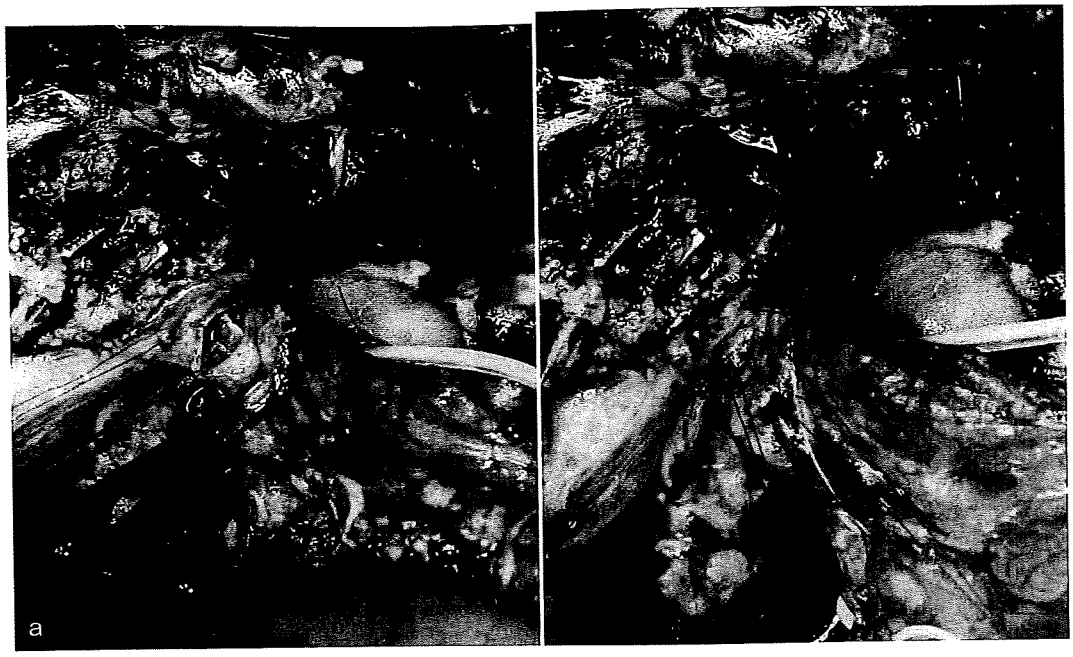

図 3　胆管胆管吻合の術中写真
a：後壁の縫合が終了したところ，胆管チューブが吻合部を超えて挿入されている。チューブは右肝管の断端から出している。
b：前壁の吻合糸を細かくかけ終わり，順に結紮しているところ。

周術期管理の安全性を考えると，胆管胆管吻合も捨てきらず，今後も体重の小さな乳幼児においても可能な限り，胆管胆管吻合を選択していくつもりである。

おわりに

肝移植における，胆管狭窄を回避するための，様々な手術手技の工夫について論じた。今後も各施設より，成績向上へ向けた提言がなされるであろうが，胆管狭窄については，ある程度長期間のフォローアップをもって，評価しなければならないと考える。様々な検証のもとに，更なる技術の向上が望まれる。

参考文献

1) Park JB, Kwon CH, Choi GS, et al. : Prolonged cold ischemic time is a risk factor for biliary strictures in duct-to-duct biliary reconstruction in living donor liver transplantation. Transplantation **86** : 1536-1542, 2008.
2) Seo JK, Ryu JK, Lee SH, et al. : Endoscopic treatment for biliary stricture after adult living donor liver transplantation. Liver Transpl **15** : 369-380, 2009.
3) Sanni A, Asher J, Wilson C, et al. : Predisposing factors for biliary complications following liver transplantation. Transplant Proc **38** : 2677-2678, 2006.
4) Tashiro H, Itamoto T, Sasaki T, et al. : Biliary complications after duct-to-duct biliary reconstruction in living-donor liver transplantation : causes and treatment. World J Surg **31** : 2222-2229, 2007.
5) Takatsuki M, Eguchi S, Kenematsu T : Which is the best timing of bile duct division in living liver donor surgery? Liver Transpl **13** : 1205, 2007.
6) Soejima Y, Fukuhara T, Morita K, et al. : A simple hilar dissection technique preserving maximum blood supply to the bile duct in living donor liver transplantation. Transplantation **86** : 1468-1469, 2008.
7) Kasahara M, Egawa H, Takada Y, et al. : Biliary reconstruction in right lobe living-donor liver transplantation : Comparison of different techniques in 321 recipients. Ann Surg **243** : 559-566, 2006.
8) Yamamoto S, Sato Y, Oya H, et al. : Risk factors and prevention of biliary anastomotic complications in adult living donor liver transplantation. World J Gastroenterol **13** : 4236-4241, 2007.
9) Bennet W, Zimmerman MA, Campsen J, et al. : Choledochoduodenostomy is a Safe Alternative to Roux-en-Y Choledochojejunostomy for Biliary Reconstruction in Liver Transplantation. World J Surg **33** : 1022-1025, 2009.
10) Sakamoto S, Egawa H, Ogawa K, et al. : The technical pitfalls of duct-to-duct biliary reconstruction in pediatric living-donor left-lobe liver transplantation : the impact of stent placement. Pediatr Transplant **12** : 661-665, 2008.
11) Shirouzu Y, Okajima H, Ogata S, et al. : Biliary reconstruction for infantile living donor liver transplanta-

tion : Roux-en-Y hepaticojejunostomy or duct-to-duct choledochocholedochostomy? Liver Transpl **14** : 1761-1765, 2008.
12) Kimura T, Hasegawa T, Ihara Y, et al. : Feasibility of duct-to-duct biliary reconstruction in pediatric living related liver transplantation : report of three cases. Pediatr Transplant **10** : 248-251, 2006.
13) Haberal M, Sevmis S, Emiroglu R, et al. : Duct-to-duct biliary reconstruction in pediatric liver transplantation : one center's results. Transplant Proc **39** : 1161-1163, 2007.
14) Liu C, Loong CC, Hsia CY, et al. : Duct-to-duct biliary reconstruction in selected cases in pediatric living-donor left-lobe liver transplantation. Pediatr Transplant, 2008.
15) Castaldo ET, Pinson CW, Feurer ID, et al. : Continuous versus interrupted suture for end-to-end biliary anastomosis during liver transplantation gives equal results. Liver Transpl **13** : 234-238, 2007.
16) Ushigome H, Sakai K, Suzuki T, et al. : Biliary anastomosis and biliary complications following living donor liver transplantation. Transplant Proc **40** : 2537-2538, 2008.
17) Yan L, Li B, Zeng Y, et al. : Preliminary experience for reducing biliary complication in adult-to-adult living donor liver transplantation using right lobe graft. Hepatol Res **37** : 305-309, 2007.
18) 池上　徹，武冨紹信，副島雄二，ほか：生体肝移植における胆管吻合部狭窄の現状．胆と膵 **29** : 131-137, 2008.
19) Yan L, Li B, Zeng Y, et al. : Introduction of microsurgical technique to biliary reconstruction in living donor liver transplantation. Transplant Proc **39** : 1513-1516, 2007.
20) Settmacher U, Steinmuller TH : Schmidt SC, et al. : Technique of bile duct reconstruction and management of biliary complications in right lobe living donor liver transplantation. Clin Transplant **17** : 37-42, 2003.
21) Alsharabi A, Zieniewicz K, Michalowicz B et al. : Biliary complications in relation to the technique of biliary reconstruction in adult liver transplant recipients. Transplant Proc **38** : 2785-2787, 2007.
22) Haberal M, Karakayali H, Sevmis S, et al. : Results of biliary reconstructions in liver transplantation at our center. Transplant Proc **38** : 2957-2960, 2006.
23) Haberal M, Sevmis S, Karakayali H, et al. : Bile duct reconstruction without a stent in liver transplantation : early results of a single center. Transplant Proc **40** : 240-244, 2008.
24) Kobayashi T, Sato Y, Yamamoto S, et al. : Long-term follow-up study of biliary reconstructions and complications after adult living donor liver transplantation : feasibility of duct-to-duct reconstruction with a T-tube stent. Transplant Proc **41** : 265-267, 2009.
25) Ikegami T, Taketoimi A, Soejima Y, et al : Characteristics of biliary reconstruction using a T-tube as compared with other methods in left-lobe adult living—donor liver transplantation. J Hepatobiliary Pancreat Surg **15** : 346-347, 2008.
26) Asonuma K, Okajima H, Ueno M, et al. : Feasibility of using the cystic duct for biliary reconstruction in right-lobe living donor liver transplantation. Liver Transpl **11** : 1431-1434, 2005.

*　　*　　*

American Journal of Transplantation 2007; 7: 1629–1632
Blackwell Munksgaard

doi: 10.1111/j.1600-6143.2007.01828.x

Whole-Liver Graft Without the Retrohepatic Inferior Vena Cava for Sequential (Domino) Living Donor Liver Transplantation

Y. Inomata[a,*], M. E. Zeledón[a], K. Asonuma[a], H. Okajima[a], T. Takeichi[a], T. Ishiko[b] and Y. Ando[c]

[a] *Department of Pediatric Surgery and Transplantation*
[b] *Department of Digestive Surgery*
[c] *Department of Diagnostic Medicine, Faculty of Medical and Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan*
* *Corresponding author: Yukihiro Inomata, yino@fc.kuh.kumamoto-u.ac.jp*

Grafts used in Domino liver transplantation (LT) obtained from living donor liver transplantation (LDLT) for familial amyloid polyneuropathy (FAP) patients have been mainly used as reduced grafts. Because of small-for-size problems seen in LDLT, using whole liver grafts could improve post-LT outcome. Eight consecutive Domino LDLT using whole livers without retrohepatic inferior vena cava (IVC) from FAP patients were retrospectively analyzed. The graft weight/recipient's body weight ratio (GWRW) in the domino recipients ranged from 1.28% to 2.4% (mean: 1.52). Multiple vascular reconstructions in the whole-liver domino LT resulted in longer than usual warm ischemia time (mean: 64 min); however immediate post-operative recovery of hepatic function was uneventful. At 8–40 months after the transplant, all the FAP patients are well and all of the domino recipients are alive. Domino LT using a whole FAP liver from a LDLT for a FAP patient presents satisfactory results, even though the transplant procedure is technically complicated.

Key words: Domino liver transplantation, familial amyloid polyneuropathy, living donor, whole liver graft

Received 25 December 2006, revised 26 February 2007 and accepted for publication 1 March 2007

Introduction

Domino liver transplantation (Domino LT) can expand the donor pool, especially in areas where deceased donor transplantation is limited, like in Japan. However, in most parts of the world, when it is performed, it is mainly in the event of a deceased donor liver transplantation (DDLT) for familial amyloid polyneuropathy (FAP) (1). When the source of the liver graft for the FAP patient is a deceased donor, the whole FAP liver can be harvested with the accompanying vessels. However, when the liver graft destined for the FAP patient comes from a living donor, the retrohepatic IVC, the portal trunk, and the peripheral hepatic arteries have to remain in the FAP patient because they are essential for the vessel reconstruction for the FAP patient; in these cases, the explanted FAP liver has multiple, relatively short vascular pedicles and biliary orifices that must be reconstructed in the domino recipient. Multiple orifice reconstruction conveys a higher chance of complications, therefore using only part of the FAP liver with more accessible vascular orifices has been the main strategy for Domino live donor liver transplantation (Domino LDLT) for FAP (2,3).

