes (68)
Graft outcomes (years)	Alive (3.4)	Alive (1.6)	Alive (1.5)	Alive (1.5)	Alive (1.5)

AST, aspartate aminotransferase; CyA, cyclosporin; FK, tacrolimus; GGT, γ-glutamyltransferase; GV, graft volume; HCV, hepatitis C virus; IL, interleukin; LDLT, living-donor liver transplantation; MELD, Model for End-Stage Liver Disease; SLV, standard liver volume; VR, viral response.

In multivariate logistic regression analysis, higher HCV RNA titer at 2 weeks after LDLT ($P = 0.026$) was the only significant factor associated with having cholestatic hepatitis C. The other factors identified in univariate analyses, including earlier peak of HCV RNA titer ($P = 0.317$), larger GV/SLV ($P = 0.382$) and cytomegalovirus infection ($P = 0.936$) were not significantly associated with cholestatic hepatitis C after LDLT. Receiver–operator curve (ROC) analysis showed that HCV RNA titer of more than 7.2 log_{10}IU/mL at 2 weeks after LDLT was the optimal cut-off for discriminating cholestatic hepatitis C after LDLT. The area under the ROC for this value was 0.989 (Fig. 1).

Histological characteristics of cholestatic hepatitis C after LDLT

The histological characteristics of the five cases of cholestatic hepatitis C are summarized in Table 1. Although hepatocyte ballooning was prominent in all of the five patients (Fig. 2), portal infiltration and cholestasis were relatively minor or absent, despite the high serum bilirubin level. Perivenulitis was observed in four cases and was significantly more common in patients with recurrent cholestatic hepatitis C than in patients with recurrent non-cholestatic hepatitis C (80.0% vs 20.5%, $P = 0.004$, Table 2). Ductular reaction was observed in four cases.