ANNALS OF TRANSPLANTATION

CASE REPORT

ISSN 1425-9524
© Ann Transplant, 2014; 19: 149-152
DOI: 10.12659/AOT.890213

Received: 2013.12.20
Accepted: 2014.01.13
Published: 2014.03.27

Urgent living donor liver transplantation for biliary atresia complicated by a strangulated internal hernia at Roux-en Y limb: A case report

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF Hajime Uchida
A Seisuke Sakamoto
B Ikumi Hamano
B Megumi Kobayashi
B Toshihiro Kitajima
B Takanobu Shigeta
B Hiroyuki Kanazawa
B Akinari Fukuda
A Mureo Kasahara

Department of Transplantation, National Center for Child Health and Development, Tokyo, Japan

Corresponding Author: Hajime Uchida, e-mail: uchida-h@ncchd.go.jp

Background: When BA patients with end-stage liver dysfunction have bowel obstruction, especially strangulated internal hernia, selecting optimal surgical therapeutic options is crucial.

Case Report: An 11-month-old female with end-stage biliary atresia (BA) was admitted for a strangulated internal hernia at the Roux-en Y limb and frequent episodes of gastrointestinal bleeding requiring blood transfusion. She was scheduled within a month to receive a portion of the liver from her blood-type identical mother. Despite intensive care, her clinical condition obviously needed a prompt surgical intervention. The operative findings at laparotomy revealed exudative moderate ascites and a dilated and ischemic afferent loop that was strangulated by a band extending from the mesentery to the transverse mesocolon. The attachment of the band was released, and gangrenous changes were recognized in the incarcerated bowel, although there were no obvious findings of intestinal perforation. After the gangrenous afferent loop was resected, the remnant afferent loop was too short to anastomose again. Following these procedures, as the patient's vital signs remained stable, we decided to simultaneously perform living donor liver transplantation (LDLT). She successfully underwent LDLT and her post-transplant course was uneventful.

Conclusions: When faced with candidates for LT as an urgent life-saving surgery, determining whether LDLT should be performed simultaneously during perioperative management is necessary to save the life of the patient.

Keywords: Liver Transplantation • Biliary Atresia • Living Donor Liver Transplantation • Strangulated Hernia

Full-text PDF: http://www.annalsoftransplantation.com/download/index/idArt/890213

1263 — 2 12

Indexed in: [Science Citation Index Expanded] Index Medicus/MEDLINE]

Background

Postoperative bowel obstruction continues to be the most common complication after laparotomy [1]. Strangulated internal hernia is a lethal condition that can lead to gangrene of the small bowel, with septic shock if not appropriately treated in time [2]. Biliary atresia (BA) is a leading indication for pediatric liver transplantation (LT), and patients with BA who undergo portoenterostomy after birth have a risk of bowel obstruction [3]. The need for relaparotomy for bowel obstruction is reported in approximately 10% of patients who undergo portoenterostomy [4]. When BA patients with end-stage liver dysfunction have bowel obstruction, especially strangulated internal hernia, selecting optimal surgical therapeutic options is crucial.

We herein present the case of a patient with BA complicated by a strangulated internal hernia at the Roux-en Y limb who successfully underwent LDLT.

Case Report

An 11-month-old female weighing 6.7 kg was transferred to our institute with disordered consciousness requiring intubation due to gastrointestinal bleeding. At 5 months of age, the patient was diagnosed with BA and portoenterostomy was performed at a local hospital. However, her clinical condition had not improved, and she exhibited growth failure (weight z-score: –2.1) and frequent episodes of gastrointestinal bleeding requiring blood transfusions, with a pediatric end-stage liver disease score of 17 [5]. The patient was referred to our institute with an indication for LT, which was scheduled within a month by receiving a portion of the liver from her blood-type identical mother.

The patient was admitted to the emergency room due to abdominal distension. Enhanced computed tomography (CT) demonstrated dilated loops of intestine with wall thickening suspicious of bowel obstruction at the Roux-en Y limb. CT also revealed narrowing of the portal venous trunk with developed collateral vessels (Figure 1). Abdominal Doppler ultrasound revealed a decreased portal venous flow and an accelerated hepatic arterial flow in the hilum of the liver. Over the course of the next 6 hours, despite the administration of gastrointestinal decompression using a nasogastric tube, the patient's abdominal distension worsened, with ascites formation and further progression of dilated intestines. Therefore, the patient's clinical condition required prompt surgical intervention. A pre-operatively laboratory evaluation showed total serum bilirubin 7.25 mg/dl, aspirate aminotransferase 95 IU/l, alanine aminotransferase 51 IU/l, albumin 3.0 g/dl, and international normalized ratio of prothrombin time 1.24. Because the patient's mother had been assessed to become a living donor without any medical problems, LDLT was considered as a surgical option.