In adult recipients, theoretically, larger liver grafts denote a better outcome for the recipient (4), so in the case of Domino LDLT for FAP patients, we have been resolute on using the whole FAP liver without splitting or reducing it, despite the fact that reconstruction of the vessels is more complicated. We hereby present the data of eight cases of Domino LDLT using a whole liver without the IVC, and our evaluation on its feasibility and efficacy.

Patients and Methods

From 1999 to 2005, 14 patients with FAP underwent LDLT at our institution. In the first three cases, the explanted FAP livers were not recovered as domino grafts; and in the following three cases, the explanted livers were shipped to another institution for the Domino LT. The remaining eight FAP livers were recovered as domino grafts and subsequently used in a Domino LT at our institution; in this study, these eight cases were retrospectively analyzed. Each transplant was reviewed with the Ethics Committee of the Faculty of Medical and Pharmaceutical Sciences of Kumamoto University.

The ages of the eight domino recipients ranged from 17 to 58 years, with a median of 40. All except two cases had primarily nonmalignant diseases (Table 1).

Before the operation, the FAP patient and the donor of the partial liver for the FAP patient underwent three-dimensional (3D) computerized tomographic (CT) examination to evaluate the anatomy of their hepatic veins, portal vein (PV) and hepatic artery (HA). They also underwent a drip infusion cholangiogram (DIC)-CT for the evaluation of the biliary tree.

All of the eight FAP patients agreed to donate their liver after fully informed consent. Each patient had both peripheral and autonomic nerve dysfunction with adequately preserved nutritional status. ABO blood type was

Table 1: LDLT for FAP and Domino LT (Kumamoto University)

LDLT for FAP				2nd recipient (Domino LT)			
FAP#	Age	Donor	Lobe of graft	Indication	Age	Graft/BW (%)	Complication
7	50	Wife	Right	HB-LC	58	1.46	–
8	31	Mother	Right	PBC	52	1.76	–
9	40	Husband	Left	HB-LC	53	1.28	Biliary stricture
10	27	Brother	Right	CAPV	34	2.40	–
11	41	Husband	Left	HB-LC, HCC	36	1.31	–
12	27	Father-in-law	Left	BA	23	1.40	Biliary stricture
13	31	Husband	Left	HB-LC, HCC	56	1.21	Biliary stricture
14	31	Husband	Left	Re-Tx	17	1.38	–

FAP#: familial amyloidotic polyneuropathy recipient number in Kumamoto University Hospital; PSC = primary sclerosing cholangitis; HB-LC = hepatitis B cirrhosis; PBC = primary biliary cirrhosis; CAPV = congenital absence of portal vein; HCC = hepatocellular carcinoma; BA = biliary atresia; Re-Tx = re-transplantation; Graft/BW = weight of the whole liver graft/recipient's body weight.

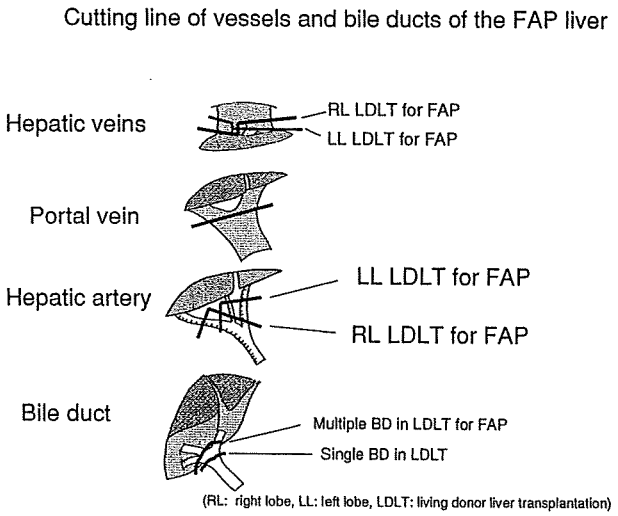

Figure 1: A schematic presentation of the cutting point of the vascular systems of the whole FAP liver graft for domino transplantation. Mild modification of the transection line was performed according to the type and number of vessels or bile ducts of the original LDLT graft for FAP patient.

compatible or identical in all except the last case (#14). Right lobe without middle hepatic vein (MHV) was transplanted in three FAP patients, and left lobe with the MHV in the other five.

Donor operation in the FAP patient (Recovery of the liver in the FAP patient)

The basic policy of the Ethics Committee in our institution concerning Domino LDLT is that the domino procedure should not endanger the safety of the original LDLT of the FAP patient. This means that the recovery of the FAP liver should not radically modify the cutting point of any vessel, thereby avoiding any variations that might make the vascular reconstruction in the LDLT for the FAP patient more dangerous or difficult.

The IVC of the FAP patient was preserved as in a typical LDLT procedure; nonetheless, while respecting the priority of safety for the FAP patient, the transection site of the vessels of the FAP liver was modestly modified for use in the Domino LT (Figure 1). In the case of the hepatic veins, when a right lobe was transplanted to the FAP patient, the stump of the right hepatic vein (RHV) of the FAP patient was left a little longer, but the middle and left hepatic veins were transected as distal as possible so that a longer vascular cuff remained on the FAP liver. On the other hand, when a left lobe was used, the length of the hepatic venous stump was treated in opposite fashion. The short and right-inferior hepatic veins (RIHV) were generally ligated and transected, but if there were large caliber veins (diameter larger than 7 mm), they were preserved for a possible reconstruction in the recipient operation.

If the PV of the partial graft for the FAP patient had multiple orifices, then the PV of the FAP liver was transected distal to the bifurcation of the left and right branch. However, if the PV of the partial liver graft had a single orifice, then the PV of the FAP liver was transected just proximal to the bifurcation (Figure 1).

The HA of the FAP liver was transected distal to the branching of the proper HA in all the cases; however, as in the case of the hepatic vein, the transection site was modified modestly according to the partial liver graft intended for the FAP patient with the aim of assisting the Domino LT while ensuring the safety of the original LDLT (Figure 1).

When multiple biliary orifices were expected in the partial liver graft for the FAP patient, FAP patient's bile duct had to be transected relatively distal in order to secure multiple orifices for duct-to-duct reconstruction. However, in cases with a single bile duct in the partial liver graft, the common hepatic duct of the FAP liver was transected to leave a single orifice (Figure 1). Duct-to-duct reconstruction was preferred for FAP recipients as well as for all other LDLT recipients in our center because it is a safe option with a lower probability of bile leak or life-threatening peritonitis in the immediate post-operative period, than hepatico-jejunostomy.

Neither a veno-venous bypass nor a temporary porto-caval shunt was used because the complete PV clamping time was within 30 min, and it did not represent a risk nor cause circulatory instability in the FAP patient. The explanted FAP liver was perfused with University of Wisconsin solution via the PV and was preserved in the same solution. If the orifices of the middle and the left hepatic veins were separated, they were unified by venoplasty in the back-table before implantation into the domino recipient.

Domino transplantation procedure in the second recipient

Once the total hepatectomy of the domino recipient was performed, the FAP liver was put into the abdominal cavity and vascular reconstruction initiated; reconstruction of the hepatic veins was performed first and then

followed by the PV anastomosis before re-perfusion. Along with the effort to obtain long RHV stumps in the domino recipient, the lateral wall of the IVC around the RHV was dissected for better mobility of the RHV stump. In the first seven cases, reconstruction of the hepatic veins began with the anastomosis of the common trunk of the middle and left hepatic veins (M + LHV) of the FAP liver. The unified M + LHV stump of the graft was anastomosed to the common trunk of its homologue vein in the recipient side in an end-to-end fashion. After completion of this anastomosis, a smaller vascular clamp was applied to the M + LHV and the side clamp of the IVC was released; then the RHV was anastomosed in an end-to-end fashion. Because the anastomosis procedure of the RHV was technically difficult in the first seven cases, the order of the anastomosis of the hepatic veins was inverted in the last case. In all the cases, the PV was anastomosed without difficulty in an end-to-end fashion, after which the graft liver was reperfused before microsurgical reconstruction of the HA. Each whole FAP liver had at least two stumps of the HA; however, if there was sufficient back flow after the anastomosis of one stump, further reconstruction was frequently judged to be unnecessary. For biliary reconstruction, duct-to-duct anastomosis was primarily considered, but if it was not possible, reconstruction using a Roux-en-Y limb was employed. Regardless of the type of biliary reconstruction, an intraluminal external biliary stent was left in each anastomosis. The number of bile duct orifices in the partial liver graft for the FAP patient was one in four cases, and two in the other four cases. In three of the single-orifice cases, a duct-to-duct reconstruction was performed, and in three of the two-orifice cases a hepatico-jejunostomy was performed. In the other of two-orifice cases, each orifice was anastomosed to the recipient's left and right hepatic duct, respectively.

Results

The FAP patients (domino donor)

Operation time of the FAP recipient (domino donor) was 677 ± 133 min (mean ± SD). There was no need for blood transfusion in any patient. Cold ischemia time was less than 2 h in all cases.

In the last FAP case (#14) the patient received an ABO incompatible transplantation; therefore, we also placed an intrahepatic arterial catheter to infuse methylpredonisolone and prostaglandin E1 to prevent the occurrence of ABO-related severe rejection (5).

There were no surgical complications in any of the FAP patients and in the follow-up period of over 8 months, all patients maintain normal liver function and have returned to their active daily life.

Domino recipients

The surgical procedure of most of the Domino LT was technically demanding due to the advanced stages of the recipient's original disease and the relative difficulty of the vessel reconstruction. Cold ischemia time ranged from 481 to 764 min, much longer than in usual LDLT; the reason for this is that the domino recipient operation routinely began after the FAP liver was taken from the FAP patient. The warm ischemia time was also longer than in our usual LDLT series; it was over 60 min in four cases, and this was because the reconstruction of the vessels was somewhat complicated. The FAP liver weighed from 660 to 1100 g and ranged from 1.21% to 2.4% of the recipient's body weight (Table 1).

Except for one case, in all the recipients the RHV and the unified M + LHV of the FAP liver were reconstructed in an end-to-end fashion. In case #5, the FAP liver had two RIHV, and one of them which was very predominant, was anastomosed directly to the wall of the IVC. In case #1, there was also a RIHV of significant size (diameter of 9 mm); however, after the reconstruction of the two major hepatic veins, its reconstruction was discarded and it was closed due to considerable difficulty.

All the FAP livers had only one PV orifice and it was reconstructed to the recipient's PV in an end-to-end fashion. In two cases (case #9 and #14), the PV of the recipient had been occluded by a thrombus. In these two cases, the portal trunk distal to the confluence of the splenic and the superior mesenteric veins had to be replaced by an iliac vein graft taken from the recipient before putting in the graft liver.

All the FAP livers had two or more HA stumps. The middle HA was always ligated. In five of eight cases, two arteries of the graft were reconstructed. There was no case with partial infarction of the graft; however, two of the three one-artery grafts had late biliary stenosis. All but one domino recipient showed very good recovery of their liver function, much better than the average of all the right lobe LDLT cases at our institution. There were no surgical complications in the early period and in CT images taken 1 month after the transplant, none of the livers showed any low-density areas or congested portions of the graft. All the domino recipients were discharged from the hospital alive.

In a follow up of 8 to 40 months, all recipients are still alive. As for long-term complications, three recipients have had biliary stricture. The onset of the strictures ranged from 6 to 8 months, and the anastomosis pattern was a single duct-to-duct anastomosis in two and a hepatico-jejunal anastomosis in one. The two patients with duct-to-duct anastomosis were treated by endoscopic retrograde biliary drainage. The third patient (#12) needed surgical revision of the anastomosis. Including these three cases, current graft function is highly preserved and all the recipients are enjoying active daily lives, except in one case of HCC recurrence.

Discussion

Domino LT has been undertaken in the setting of LDLT for FAP patients in Japan, where the chance of DDLT is quite limited (2). However, livers obtained from LDLT for FAP patients will always lack the retro-hepatic IVC and have multiple vessel and bile duct orifices; due to this reason, previously reported Domino LDLT for FAP have exclusively used partial livers, because either right or left lobes, can be handled just like partial liver grafts in regular LDLT. Evidently, splitting of whole FAP livers taken during LDLT theoretically permits the expansion of the donor pool; however,

during our previous experience in other institutions, two domino recipients who received the smaller left lobe graft after splitting, died post-transplantation (6). The consistently smaller size of whole FAP livers might be one of the reasons for this mortality: FAP patients generally have a smaller body size due to some degree of nutritional or digestive impairment and this is may lead to smaller than average-sized livers. Furthermore, domino recipients are generally selected among candidates with more critical preoperative conditions, so splitting or reduction of an otherwise small liver may result in an inadequate sized graft for a domino recipient in a very poor condition.

The results of the present study on the use of whole liver Domino LT using livers taken during LDLT for FAP patients showed increased technical difficulty during the operation, but good post-operative functional recovery. This benefit might be related to the larger size of the graft. In most Domino LDLT, using only the right lobe after reduction is still the conventional method; however, if there is any concern about the graft volume for the second recipient, the whole liver can be safely used.

The central point of Domino LT is that the procedure should not pose a negative influence on the safety of the original LT, especially in LDLT. Therefore, with safety weighing in favor of the FAP patient, the fact that each hepatic vessel and biliary duct is unevenly shared between the FAP patient and the domino recipient, is a disadvantage that has to be compensated by technical innovations in the reconstruction of the domino recipients. The most difficult part of Domino LT using the whole FAP liver without the retrohepatic vena cava is the reconstruction of the hepatic veins, especially the RHV. The difficulty consists in obtaining a suitable operative field for the reconstruction, because the relatively short hepatic veins are hidden under the liver parenchyma. Extensive mobilization of the caval wall around the RHV of the domino recipient is essential to facilitate reconstruction. The change of the order of reconstruction (first RHV, followed by M + LHV) in the last case made it somewhat easier.

Except in the two cases with preexisting PV thrombosis, reconstruction of the PV presented no additional obstacles.

For the reconstruction of the HA, the presence of relatively short, multiple stumps in the whole FAP liver, added difficulty to the reconstruction. Kubota et al. has reported a simple back-table procedure justifying single HA reconstruction (7), but our group prefers to wait after the reconstruction of the dominant HA, before deciding if a second anastomosis is necessary. Unfortunately, two of three cases with single HA reconstructions were complicated with late biliary stenosis of the anastomosis site. A comparatively reduced blood supply might be related to the occurrence of this complication. From this preliminary data of our series we consider that bilateral HA anastomosis should be recommended as the standard procedure in whole liver Domino LT.

The incidence of biliary stricture (3/8 cases) was higher than in our entire series of LDLT, and it was also higher than that of other larger series of right lobe LDLT (8). It is possible that the longer length and deep dissection around the biliary stumps, as well as a hemilateral HA anastomosis may have contributed to the biliary stricture seen in these three cases. Therefore, in addition to the bilateral reconstruction of HA, a separate anastomosis of the left and right hepatic ducts, with shorter stumps might be helpful to prevent late strictures. The incidence of this complication was not apparently influenced by the original disease or conditions of the domino recipients.

From the present experience, although the whole liver domino LDLT is technically demanding, any contraindication about the anatomical variations or donor–recipient matching could not be suspected.

In conclusion, our preliminary results show that whole liver Domino LT without the retro-hepatic IVC is effective and safe without compromising the safety of the LDLT for FAP patients.

Acknowledgement

This work was supported in part by 'Grants-in-aid for Scientific Research C' from the Japan Society for the Promotion of Science (JSPS) and by Health Science Research Grants from the Ministry of Health, Labor and Welfare of Japan.

References

1. Monterio E, Perdigoto R, Furtado AL. Liver transplantation for familial amyloid polyneuropathy. Hepatogastroenteroly 1998; 45: 1375–1380.
2. Nishizaki T, Kishikawa Y, Yoshizumi T et al. Domino liver transplantation from a living related donor. Transplantation 2000; 70: 1236–1239.
3. Hashikura Y, Ikegami T, Nakazawa Y et al. Domino liver transplantation in living donors. Transplant Proc 2005; 37: 1076–1078.
4. Kiuchi T, Kasahara M, Uryuhara K et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation 1999; 67: 321–327.
5. Yoshizawa A, Sakamoto S, Ogawa K et al. New protocol of immunosuppression for liver transplantation across ABO barrier: The use of Rituximab, hepatic arterial infusion, and preservation of spleen. Transplant Proc 2005; 37: 1718–1719.
6. Inomata Y, Nakamura T, Uemoto S et al. Domino split-liver transplantation from a living donor: Case reports of in situ and ex situ splitting. Liver Transplant 2001; 7: 150–153.
7. Kubota K, Makuuchi M, Takayama T et al. Simple test on the back table for justifying single hepatic-arterial reconstruction in living related liver transplantation. Transplantation 2000; 70: 696–697.
8. Kasahara M, Egawa H, Takada Y et al. Biliary reconstruction in right lobe living-donor liver transplantation: Comparison of different techniques in 321 recipients. Ann Surg 2006; 243: 559–566.

ORIGINAL ARTICLE

Biliary Reconstruction for Infantile Living Donor Liver Transplantation: Roux-en-Y Hepaticojejunostomy or Duct-to-Duct Choledochocholedochostomy?

Yasumasa Shirouzu, Hideaki Okajima, Satsuki Ogata, Yuki Ohya, Yukika Tsukamoto, Hidekazu Yamamoto, Takayuki Takeichi, Lee Kwang-Jong, Katsuhiro Asonuma, and Yukihiro Inomata
Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University, Kumamoto, Japan

Hepaticojejunostomy is a standard biliary reconstruction method for infantile living donor liver transplantation (LDLT), but choledochocholedochostomy for infants is not generally accepted yet. Ten pediatric recipients weighing no more than 10 kg underwent duct-to-duct choledochocholedochostomy (DD) for biliary reconstruction for LDLT. Patients were followed up for a median period of 26.8 months (range: 4.0-79.0 months). The incidence of posttransplant biliary complications for DD was compared with that for Roux-en-Y hepaticojejunostomy (RY). No DD patients and 1 RY patient (5%) developed biliary leakage ($P > 0.05$), and biliary stricture occurred in 1 DD patient (10%) and none of the RY patients ($P > 0.05$); none of the DD patients and 5 RY patients (25%) suffered from uncomplicated cholangitis after LDLT ($P > 0.05$), and 1 DD patient (10%) and 2 RY patients (10%) died of causes unrelated to biliary complications. In conclusion, both hepaticojejunostomy and choledochocholedochostomy resulted in satisfactory outcome in terms of biliary complications, including leakage and stricture, for recipients weighing no more than 10 kg. *Liver Transpl 14:1761-1765, 2008.* © 2008 AASLD.

Received April 8, 2008; accepted June 26, 2008.

Liver transplantation is an established curative therapy for children with end-stage chronic liver disease or acute liver failure. Outcomes following liver transplantation for children have significantly improved over the past 2 decades because of advances in surgical procedures, preservation technology, immunosuppressants, and perioperative management.[1]

However, despite refinements in surgical techniques for living donor liver transplantation (LDLT), biliary complications are still associated with significant morbidity and mortality.[2] Duct-to-duct choledochocholedochostomy (DD) and Roux-en-Y hepaticojejunostomy (RY) are now generally accepted procedures for biliary reconstruction in adult-to-adult LDLT.[3,4] However, RY has remained the standard method for pediatric LDLT because of the dominance of biliary atresia and technical difficulties related to the size and fragility of recipients' bile ducts. Only a few reports can be found in the literature on pediatric LDLT using DD,[5,6] and to the best of our knowledge, there have been no studies focused on DD for infantile LDLT. This is therefore the first report to investigate the viability of DD in LDLT for infants weighing no more than 10 kg.

PATIENTS AND METHODS

Patients

Between February 2001 and January 2008, 57 pediatric patients (less than 15 years old) underwent 60 LDLTs at Kumamoto University Hospital. Thirty-four of these pediatric recipients (59.6%) weighed no more

Abbreviations: CIT, cold ischemic time; DD, duct-to-duct choledochocholedochostomy; GRWR, graft-to-recipient weight ratio; LDLT, living donor liver transplantation; RY, Roux-en-Y hepaticojejunostomy; WIT, warm ischemic time.

Address reprint requests to Yasumasa Shirouzu, M.D., Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University, 1-1-1 Honjyo, Kumamoto, 860-8556, Japan. Telephone: 81-96-373-5616; FAX: 81-96-373-5616; E-mail: shirouzu@fc.kuh.kumamoto-u.ac.jp

DOI 10.1002/lt.21599
Published online in Wiley InterScience (www.interscience.wiley.com).

TABLE 1. Demographic Data for the Recipients

	DD (n = 10)
Age (months)	12.2 ± 9.9 (3–33)
Sex (male/female)	7/3
Body weight (kg)	7.3 ± 2.4 (3.3–10)
Diagnosis	
Fulminant hepatic failure	4
Byler	2
Methylmalonic acidemia	2
Cryptogenic cirrhosis	1
Hepatoblastoma	1
Blood combination	
Identical	7
Compatible	0
Incompatible	3
Follow-up (months)	26.8 ± 27.5 (4.0–79.0)

Abbreviation: DD, duct-to-duct choledochocholedochostomy.

TABLE 2. Demographic Data for the Operations

	DD (n = 10)
Operation time (minutes)	494.2 ± 71.8 (401–600)
CIT (minutes)	56.3 ± 40.5 (19–147)
WIT (minutes)	40.0 ± 6.7 (33–52)
Blood loss (g)	250.6 ± 259.9 (35–915)
Graft type	
Lateral	5
Monosegment	2
Reduced monosegment	3
Graft weight (g)	221.8 ± 46.4 (115–265)
GRWR (%)	3.2 ± 0.9 (2.2–5.2)

Abbreviations: CIT, cold ischemic time; DD, duct-to-duct choledochocholedochostomy; GRWR, graft-to-recipient weight ratio; WIT, warm ischemic time.

than 10 kg at the time of LDLT. Ten of them who were treated with DD and survived more than 1 month after LDLT were included in this study. Their demographic data are shown in Table 1. The subjects were followed up until May 2008 for a median period of 26.8 months (range: 4.0-79.0 months). Three ABO-incompatible transplants were included in this group.

Operations

Operative details are summarized in Table 2. All patients received grafts from either their mother or father. In principle, the left-lateral segment was used as the graft, and the reduction of this graft was considered when the graft-to-recipient weight ratio estimated by preoperative computed tomography volumetry was larger than 4%.