Figure 1. Enhanced abdominal computed tomography demonstrated dilated loops of intestine with wall thickening at the Roux-Limb (white arrow) and narrowing of the portal venous trunk (white arrowhead).

Figure 2. The operative findings at laparotomy revealed a dilated and ischemic afferent loop that was strangulated by a band (white arrow) extending from the mesentery to the transverse mesocolon.

The operative findings at laparotomy revealed exudative moderate ascites and a dilated and ischemic afferent loop that was strangulated by a band extending from the mesentery to the transverse mesocolon (Figure 2). The attachment of the band was released, and gangrenous changes were recognized in

the incarcerated bowel, although there were no obvious findings of intestinal perforation. After the gangrenous afferent loop was resected, the remnant afferent loop was too short to anastomose again. Following these procedures, because the patient's vital signs remained stable, we decided to simultaneously perform LDLT. A liver graft left sector weighing 256 g, representing 3.82% of the graft-to-recipient weight ratio, was procured. The operation employed a standard LDLT technique, and portal vein anastomosis was performed with the branch patch technique, because a sufficient hepatopetal flow was obtained following devascularization of the collateral vessels. The operation lasted 8 hours and 25 minutes, with a blood loss of 73.5 ml/kg. The explanted liver showed marked cholestasis with intrahepatic bile duct proliferation, fibrosis, and cirrhosis, which were consistent with extrahepatic biliary atresia. Immunosuppressive treatment was initiated with tacrolimus and low-dose steroids. As the presence of *Streptococcus oralis* in a blood culture collected at the time of admission was confirmed on postoperative day 2, antimicrobial therapy with vancomycin and piperacillin-tazobactam was therefore cautiously continued for 10 days after LDLT. The patient's postoperative course was uneventful, except for an episode of a graft rejection, and she was discharged on postoperative day 42 without any surgical complications. She was found to be doing well 1 year after the LDLT.

Discussion

The present patient developed several medical problems before undergoing LDLT, which complicated our decision-making regarding the optimal surgical therapeutic options; specifically, whether LDLT should be performed simultaneously. The patient's primary medical problem was deterioration in the liver function, which resulted in poor portal flow, gastrointestinal bleeding, and malnutrition. Long-term fasting for LDLT because of gastrointestinal bleeding is too invasive for unstable patients and can result in a high mortality rate [6]. Post-transplant immunosuppression, as well as preoperative malnutrition, can increase the risk of bacterial infection following LDLT [7]. On the other hand, in the operative findings at laparotomy, the entire afferent loop had become gangrenous. In this situation, external biliary drainage through the previous Roux-en Y limb with or without resection of the gangrenous intestines and redo hepatic portoenterostomy were considered as the other therapeutic option. However, the possibility of bacterial translocation from the gangrenous intestines was high enough to trigger sepsis and there is a high complication rate after redo hepatic portoenterostomy under such severe end-stage liver disease (ESLD). Moreover, among patients with a strangulating obstruction, intestinal perforation can occur more readily and severely in those with ESLD [8]. In the present case, the patient's vital signs were stable during surgery, and there were no findings of intestinal perforation; therefore, LDLT was performed simultaneously rather than at a second surgery, with careful consideration of the patient's chances for survival between these 2 therapeutic surgeries. Because the patient's mother had been assessed to become a living donor without any medical problems, LDLT was considered to achieve timely surgical intervention in this case; nevertheless, other types of surgical intervention should have been considered. Regarding infection control after LDLT, unfortunately, our patient had a positive blood culture collected before LDLT. However, the patient did not have any infectious complications after the procedure due to the administration of appropriate antibiotic therapy proposed by infectious disease specialists. For such unstable patients with immunosuppression, daily consultations with infectious disease specialists are crucial. Moreover, bacterial cultures should be performed promptly when any suspicious signs of infectious disease are detected, at which time appropriate antibiotic therapy must be initiated.