Every donor candidate underwent preoperative drip infusion cholecystocholangiography/computed tomography. Intraoperative cholangiography was performed throughout the hilar dissection for better visualization of the biliary anatomy. Two small vascular clips were placed on the left hepatic duct around 5 mm distant from the bifurcation to facilitate identification of the optimal site for transection of the left hepatic duct. The left hepatic duct was sharply transected at the planned site after it was determined that there were no right posterior branches originating from the more proximal left hepatic duct. Hepatic resection of the donor was performed with an ultrasonic dissector and bipolar electrical cautery without inflow occlusion. One of the left-lateral segments, the monosegment or the reduced monosegment, was used as the graft; the size was chosen according to the graft-to-recipient weight ratio estimation. The graft liver was removed after vascular clamping, and this was followed by in situ perfusion with University of Wisconsin solution (ViaSpan, Bristol-Myers Squibb Co., New York, NY) or histidine-tryptophan-ketoglutarate solution (Custodiol, Odyssey Pharmaceuticals, Inc., East Hanover, NJ) through the left portal vein.

In recipients with an intact extrahepatic biliary tract, the bile duct was usually divided above the bifurcation. Pericholangic connective tissue was preserved as much as possible to maintain an adequate blood supply to the bile duct. However, adequate mobilization was also performed to reduce the tension at the anastomotic site. The liver graft was implanted in an orthotopic manner following total hepatectomy, with the inferior vena cava in the recipient being preserved. The hepatic artery was then anastomosed with a microsurgical technique. Before biliary reconstruction, the blood supply of the stump of the recipient's bile duct was confirmed by monitoring of the bleeding at the stump. Minute interrupted suturing was performed with 6-0 absorbable sutures (polydioxanone; Ethicon, Somerville, NJ). After completion of the suture of the posterior row, an external transanastomotic stent tube (4-French pancreatic duct tube; Sumitomo Bakelite, Tokyo, Japan) was placed and anchored with an absorbable stitch at the edge of the anterior wall of the graft hepatic duct. The tube was then passed through the remnant cystic duct or the common bile duct. After completion of the anterior wall, surrounding tissue of the recipient's bile duct wall was anchored to the hilar plate of the graft liver with 4 to 6 absorbable stitches in order to reduce the tension of the anastomosis (Fig. 1).

Immunosuppression

Immunosuppression was performed with tacrolimus and low-dose steroids. Target trough levels of tacrolimus were 12 to 15 ng/mL for the first 2 weeks, approximately 10 to 12 ng/mL for the following 2 weeks, and 5 to 10 ng/mL thereafter. Steroid treatment was initiated with an injection of 10 mg/kg methylprednisolone prior to graft reperfusion during surgery, and it was usually

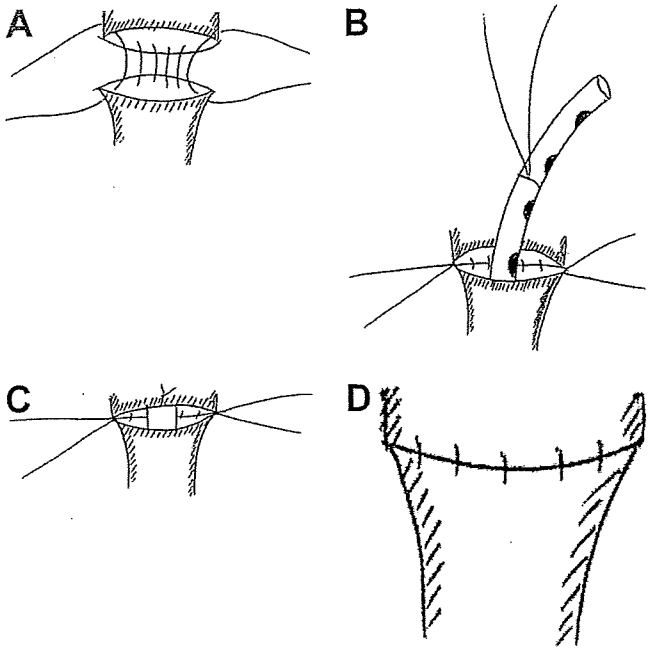

Figure 1. (A) A stay suture was placed at both ends of the anastomotic orifice. Minute interrupted suturing of the posterior row was performed. (B) An external transanastomotic stent tube was then passed through the remnant cystic duct or the common bile duct. (C) The stent was inserted into the intrahepatic duct and anchored with an absorbable stitch at the anterior wall of the stump of the graft hepatic duct. (D) Finally, the interrupted suture of the anterior wall was completed.

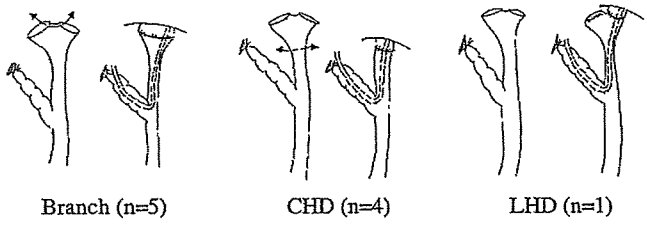

Figure 2. Three types of orifices of the bile duct were prepared for the duct-to-duct choledochocholedochostomy recipients. Branch was used in 5 cases, CHD was used in 4 cases, and LHD was used in 1 case.

tapered off until complete withdrawal around 3 to 6 months after the transplant.

Study Design and Outcome Parameters

Medical records of the LDLTs of the 10 patients who weighed no more than 10 kg and were treated with DD were reviewed, and the perioperative characteristics and the method of biliary reconstruction were examined. Posttransplant biliary complications in the DD recipients were compared with those in the 20 recipients who weighed no more than 10 kg and were treated with RY.

Biliary leakage was diagnosed by cholangiography using the external stent tube when a yellowish discharge with a higher bilirubin level than that in serum was obtained from the abdominal drain. Postoperative cholangiography using the external stent tube was routinely carried out for an early diagnosis of any biliary complication 1 month after LDLT. When liver function test results suggested biliary stricture and/or ultrasonography and computed tomography scanning showed a dilated bile duct, either percutaneous transhepatic cholangiography or endoscopic retrograde cholangiopancreatography was conducted. Posttransplant uncomplicated cholangitis was defined as fever accompanied by an elevation of the serum level of C reactive protein and/or leukocytosis in addition to the liver function test showing a cholestatic pattern without echographic evidence of biliary tract dilatation.[7]

TABLE 3. Biliary Complications

	DD (n = 10)	RY (n = 20)	*P*
Leakage	0	1 (5%)	0.472
Stricture	1 (10%)	0	0.1503
Uncomplicated cholangitis	0	5 (25%)	0.0833

Abbreviations: DD, duct-to-duct choledochocholedochostomy; RY, Roux-en-Y hepaticojcjunostomy.

Statistics

Data were expressed as means ± standard deviation. Fisher's exact test, unpaired *t* tests, and the log-rank test were used for statistical analysis. *P* values less than 0.05 were regarded as significant.

RESULTS

There were no multiple bile ducts of the graft liver. Figure 2 shows the biliary reconstruction in DD. Three different types of orifices of the recipient's bile duct were used. *Branch* corresponded to the anastomosis of the graft hepatic duct to the bifurcation of the right and left branches of the recipient's hepatic duct; it was most frequently employed to reconstruct the biliary tract in small infants. Common hepatic duct (*CHD*) involved the end-to-end anastomosis between the graft hepatic duct and the recipient common hepatic duct, and left hepatic duct (*LHD*) was the anastomosis between the graft hepatic duct and the recipient left hepatic duct. CHD was used in 4 recipients, and LHD was used in only 1 recipient.

The biliary stent tube was left in place for a median period of 121.8 (94-155) days even if the postoperative cholangiogram showed no evidence of biliary leakage or stricture during postoperative month 1. Meanwhile, the stent tube in the RY group was pulled out on mean postoperative day 114.5 (86-175; versus DD, $P > 0.05$).

The occurrence of biliary complications is shown in Table 3. Leakage developed in only 1 RY patient, who produced a peritoneal discharge with a high amylase level (11,392 U/L) and a high total bilirubin level (49.4 mg/dL) on postoperative day 15, and a diversion enter-

ostomy of the Roux-en-Y limb was made to isolate the biliary anastomosis from the regurgitation of intestinal juice. Biliary stricture occurred in only 1 DD patient. A cholangiogram performed in postoperative month 6 showed complete obstruction of the graft bile duct proximal to the biliary anastomosis, and the patient underwent revision of hepaticojejunostomy 250 days after LDLT following percutaneous transhepatic cholangiodrainage. The area of fibrotic obstruction of the graft hepatic duct was located proximally to the anastomosis in the graft liver, and it seemed to be caused by poor blood supply to the graft hepatic duct. This patient is doing well 1.3 years after LDLT. Five of the RY patients suffered 10 episodes of uncomplicated cholangitis lasting from 4 to 27 months after LDLT, but none of the DD patients did ($P > 0.05$). The 5 RY cases were all successfully treated with antibiotics, but 2 of them suffered such episodes more than twice.

One DD patient died of recurrence of hepatoblastoma, and 2 RY patients died of respiratory failure following severe pneumonia. Biliary complications were not related to the mortality of the infants weighing no more than 10 kg in this series.

DISCUSSION

We employed DD for biliary reconstruction, even for pediatric LDLT in principle, when the recipient's common bile duct was available. We have previously reported the results of duct-to-duct reconstruction for LDLT for pediatric patients, including infants.[8] The present report is a small (but so far the largest) retrospective study of the viability of the duct-to-duct technique for infantile LDLT. Our findings demonstrate that DD can produce a satisfactory outcome comparable to that for hepaticojejunostomy in biliary reconstruction.

We employed 3 DD types for small infants. We believe that using the bifurcation of the right and left branches of the recipient's hepatic duct for biliary anastomosis is the best method to facilitate acceptance of the larger graft bile duct of the adult donor. The common hepatic duct was chosen as the anastomotic orifice when the caliber of the graft left hepatic duct was relatively small. When the graft hepatic duct was smaller and/or the anastomosis to the recipient's common hepatic duct might generate much tension, the left hepatic duct was chosen as the recipient's anastomotic site. The small caliber of the pediatric bile duct did not interfere with our performance of duct-to-duct anastomosis.

An external biliary stent tube was placed in all pediatric recipients enrolled in this study. Use of the anastomotic stent is somewhat controversial. Marcos et al.[9] reported that biliary complications occurred in 33% of patients without stents versus 4% of those with stents for adult-to-adult LDLT with RY. Furthermore, the incidence of biliary complications in duct-to-duct anastomosis has also been reported to be significantly lower with a stent tube used as a splint for the anastomosis than with only a drainage tube without stenting.[10] On the other hand, Lin et al.[11] reported that whether or not a T-tube was used for choledochocholedochostomy after liver transplantation had no effect on biliary complications. Because the stent tube sometimes occupies the small lumen of the infant hepatic duct, some infants who underwent DD suffered from acholic stool until the tube was pulled out. However, liver function test results for every case in our series showed excellent recovery from LDLT (data not shown).

The reported incidence of biliary complications, including leakage and stricture, varies from 14% to 38% for pediatric liver transplantation.[12-14] Biliary reconstruction procedures employed by us produced a good outcome for infantile LDLT, with an incidence of biliary complications of 10%. The low incidence of anastomotic leakage may be partially due to anchoring the recipient's bile duct to the graft hilar plate to reduce the tension on the anastomosis. The extrahepatic ducts are surrounded by a vascular plexus, which is composed of branches arising directly from the right and left hepatic arteries.[15] ABO-incompatible liver transplantation has an increased risk of hepatic artery thrombosis and diffuse biliary stricture caused by a primary antibody-mediated injury to the bile duct epithelium or ischemia following endothelial injury of the hepatic artery.[16] Three infants received ABO-incompatible grafts in the present study. However, LDLT can be carried out with relative safety during infancy before the onset of isohemagglutinin production.[17] None of the recipients in our study weighing no more than 10 kg developed hepatic artery complications. Also, postoperative cholangiogram using the external stent tube showed no abnormal findings such as diffuse biliary strictures in the infantile recipients, although it was not after a long term. A stricture of the graft hepatic duct localized proximally to the anastomotic site developed in only 1 DD patient who had received an ABO-identical graft because shortening of the donor extrahepatic duct segment seemed to improve circulatory status around the biliary anastomosis. Moreover, a special feature of our technique, that is, harvesting the lateral segment graft while leaving as much peribiliary tissue as possible on the stump of the graft hepatic duct, may have contributed to the low incidence of biliary complications for infantile LDLT using not only hepaticojejunostomy but also DD. However, additional and longer follow-up is needed to evaluate late biliary complications, including biliary stricture, in DD.

Cholangitis is a common complication in the early period following liver transplantation and sometimes recurs even years after surgery.[18,19] In the case of hepaticojejunostomy, the sphincteric mechanism that prevents reflux of intestinal contents is bypassed, and the reflux of intestinal contents is an important pathogenic mechanism.[20,21] In fact, posttransplant cholangitis was usual in our RY cases, although it was successfully treated with antibiotics. On the other hand, there were no episodes of cholangitis for DD with the sphincteric mechanism kept intact.

In conclusion, our surgical technique using DD in recipients weighing no more than 10 kg produced excellent outcomes with a low incidence of biliary complications, including leakage and stricture. DD may be-

come the standard method for biliary reconstruction even in infantile LDLT in the future if the recipient's common bile duct is available.

REFERENCES

1. Ferreira CT, Vieira SM, Silveira TR. Liver transplantation. J Pediatr 2000;76:S198–S208.
2. Egawa H, Inomata Y, Uemoto S, Asonuma K, Kiuchi T, Fujita S, et al. Biliary anastomotic complications in 400 living related liver transplantations. World J Surg 2001; 25:1300-1307.
3. Sugawara Y, Makuuchi M, Sano K, Ohkubo T, Kaneko J, Takayama T. Duct-to-duct biliary reconstruction in living-related liver transplantation. Transplantation 2002;73: 1348-1350.
4. Soejima Y, Shimada M, Suehiro T, Kishikawa K, Minagawa R, Hiroshige S, et al. Feasibility of duct-to-duct biliary reconstruction in left-lobe adult-living-donor liver transplantation. Transplantation 2003;75:557-559.
5. Kimura T, Hasegawa T, Ihara Y, Nara K, Sasaki T, Dono K, et al. Feasibility of duct-to-duct biliary reconstruction in pediatric living related liver transplantation: report of three cases. Pediatr Transplant 2006;10:248-251.
6. Haberal M, Sevmis S, Emiroglu R, Karakayali H, Arslan G. Duct-to-duct biliary reconstruction in pediatric liver transplantation: one center's results. Transplant Proc 2007;39:1161-1163.
7. Lopez-Santamaria M, Martinez L, Hierro L, Gamez M, Murcia J, Camarena C, et al. Late biliary complications in pediatric liver transplantation. Pediatr Surg 1999;34:316-320.
8. Okajima H, Inomata Y, Asonuma K, Ueno M, Ishiko T, Takeichi T, et al. Duct-to-duct biliary reconstruction in pediatric living donor liver transplantation. Pediatr Transplant 2005;9:531-533.
9. Marcos A, Ham JM, Fisher RA, Olzinski AT, Posner MP. Surgical management of anatomical variations of the right lobe in living donor liver transplantation. Ann Surg 2000; 231:824-831.
10. Ishiko T, Egawa H, Kasahara M, Nakamura T, Oike F, Kaihara S, et al. Duct-to-duct biliary reconstruction in living donor liver transplantation utilizing right lobe graft. Ann Surg 2002;236:235-240.
11. Lin CH, Yu JC, Chen TW, Chuang CH, Tsai YC, Chen SY, Hsieh CB. The experience of biliary tract complications after liver transplantation. Transplant Proc 2007;39: 3251-3256.
12. Letourneau JG, Hunter DW, Ascher NL, Roberts JP, Payne WD, Thompson WM, et al. Biliary complications after liver transplantation in children. Radiology 1989;170:1095-1099.
13. Egawa H, Uemoto S, Inomata Y, Shapiro AM, Asonuma K, Kiuchi T, et al. Biliary complications in pediatric living related liver transplantation. Surgery 1998;124:901-910.
14. Kling K, Lau H, Colombani P. Biliary complications of living related pediatric liver transplant patients. Pediatr Transplant 2004;8:178-184.
15. Stapleton GN, Hickman R, Terblanche J. Blood supply of the right and left hepatic ducts. Br J Surg 1998;85:202-207.
16. Sanchez-Urdazpal L, Batts KP, Gores GJ, Moore SB, Sterioff S, Wiesner RH, Krom RA. Increased bile duct complications in liver transplantation across the ABO barrier. Ann Surg 1993;218:152-158.
17. Egawa H, Oike F, Buhler L, Shapiro AM, Minamiguchi S, Haga H, et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. Transplantation 2004;77:403-411.
18. Vrochides D, Fischer SA, Soares G, Morrissey PE. Successful treatment of recurrent cholangitis complicating liver transplantation by Roux-en-Y limb lengthening. Transpl Infect Dis 2007;9:327-331.
19. Orlando G, Blairvacq JS, Otte JB, Goffette P, Ciccarelli O, Sempoux C, Lerut J. Successful treatment of recurrent cholangitis after adult liver transplantation with a Tsuchida antireflux valve. Transplantation 2004;77: 1307-1308.
20. Sung JY, Costerton JW, Shaffer EA. Defense system in the biliary tract against bacterial infection. Dig Dis Sci 1992; 37:689-696.
21. Chuang JH, Lee SY, Chen WJ, Hsieh CS, Chang NK, Lo SK. Changes in bacterial concentration in the liver correlate with that in the hepaticojejunostomy after bile duct reconstruction: implication in the pathogenesis of postoperative cholangitis. World J Surg 2001;25:1512-1518.

Journal of Pediatric Surgery (2009) 44, E15–E18

ELSEVIER

Journal of
Pediatric
Surgery

www.elsevier.com/locate/jpedsurg

Repair of huge incisional hernias intentionally made during infantile living donor liver transplantation

Takahiro Murokawa*, Yukihiro Inomata, Katsuhiro Asonuma, Satsuki Ogata, Yukika Tsukamoto, Lee Kwang-Jong, Takayuki Takeichi, Hidekazu Yamamoto, Hideaki Okajima

Department of Pediatric Surgery and Transplantation, School of Medicine, Kumamoto University, Kumamoto 860-8556, Japan

Received 17 October 2008; accepted 20 November 2008

Key words:
Repair;
Incisional hernia;
Infant;
Living donor liver;
Transplantation;
Large-for-size graft

Abstract

Background and Purpose: In some small infants who are transplanted with a large-for-size graft by living donor liver transplantation, an incisional hernia is intentionally made to decrease the tension on the graft. The procedure and timing for repair of this type of hernia were retrospectively evaluated.

Patients and Methods: Repair was carried out in 3 patients at 4 to 11 years after living donor liver transplantation. The preoperative, perioperative, and postoperative statuses were analyzed in each patient.

Result: Fascial closure was possible in all 3 patients. In 2 patients, separation of a component of the rectus sheath or a lower part of the major pectoral muscle was required for approximation of the fascia. One recipient had transient bile leakage that was treated successfully.

Conclusion: An intentionally made hernia should be as small as possible to facilitate easy primary closure at a later date. A procedure resembling the "clam-shell opening" method, which used a partly separated and extended sheet of the fascia, was feasible to avoid the requirement for an artificial mesh. Preschool ages may be suitable for easier approximation of the fascia.

The problems associated with size-mismatched large grafts include the inability to accommodate the graft in the small abdominal domain of the recipient and the inability to provide sufficient blood supply to the liver graft [1]. In the setting of pediatric living donor liver transplantation (LDLT), the size of the graft can generally be adjusted according to the size of the recipient. However, large-for-size grafts cannot be avoided in some cases, especially with small infants. Primary closure of the laparotomy wound is sometimes difficult and harmful. Skin closure without fascial approximation is a simple and conventional method for such cases. However, if the wound is not repaired during the immediate posttransplant period, the residual ventral hernia becomes more prominent as the patient grows up and may interfere with the patient's active normal life. Detailed repair procedures for this type of ventral hernia without use of prosthetic materials at a long time after LDLT have not yet been described. In this

* Corresponding author. Tel.: +81 96 373 5616; fax: +81 96 373 5783.
E-mail address: takmuro@gmail.com (T. Murokawa).

0022-3468/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.jpedsurg.2008.11.048

Table 1 Patient characteristics

	Case 1	Case 2	Case 3
Primary diseases	FHF	Biliary atresia	Hemochromatosis
Age at LDLT	5 mo	6 mo	43 d
Body weight at LDLT (g)	5000	5540	4056
Graft segment	Segment 3	Segment 2 + 3	Segment 2 + 3
Weight of graft (g)	260	310	210
GRWR (%)	5.2	5.6	5.2
Age at hernial repair (y)	5	4	11
Height (cm)/body weight (kg) at repair	106/12.8	105/19.3	149/36.5
Maximum diameter of fascial defect (cm)	9.8	13	12

FHF indicates fulminant hepatic failure; GRWR, graft weight/recipient body weight ratio.

article, 3 cases are presented to evaluate the feasibility of the repair procedures.

1. Patients

Three patients underwent repair of a ventral hernia that had been intentionally made during LDLT in their infantile period. The primary diseases comprised fulminant hepatic failure of unknown origin, biliary atresia, and neonatal hemochromatosis (Table 1). The patients underwent LDLT from 43 days to 6 months of age, and their body weights at the time of LDLT ranged from 4 to 5.5 kg. The grafts at the LDLT were a monosegment (segment 2) in 1 case and a left lateral segment in the other 2 cases. The graft weight/recipient body weight ratios ranged from 5.2% to 5.6%. The operative procedures are described below on a case-by-case basis.

2. Case 1

The repair was carried out at 5 years after the original LDLT. In this case, the original graft was a monosegment, and the laparotomy wound had been partly closed leaving the central part of the inverted T-shaped incision. A relaparotomy was performed using the full length of the operative scar. The liver graft protruded as the hernia content and adhered just under the skin.

The adhesion was released meticulously. Dissection of the skin layer, including the subcutaneous tissue from the fascial layer, was extended sufficiently widely to the area peripheral to the hernia. After this dissection, approximation of the fascia was primarily possible without too much tension. Although she was complicated with pulmonary atelectasis, she was discharged from the hospital at 2 weeks after the repair without any graft dysfunction. There has been no recurrence of the hernia during follow-up for 3 years.