Previous studies have suggested that a decreased portal venous flow is often observed in patients affected by BA before LT, which is indicative of a poor prognosis [9]. Patients with BA often exhibit a sclerotic portal venous trunk due to inflammation of the hepatoduodenal ligament and recurrent cholangitis, as previously reported in approximately 80% of patients with BA who undergo portoenterostomy at the time of LDLT [10]. Technical difficulties in performing portal venous reconstruction at the time of LT may lead to serious morbidity and mortality [11]. Moreover, the use of LT in children younger than 1 year of age is associated with an especially high risk of morbidity and mortality due to the need for difficult technical approaches [12]. In the present case, although the preoperative radiological findings revealed narrowing of the portal venous trunk with a decreased blood flow, the portal venous trunk macroscopically appeared to be patent without sclerotic changes. Portal vein reconstruction was performed using the branch patch technique with a sufficient hepatopetal flow.

Conclusions

The patient presented herein was able to survive because a hepatic graft from a living donor was quickly obtained. When faced with candidates for LT as an urgent life-saving surgery, determining whether LDLT should be performed simultaneously during perioperative management is necessary to save the life of the patient. Selecting optimal surgical therapeutic options is crucial in patients with strangulated bowel obstruction at the Roux-en Y limb with end-stage BA.

Conflicts of interest

There are no conflicts of interest from any of the authors.

References:

1. Chen CL, Concejero A, Wang CC et al: Living donor liver transplantation for biliary atresia: a single-center experience with first 100 cases. Am J Transplan, 2006; 6: 2672–79
2. Newsom BD, Kukora JS: Congenital and acquired internal hernia: unusual causes of small bowel obstruction. Am J Surg, 1986; 152: 279–85
3. Lubawski J, Saclarides T: Postoperative ileus: strategies for reduction. Ther Clin Risk Manag, 2008; 4: 913–17
4. Lai HS, Chen WJ, Chen CC et al: Long-Term prognosis and factors affecting biliary atresia from experience over a 25 year period. Chang Gung Med J, 2006; 29: 3
5. Wlesner RH, Mcdiamid SV, Kamath PS et al: MELD and PELD: application of survival models to liver allocation. Liver Transpl, 2001; 7: 567–80
6. Varma V, Mehta N, Kumaran V, Nundy S: Indications and contraindications for liver transplantation. Int J Hepatol. 2011; 2011: 121862
7. Iida T, Kaido T, Yagi S et al: Posttransplant bacteremia in adult living donor liver transplant recipients. Liver Transpl, 2010; 16: 1379–85
8. Baliga P, Merion RM, Turcotte JG et al: Preoperative risk factor assessment in liver transplantation. Surgery, 1992; 112: 704–10
9. Diem HV, Evrard V, Vinh HT et al: Pediatric liver transplantation for biliary atresia: results of primary grafts in 328 recipients. Transplantation, 2003; 75: 1692–97
10. de Magnée C, Bourdeaux C, de Dobbeleer F et al: Impact of pre-transplant liver hemodynamics and portal reconstruction techniques on post-transplant portal vein complications in pediatric liver transplantation: a retrospective analysis in 197 recipients. Ann Surg, 2011; 254: 55–61
11. Kiuchi T, Kasahara M, Uryuhara K et al: Impact of graft size mismatching on graft prognosis in liver transplantation from living donors. Transplantation, 1999; 27: 321–27
12. Knazawa H, Sakamoto S, Fukuda A et al: Portal vein reconstruction in pediatric living donor liver transplantation for patients younger than 1 year with biliary atresia. J Pediatr Surg, 2012; 47: 523–27

Pediatr Transplantation 2015: 19: E70–E74

Pediatric Transplantation
DOI: 10.1111/petr.12446

Successful living domino liver transplantation in a child with protein C deficiency

Matsunami M, Ishiguro A, Fukuda A, Sasaki K, Uchida H, Shigeta T, Kanazawa H, Sakamoto S, Ohta M, Nakadate H, Horikawa R, Nakazawa A, Ishige M, Mizuta K, Kasahara M. (2015) Successful living domino liver transplantation in a child with protein C deficiency. *Pediatr Transplant*, 19: E70–E74. DOI: 10.1111/petr.12446.

Abstract: PC is produced in the liver and inhibits blood coagulation by catalyzing active factors V and VIII. PC deficiency causes abnormal blood clotting that is difficult to regulate by anticoagulative treatments. Four reports of PC deficiency treated with LTx have been published; however, no report of DLT as a therapy for PC deficiency is available. We describe a case of a 23-month-old girl who received DLT for compound heterozygous PC deficiency. Her PC activity was below 5%. She developed intracranial lesion and frequent refractory purpura fulminans. Both her parents had heterozygous mutations of PC genes and were excluded as living donors. Furthermore, she was a low priority on the waiting list of deceased-donor transplantation. We performed living DLT using the liver from a patient with MSUD. Activated PC concentrate safely supported the perioperative period. After DLT, she maintained normal PC activities and BCAA levels. This is the first case of PC deficiency successfully treated by living DLT with MSUD. We propose that DLT using liver from patients with MSUD is a treatment option for PC deficiency.

Masatoshi Matsunami[1], Akira Ishiguro[2], Akinari Fukuda[1], Kengo Sasaki[1], Hajime Uchida[1], Takanobu Shigeta[1], Hiroyuki Kanazawa[1], Seisuke Sakamoto[1], Motoki Ohta[3], Hisaya Nakadate[2], Reiko Horikawa[4], Atsuko Nakazawa[5], Mika Ishige[6], Koichi Mizuta[7] and Mureo Kasahara[1]

[1]Transplantation Center, National Center for Child Health and Development, Tokyo, Japan, [2]Division of Hematology, National Center for Child Health and Development, Tokyo, Japan, [3]Department of Pediatrics, Shiga University of Medical Science, Shiga, Japan, [4]Division of Endocrinology and Metabolism, National Center for Child Health and Development, Tokyo, Japan, [5]Division of Clinical Pathology, National Center for Child Health and Development, Tokyo, Japan, [6]Department of Pediatrics,Nihon University School of Medicine, Tokyo, Japan, [7]Department of Transplant Surgery, Jichi Medical University, Tochigi, Japan

Key words: protein C deficiency – protein C concentrate – domino liver transplantation – neonatal purpura fulminans – maple syrup urine disease

Masatoshi Matsunami, MD, Transplantation Center, National Center for Child Health and Development, 2-10-1 Okura, Setagaya-ku, Tokyo 157-8535, Japan
Tel.: +81 3 3416 0181
Fax: +81 3 3416 2222
E-mail: matsunami-m@ncchd.go.jp

Accepted for publication 23 January 2015

Abbreviations: 3D-CT, three-dimensional computerized tomography; aPTT, activated partial thromboplastin time; BCAA, branched-chain amino acid; DDLT, deceased-donor liver transplantation; DLT, domino liver transplantation; FAP, familial amyloidotic polyneuropathy; FFP, fresh frozen plasma; HA, hepatic artery; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HV, hepatic vein; LDLT, living-donor liver transplantation; LHV, left hepatic veins; LTx, liver transplantation; M + LHV, middle and left hepatic veins; MHV, middle hepatic vein; MSUD, maple syrup urine disease; PBC, primary biliary cirrhosis; PC, protein C; PFIC, progressive familial intrahepatic cholestasis; PSC, primary sclerosing cholangitis; PT, prothrombin time; PV, portal vein; RHV, right hepatic vein.

PC is a vitamin K-dependent serine protease synthesized and secreted by liver cells as a zymogen. Activated PC downregulates the blood coagulation cascade through its proteolytic effect on factors Va and VIIIa, in conjunction with its cofactor protein S (1, 2). PC deficiency is inherited as an autosomal dominant disorder, and in most cases, derived from heterozygous mutations. Homozygous or compound heterozygous patients often suffer severe and life-threatening complications from birth. The most common presentation is purpura fulminans, a rapidly progressing hemorrhagic necrosis of the skin, which

can occur within the first few hours of birth (1, 2). Cerebral and ophthalmic damage can occur in utero, and children are often born with visual impairment. In addition, patients may suffer from venous thromboembolism (3).

Historically, the standard treatment for PC deficiency was the replacement of FFP and administration of warfarin (1, 2). In 1983, the first case of severe PC deficiency associated with neonatal purpura fulminans successfully managed by warfarin was reported (4). Since 1988, the use of PC concentrate has been widely adopted and reports of successful long-term management using PC concentrate have been published (5). Warfarin, FFP, and PC concentrate, however, are associated with thrombosis and can sometimes be difficult to administer. Hence, there is rising interest in LTx as a therapy for PC deficiency, as the liver produces PC (6–9).

To date, there are 10 reports of DLT of a recipient's metabolically defective, but structurally and functionally normal, explanted liver into a second recipient. The most common indication for DLT is FAP. With an increasing need for organs, livers explanted from patients with rare metabolic diseases, such as primary hyperoxaluria, acute intermittent porphyria, MSUD, and homozygous familial hypercholesterolemia, are being used for DLT (10–12). DLT has since become an option for increasing organ availability.

Here, we report the case of a child with severe compound heterozygous PC deficiency who was successfully treated by DLT using liver from an MSUD patient.