3. Case 2

The repair was carried out at 4 years after the original LDLT. The laparotomy at the initial LDLT was only closed by skin for the whole length of the wound. Wide dissection of the skin layer alone, as performed in case 1, was not sufficient for primary closure of the fascia because the fascial end was far separated and the peritoneal domain had not

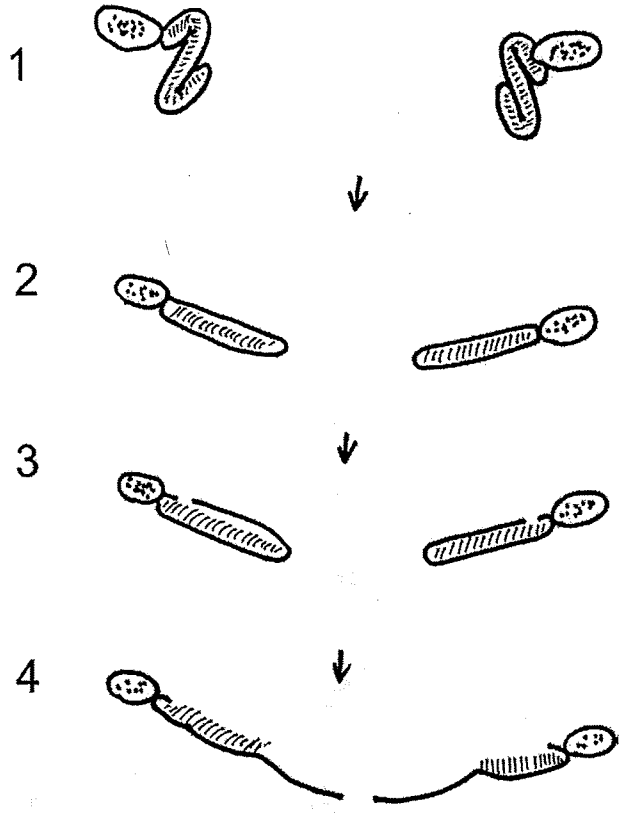

Fig. 1 Clam-shell opening method for extension of the fascial sheet using the anterior rectus sheath. The shrunken rectus muscle is stretched (1 and 2). The anterior sheath is then incised along the lateral border (3). The anterior sheath on both sides is reflected (4) to cover the hernial defect.

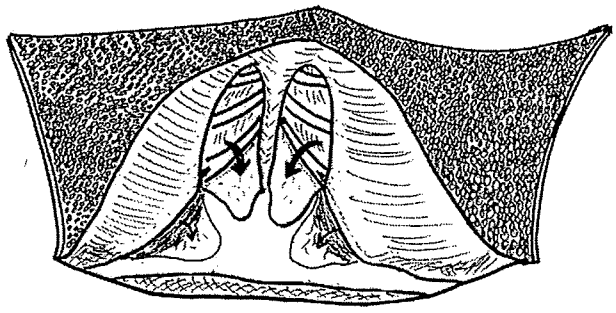

Fig. 2 Use of part of the bilateral major pectoral muscle to cover the cranial end of a hernial defect (large arrows). In this case (case 3), the clam-shell opening method (small arrows) is not sufficient for complete coverage of the hernial defect.

expanded much during the long interval from the original LDLT. After dissection of the skin layer, both sides of the epigastric anterior rectus sheath were longitudinally incised along the lateral border and reflected to the medial direction (Fig. 1). This "clam-shell opening" method enabled primary closure of the hernial defect. There has been no recurrence of the hernia during follow-up for 2 years.

4. Case 3

The repair was carried out at 11 years after the original LDLT was performed for neonatal hemochromatosis at 43 days of age. The graft was a whole left lateral segment because the innovation of graft size reduction had not yet been applied at the time of the original LDLT. The maximum diameter of the fascial defect was 12 cm. The abdominal rectus muscle was very atrophic, especially at the cranial part, and had shrunk around the costal arch. The same procedure used for the case 2 was applied, but it was not sufficient to obtain full coverage of the hernia. At the cranial portion of the midline wound, the sternocostal part of the major pectoral muscle was transected and reflected as a complementary sheet (Fig. 2). After this elongation of the fascial sheet, primary closure was possible without tension on the graft.

Although bile leakage was caused by capsular injury to the liver graft during the dissection, it was successfully treated by drainage. The appearance of the abdomen improved satisfactorily, and there has been no recurrence of the hernia during follow-up for 1 year (Fig. 3).

5. Discussion

Temporary abdominal closure using prosthetic materials has been reported in pediatric liver transplantation in infants with size-mismatched grafts [2-7]. Although the reported incidence is not very high, such artificial materials are associated with the risk of infectious complications under the immunosuppression status after transplantation. Delayed primary closure requires repeated operations under the relatively unstable status in the immediate postoperative period [5]. Other disadvantages include leakage of intraperitoneal fluid, interference with postoperative ultrasonic studies, and the necessity for meticulous postoperative wound care and repeated surgery until the final closure [2]. Although a bioengineered skin equivalent has been reported for management of difficult abdominal skin defects after multivisceral transplantation in the pediatric generation, it is not available in all institutions [8]. Use of dermal homografts has also been reported as a tool for wound closure, but this is not possible in LDLT [9]. Skin closure leaving an incisional ventral hernia is an alternative method for wound closure in cases with large-for-size grafts. The possible risks associated

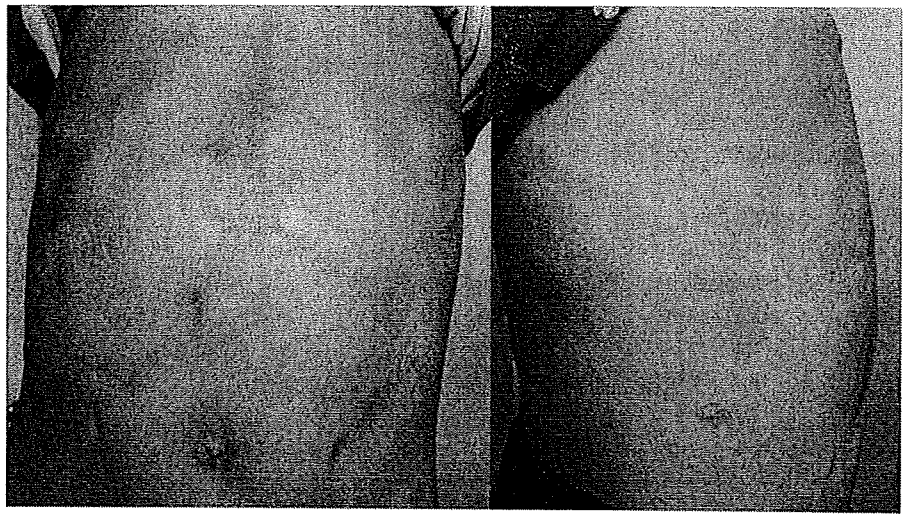

Fig. 3 Postoperative appearance of the abdomen in case 3. The external appearance was a prominent hernia through the fascial defect. The hernia is normalized after the repair.

with the use of prosthetic materials can be avoided with this skin closure technique. The major disadvantage of this technique is the cosmetic appearance. Prominent protrusion of the liver graft or abdominal viscera through the hernia can sometimes disturb the quality of life of the recipients after they grow up. Procedures for repair of congenital giant omphaloceles have been introduced in the field of ordinary pediatric surgery, but techniques for later repair of skin closure after LDLT have not yet been described.

How and when should such huge ventral hernias after LDLT be repaired? Among the 3 cases presented here, only one was able to undergo simple closure without any elongation technique for the fascia. The peripheral portion of the laparotomy wound in case 1 had been closed using the fascia, and only the central part was left as the hernia. This partial closure was definitely a favorable factor for facilitating easier closure during later repair. In the other 2 cases, we chose not to use prosthetic materials for repair of the ventral hernias because of the possible risk of infection under immunosuppression. Instead, elongation of the fascial sheet using the anterior rectus sheath or part of the major pectoral muscle was applied. Although this is technically a little complicated and has some possibility of mild surgical complications, the technique was successful in accomplishing primary closure in cases 2 and 3. The elongation technique, which can be referred to as a clam-shell opening procedure, was very useful because the rectus muscle had become atrophic and could not be stretched without such a procedure. Van Eijck et al [10] reported a procedure called the "abdominal component separation" technique for secondary repair of giant omphaloceles. They incised the aponeurosis of the external oblique muscle along the rectus muscle to facilitate expansion of the oblique muscle layer. This technique may be difficult to use after LDLT because the wound after LDLT is an inverse T-shape and not round as in the case of an omphalocele. Repair of an incisional hernia using a polypropylene mesh after liver transplantation has also been reported, but the procedure could not avoid low rates of bowel injury, infection, and recurrence [11].

In the present series, the timing of the repair ranged from 4 to 11 years after the original LDLT. In cases without partial closure of the fascia at the original LDLT, the abdominal capacity would not increase very much even as the patients grow older and the abdominal wall would become less flexible as the recipients reach school age. In addition, interference with the patients' quality of life becomes more serious during school life. Therefore, based on our limited number of cases, we consider that the best timing for hernia repair after LDLT should be before the patient reaches school age.

In conclusion, fascial elongation, referred to as a clam-shell opening method, using the reflected anterior rectus sheath or pectoral major muscle was feasible for primary closure of a huge ventral hernia intentionally made at the time of infantile LDLT. The repair should be done before the patient reaches school age for easier primary closure.

References

[1] Kiuchi T, Kasahara M, Uryuhara K, et al. Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation 1999;67(2):321-7.

[2] Ong TH, Strong R, Zahari Z, et al. The management of difficult abdominal closure after pediatric liver transplantation. J Pediatr Surg 1996;31(2):295-6.

[3] Shun A, Thompson JF, Dorney SFA, et al. Temporary wound closure with expanded polytetrafluoroethlene in pediatric liver transplantation. Clin Tranpl 1992;6:315-7.

[4] Machens HG, Ringe B, Zimmer G, et al. A new procedure for abdominal wound closure after pediatric liver transplantation: the "sand-witch" technique. Surgery 1994;115:255-6.

[5] Soin AS, Friend PJ, Noble-Jamieson G, et al. Successful use of size mismatched liver allografts in children by delayed primary closure of the abdominal wall. Br J Surg 1996;83:1530-1.

[6] de Ville de Goyet J, Struye de Swielande Y, Reding R, et al. Delayed primary closure of the abdominal wall after cadaveric and living donor liver graft transplantation in children: a safe and useful technique. Transplant Int 1998;11:117-22.

[7] Jones WT, Ratner I, Abrahamian G, et al. Use of a silastic silo for closure of the abdominal wall in a pediatric patient receiving a cadaveric split liver. J Pediatr Surg 2003;38:E20-2.

[8] Drosou A, Kirsner RS, Kato T, et al. Use of a bioengineered skin equivalent for the management of difficult skin defects after pediatric multivisceral transplantation. J Am Acad Dermatol 2005; 52:854-8.

[9] Charles CA, Kato T, Tzakis AG, et al. Use of a living dermal equivalent for a refractory abdominal defect after pediatric multivisceral transplantation. Dermatol Surg 2004;30:1236-40.

[10] van Eijck FC, de Blaauw I, Bleichrodt RP, et al. Closure of giant omphaloceles by the abdominal wall component separation technique in infants. J Pediatr Surg 2008;43:246-50.

[11] Müller V, Lehner M, Klein P, et al. Incisional hernia repair after orthotopic liver transplantation: a technique employing an inlay/onlay polypropylene mesh. Langenbecks Arch Surg 2003;388:167-73.

Forkhead Box P3 (FOXP3) mRNA Expression Immediately After Living-Donor Liver Transplant

Rieko Sakamoto,[1] Katsuhiro Asonuma,[1] Manuel E. Zeledon Ramirez,[2] Kazuhiko Yoshimoto,[1] Aya Nishimori,[1] Yukihiro Inomata[1]

Abstract

Objectives: **The forkhead box P3 (FOXP3) gene is considered to be the master gene of regulatory T cells. The significance of regulatory T cells in liver transplant has been investigated in previous reports, but quantitative FOXP3 messenger RNA (mRNA) expression after living-donor liver transplant has not been assessed. The objective of this study was to determine whether the human FOXP3 gene is a good marker for regulatory activity in T cells in living-donor liver transplant recipients during the immediate posttransplant period.**

Materials and Methods: **In peripheral blood mononuclear cells of 15 living-donor liver transplant recipients during the first month after transplant, we measured the population of $CD4^+CD25^+$ T cells using flow-assisted cell sorting and the expression of FOXP3 mRNA using real-time polymerase chain reaction.**

Results: **Fold induction of FOXP3 mRNA significantly increased on postoperative day 7 (3.3-fold) compared with the reference preoperative value ($P < .01$) but returned to baseline by 28 days after transplant. The population of $CD4^+CD25^+$ T cells did not change significantly. Expression of FOXP3 mRNA on days 14, 21, and 28 were lower in recipients with acute cellular rejection within 60 days after living-donor liver transplant.**

Conclusions: **Increased expression of FOXP3 mRNA immediately after living-donor liver transplant might be influenced by activation of T cells including regulatory T cells and other T cells. However, after stabilization of these activation profiles, it seems likely that FOXP3 mRNA expression is associated with graft acceptance. Further studies are necessary with measurement of FOXP3 mRNA expression at appropriate sampling points.**

Key words: *Forkhead box P3, Regulatory T cells, Liver transplant, Acute cellular rejection, Immunosuppression*

Regulatory T cells are a subset of CD4+ T cells with suppressive function. Because regulatory T cells constitutively express CD25 (interleukin 2 [IL-2] receptor, alpha chain), $CD4^+CD25^+$ T cells isolated from blood have been used in vitro as regulatory T cells, as initially reported using mice (1, 2). A comparable subset of regulatory T cells exists in humans, with some differences from those in mice (3, 4). This subset is reportedly related to transplant tolerance (5, 6). Demirkiran and colleagues reported that the population of $CD4^+CD25^+$ T cells in peripheral blood decreased after liver transplant and that this reduction was associated with immunosuppression (7, 8).

On the other hand, it has been reported that the forkhead box P3 (FOXP3) gene, whose defect is related to an autoimmune disease in mice (scurfy mice), is the master control gene of regulatory T cells in mice (9, 10). In addition, naive T cells transduced with the FOXP3 gene acquire suppressive function in mice (10). However, in studies of the human FOXP3 gene, Allan and colleagues reported that human naive T cells transduced with the FOXP3 gene did not show suppressive function and that FOXP3 protein is expressed in activated human effector T cells, which do not necessarily have suppressive function (11, 12). Therefore, it is necessary to determine whether or not the human

From the [1]Department of Pediatric Surgery and Transplantation, Kumamoto University, Japan
[2]Department of Surgery, Hospital Calderon Guardia, Costa Rica
Acknowledgement: This work was supported by grant-in-aid for scientific research No.19591488 from the Ministry of Education, Culture, Sports, Science and Technology-Japan.
Address reprint requests to: Rieko Sakamoto, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan
Phone: +81 963735616 Fax: +81 963735783 E-mail: rieko-ha@nifty.com

Experimental and Clinical Transplantation (2009) 1: 8-12

FOXP3 gene is a good marker for regulatory activity in T cells.

In this study, we measured the population of $CD4^+CD25^+$ T cells and FOXP3 mRNA expression in peripheral blood mononuclear cells of living-donor liver transplant recipients during the immediate posttransplant period.

Materials and Methods

Fifteen recipients who underwent living-donor liver transplant at our institution between March 2005 and May 2006 were enrolled in the study. Informed consent was obtained from all recipients. And the study was approved by the ethics committee of our institute. Tacrolimus and steroids were administered to all recipients. Corticosteroids were administered during and progressively decreased after transplant. Methylprednisolone was administered intravenously at a dosage of 10 mg/kg immediately before reperfusion; at 1 mg/kg on postoperative days 1, 2, and 3; at 0.5 mg/kg on postoperative days 4, 5, and 6; and at 0.3 mg/kg on postoperative day 7. Steroid therapy was then changed to oral prednisolone at a dosage of 0.3 mg/kg. Blood sampling was performed on the day before living-donor liver transplant (day 0) and on postoperative days 7, 14, 21, and 28.

Peripheral blood was collected using EDTA tubes. Peripheral blood mononuclear cells were isolated using Ficoll-Paque (Amersham Biosciences, Piscataway, NY, USA) gradient centrifugation and washed twice in Royal Park Memorial Institute 1640 solution (Sigma, St. Louis, MO, USA).

Total RNA (0.5 μg) from the peripheral blood mononuclear cells was reverse transcribed with a high capacity complementary DNA (cDNA) archive kit (Applied Biosystems, Foster City, CA, USA). Real-time polymerase chain reaction (RT-PCR) was performed for FOXP3 or 18s ribosomal RNA (rRNA) using Pre-developed TaqMan Assay Reagents (Applied Biosystems) with primer pairs FOXP3 forward 5'-TGCCTCCTCTTCTTCCTTGAAC-3' and reverse 5'-GGGCGTGGGCATCCA-3' with TaqMan MGB probes (Assays-on-demand TM gene expression assay) for 5'-6FAM-ATCCGCTGGGCCATCCTGGAGGCT-C-3' (FOXP3), and specific primer pairs and a TaqMan MGB probe for 18s rRNA (TaqMan Pre-Developed Assay Reagents Human 18s rRNA, Applied Biosystems). Real-time PCR was carried out in an Applied Biosystems PRISM 7000 Sequence detection system with cycling of 2 minutes at 50°C and 10 minutes at 95°C, followed by 40 cycles of 15 seconds at 95°C and 1 minute at 60°C. Fold induction was determined by using $\Delta\Delta$Ct method and was expressed as $2^{-(\Delta\Delta Ct)}$, where $\Delta\Delta Ct = (Ct_{FOXP3}\text{-}Ct_{18s})_{sample}\text{-}(Ct_{FOXP3}\text{-}Ct_{18s})_{reference}$. For each patient, the FOXP3 mRNA fold induction of samples from postoperative days 7, 14, 21, and 28 was divided by the FOXP3 fold induction on day 0, and the values were reported as the relative FOXP3 fold induction.

Fresh peripheral blood mononuclear cells were stained with fluorescently labeled antibodies, CD3-allophycocyanin (Biosciences Pharmingen, San Diego, CA, USA), CD4-phycoerythrin (Biosciences Pharmingen), and CD25-fluorescein isothiocyanate (Becton Dickinson Biosciences, Franklin Lakes, NJ, USA) for 30 minutes at 4°C. The cells were washed twice with phosphate-buffered saline containing 2% bovine serum albumin and 0.2% azide and fixed in 0.5 mL 2% paraformaldehyde in phosphate-buffered saline. The percentage of $CD3^+CD4^+CD25^+$ cells in peripheral blood mononuclear cells was analyzed with a flow cytometer (FACS Calibur; Becton Dickinson Biosciences).

The serum trough level of tacrolimus and the concentration of total bilirubin were measured on day 0 and postoperative days 7, 14, 21, and 28. Acute cellular rejection was confirmed by histologic examination of liver biopsies using the Banff classification (13), with a rejection activity index of 4 or more defined as rejection.

The Mann-Whitney test and 1-factor repeated measures analysis of variance were used for statistical analyses. Significant differences identified by analysis of variance were followed by Ryan's method for post hoc comparisons. *P* values less than .05 were considered statistically significant.

Results

The age of the 15 recipients at the time of living-donor liver transplant ranged from 6 months to 65 years (Table). The group consisted of 7 patients with biliary atresia, 5 with hepatitis C virus-related disease, and one each with familial amyloid polyneuropathy, primary biliary cirrhosis, and hepatitis B virus-related disease. Total bilirubin decreased gradually after living-donor liver transplant (Figure 1a), and all recipients had good graft function in the early postoperative period.

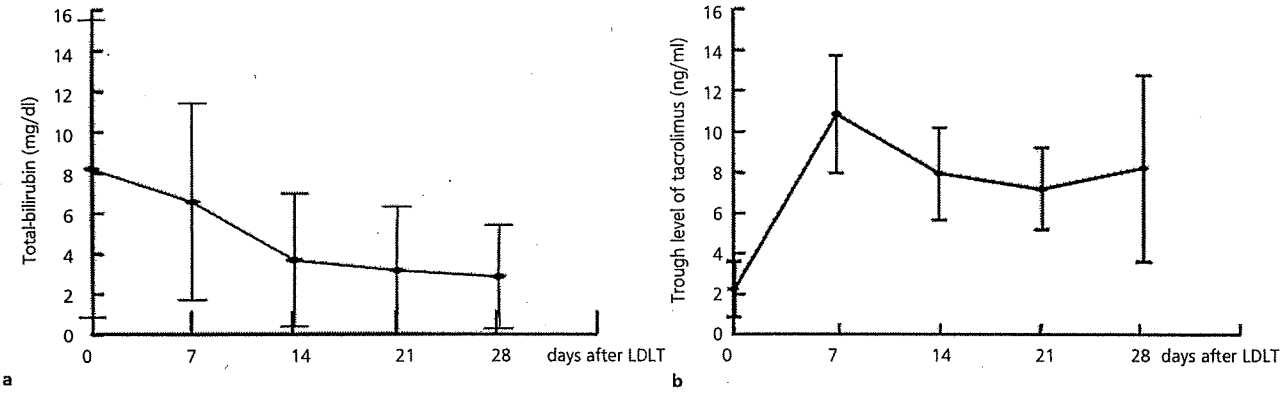

Figure 1. Total bilirubin levels and trough level of tacrolimus in the first 28 days after living-donor liver transplant.
The bars show the standard deviation. **Figure 1a.** Mean total bilirubin level decreased gradually after living-donor liver transplant. **Figure 1b.** Trough levels of tacrolimus were maintained between 8 and 12 ng/mL.

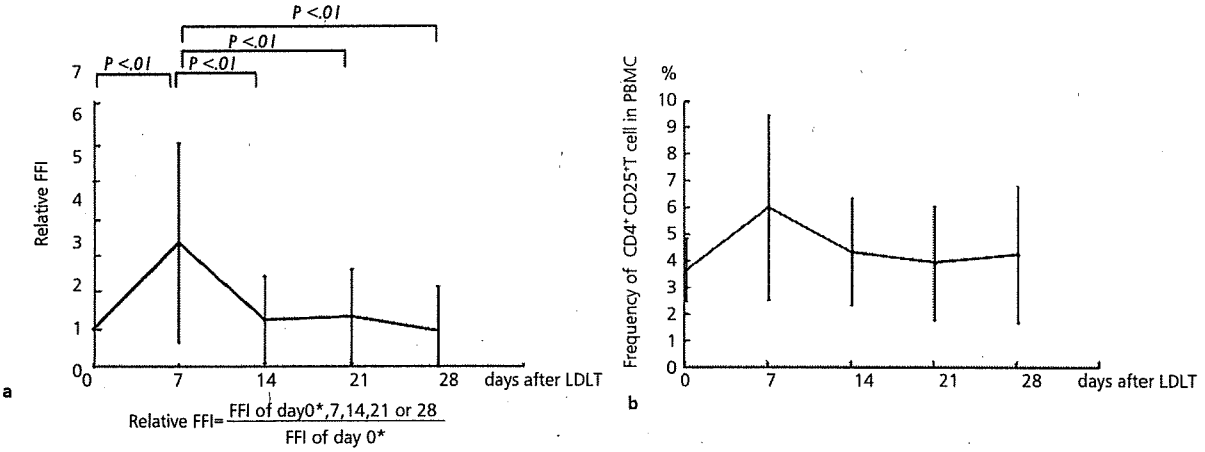

Figure 2. Mean relative FOXP3 fold induction and mean frequency of $CD4^+CD25^+$ T cells in the first month ffter living-donor liver transplant.
Figure 2a. Mean relative FOXP3 fold induction on postoperative day 7 was significantly higher than the reference preoperative level ($P < .01$).
Figure 2b. There were no significant differences between the sampling points ($P = .19$) for the proportion of $CD4^+CD25^+$ T cells. The bars show the standard deviation.
Abbreviations: FOXP3, forkhead box P3; mRNA, messenger RNA; rRNA, ribosomal RNA; *day 0 = the day immediately before living-donor liver transplant.

Table. Characteristics of 15 LDLT Patients

Patient	Disease	Age, y	Sex	ACR within 60 days	FFI peak on day 7
1	BA	0	F	-	+
2	BA	0	M	+ (POD37)	+
3	BA	4	M	-	-
4	BA	16	M	-	-
5	BA	22	F	-	+
6	BA	23	F	- (POD40)	+
7	FAP	24	F	-	-
8	BA	32	M	-	+
9	HCV	46	M	+ (POD23)	+
10	PBC	49	M	-	+
11	HCV	53	M	-	-
12	HBV	54	F	+ (POD20)	+
13	HCV	56	F	-	+
14	HCV	61	F	-	-
15	HCV	65	M	-	-

Abbreviations: ACR, acute cellular rejection; BA, biliary atresia; FAP, familial amyloid polyneuropathy; FFI, forkhead box P3 (FOXP3) messenger RNA fold induction; HBV, hepatitis-B-virus–related disease; HCV, hepatitis-C-virus–related disease; LDLT, living-donor liver transplant; PBC, primary biliary cirrhosis; POD, postoperative day.

Trough levels of tacrolimus were maintained between 8 and 12 ng/mL (Figure 1b). Although the trough level of tacrolimus reached a peak on postoperative day 7, it was not significantly different from the values at other sampling points.

Mean relative FOXP3 fold inductions at each sampling point are shown in Figure 2a. Mean relative FOXP3 fold induction was significantly increased on postoperative day 7 (3.3-fold) compared with the reference preoperative value ($P < .01$); it returned to baseline by 28 days after transplant. This relative FOXP3 fold induction peak on postoperative day 7 was observed in 9 of the 15 patients (Table). Mean population of $CD4^+CD25^+$ T cells increased on postoperative day 7, but this was not significantly different compared with the other sampling points ($P = .19$; Figure 2b).

The recipients were divided into 2 groups according to whether they developed acute cellular rejection within 60 days after living-donor liver transplant (Table). The 4 patients with acute cellular rejection all had peaks of relative FOXP3 fold induction on postoperative day 7, compared to 5 patients with FOXP3 peaks of 11 patients without acute cellular rejection. Relative FOXP3 fold induction on postoperative days 14, 21, and 28 showed a tendency to be lower in the 4 patients with acute cellular rejection than in the 11 patients without acute cellular rejection (day14:$P = .093$, day 21:$P = .057$, day 28:$P = .16$, Figure 3). The population of $CD4^+CD25^+$ T cells did not show any significant differences between the acute cellular rejection group and the group without acute cellular rejection (data not shown).

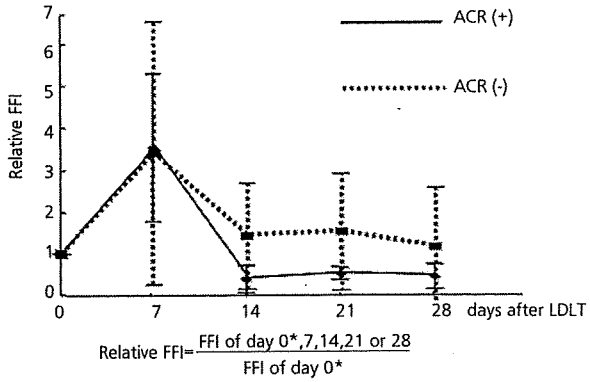

Figure 3. Mean relative FOXP3 fold induction in recipients with and without acute cellular rejection.
Mean relative FOXP3 fold induction on postoperative days 14, 21, and 28 showed a tendency to be lower in the 4 patients with acute cellular rejection (+) than in the 11 patients without acute cellular rejection (–).
Abbreviations: FOXP3, forkhead box P3; *day 0 = the day immediately before living-donor liver transplant.

Discussion

The FOXP3 gene is considered to be the master control gene of regulatory T cells in mice; however, it is debated whether the human FOXP3 gene also controls the activity of regulatory T cells because there are some differences between the mouse and human FOXP3 genes (14). Therefore, it is important to evaluate whether the FOXP3 gene can be a marker for immunologic status in living-donor liver transplant recipients. Although the population of $CD4^+CD25^+$ T cells did not show any significant changes during the first month after living-donor liver transplant in our study, FOXP3 mRNA was significantly increased on postoperative day 7 and subsequently returned to preoperative values.

However, the mechanism underlying these results is unclear. Morgan and colleagues found in an in vitro study that both activated $CD4^+CD25^+$ T cells and activated $CD8^+$ T cells expressed FOXP3 mRNA (15). They also reported that the production of FOXP3 in human cells, unlike in murine cells, is greatly affected by activation of various types of T cells. Moreover, recent studies have found that $CD25^-$ T cells activated by anti-CD3/CD28 monoclonal antibody expressed FOXP3 protein and that these T cells did not show any suppressive function (12, 16). In the clinical setting, it is conceivable that the increase in FOXP3 mRNA expression on postoperative day 7 reflected the activation of T cells, not only of regulatory T cells but also of other T cells, as part of the immune response that occurs after transplant. Moreover, this peak of FOXP3 mRNA expression may not necessarily be related to the regulatory function against allograft, because all 4 recipients who developed acute cellular rejection within 60 days after living-donor liver transplant had the relative FOXP3 fold induction peak on postoperative day 7.

Average trough level of tacrolimus increased on postoperative day 7 (although not to a statistically significant degree). This indicates that this high concentration of tacrolimus stabilized immune system reactivity and therefore allowed FOXP3 mRNA expression to return to preoperative values. The FOXP3 fold induction on postoperative days 14, 21, and 28 was lower in the patients who developed acute cellular rejection within 60 days after living-donor liver transplant. This finding suggests that the change in FOXP3 fold induction after stabilizing T-cell activation immediately after living-donor liver transplant is associated with graft acceptance.

We have used intracellular staining and fluorescently labeled antibodies to identify FOXP3 protein in previous studies (17) and obtained reliable results for assessing the relation between regulatory T cells and immunologic tolerance in living-donor liver transplant recipients. Serial and dynamic analysis of FOXP3 protein levels before and after living-donor liver transplant, including the time of rejection episodes, is necessary in future studies.

In conclusion, FOXP3 mRNA expression increased immediately after living-donor liver transplant, probably because of immune reaction

with T cells, which was not associated with immunologic tolerance. However, the subsequent decrease in FOXP3 mRNA expression after stabilizing T-cell activation might be associated with graft acceptance. We must investigate appropriate sampling points for measuring FOXP3 mRNA expression to determine whether it can be used as a marker for graft acceptance after living-donor liver transplant.

References

1. Bluestone JA, Abbas AK. Natural versus adaptive regulatory T cells. Nat Rev Immunol. 2003;3(3):253-257.
2. Sakaguchi S, Sakaguchi N, Shimizu J, et al. Immunologic tolerance maintained by CD25+ CD4+ regulatory T cells: their common role in controlling autoimmunity, tumor immunity, and transplantation tolerance. Immunol Rev. 2001;182:18-32.
3. Jonuleit H, Schmitt E, Stassen M, et al. Identification and functional characterization of human CD4(+)CD25(+) T cells with regulatory properties isolated from peripheral blood. J Exp Med. 2001;193(11):1285-1294.
4. Valmori D, Merlo A, Souleimanian NE, et al. A peripheral circulating compartment of natural naive CD4 Tregs. J Clin Invest. 2005;115(7):1953-1962.
5. Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol. 2003;3(3):199-210.
6. Yoshizawa A, Ito A, Li Y, et al. The roles of CD25+CD4+ regulatory T cells in operational tolerance after living donor liver transplantation. Transplant Proc. 2005;37(1):37-39.
7. Demirkiran A, Kok A, Kwekkeboom J, et al. Decrease of CD4+CD25+ T cells in peripheral blood after liver transplantation: association with immunosuppression. Transplant Proc. 2005;37(2):1194-1196.
8. Demirkiran A, Kok A, Kwekkeboom J, et al. Low circulating regulatory T-cell levels after acute rejection in liver transplantation. Liver Transpl. 2006;12(2):277-284.
9. Sakaguchi S. The origin of FOXP3-expression CD4+ regulatory T cells: thymus or periphery. J Clin Invest. 2003;112(9):1310-1312.
10. Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science. 2003;299(5609):1057-1061.
11. Allan SE, Passerini L, Bacchetta R, et al. The role of 2 FOXP3 isoforms in the generation of human CD4+ Tregs. J Clin Invest. 2005;115(11):3276-3284.
12. Allan SE, Crome SQ, Crellin NK, et al. Activation-induced FOXP3 in human T effector cells does not suppress proliferation or cytokine production. Int Immunol. 2007;19(4):345-354.
13. Ormonde DG, de Boer WB, Kierath A, et al. Banff schema for grading liver allograft rejection: utility in clinical practice. Liver Transpl Surg. 1999;5(4):261-268.
14. Ziegler SF. FOXP3: not just for regulatory T cells anymore. Eur J Immunol. 2007;37(1):21-23.
15. Morgan ME, van Bilsen JH, Bakker AM, et al. Expression of FOXP3 mRNA is not confined to CD4+CD25+ T regulatory cells in humans. Hum Immunol. 2005;66(1):13-20.
16. Gavin MA, Torgerson TR, Houston E, et al. Single-cell analysis of normal and FOXP3-mutant human T cells: FOXP3 expression without regulatory T cell development [published correction appears in Proc Natl Acad Sci U S A. 2006;103(24):9373]. Proc Natl Acad Sci U S A. 2006;103(17):6659-6664.
17. Bloom DD, Chang Z, Fechner JH, et al. CD4+ CD25+ FOXP3+ regulatory T cells increase de novo in kidney transplant patients after immunodepletion with Campath-1H. Am J Transplant. 2008;8(4):793-802.