Case presentation

The patient was a 23-month-old girl (body weight: 9.5 kg) who was delivered by C-section because of fetal heart rate deceleration (37w 6d, 2048 g). She was the only child in the family. Multiple cerebral bleeding, subcutaneous bleeding, purpuric skin lesions on lower limbs, and vitreous hemorrhages (ultimately resulting in complete blindness) were observed shortly after birth (Fig. 1). PC activity of the patient was <5%. Genetic test performed on a blood sample confirmed that she inherited an inability to produce PC. Two heterozygous missense mutations of PC in the proband were identified c.296G>A, p.E68K and c.1109G>A, p.V339M. A low PC activity and low antigen level led to the diagnosis of compound heterozygous PC deficiency. The PC activity of the parents was also low (66% and 77%, respectively). The patient's grandmother died of cerebral bleeding, but the details were unclear. At one month of life, an ophthalmectomy was performed due to bilateral vitreous hemorrhages. Initially, the patient was managed with warfarin (0.07 mg/kg/day) and heparin (225 U/kg/day). At 14 months of age, she

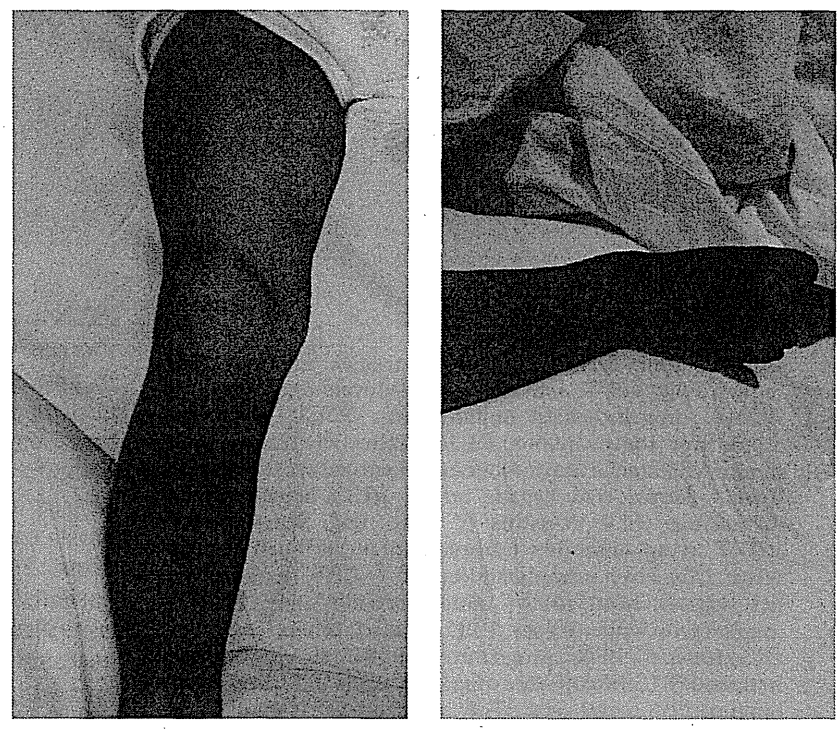

Fig. 1. Skin lesions were observed on the right foot and ankle arising from peripheral thrombotic events.

suffered from symptomatic West syndrome because of past cerebral bleeding; warfarin was changed to dabigatran to control phenobarbital. However, frequent episodes of bleeding and thrombosis such as subcutaneous hematoma persisted, and FFP and activated PC concentrate were required. In addition, there was an increasing difficulty of venous line access. She was evaluated as a candidate for LTx. Both her parents had heterozygous mutations of PC genes and were excluded as living donors. Furthermore, she was a low priority on the waiting list of deceased-donor transplantation. A decision for DLT was made.

At 22 months of age, the patient was transferred to our hospital for LTx. Pre-operatively, therapy with FFP (120 mL) was instituted on alternate days until operation. There were no thrombosis and bleeding episodes before operation. In addition, she was also given dabigatran (36 mg/kg/day) and was monitored to maintain an aPTT of 60–80 s. The final dose (360 mg) was administered 48 h before induction of anesthesia and the initiation of intravenous heparin. Soon after, thrombocytopenia was recognized; heparin was discontinued, and continuous intravenous infusion of activated PC concentrate (300 U/h) commenced.

At 23 months of age, the patient underwent DLT. The first recipient was a 12-month-old-girl with MSUD, who required LDLT of the left lateral segment from a living donor because of high leucine acid, frequent metabolic acidosis, and growth impairment. As size matching between the MSUD patient and our patient was suitable, the whole liver was used as the domino graft. The whole MSUD liver was placed in the abdominal cavity and vascular reconstruction initiated. We decided the transection site of the vessels based on 3D-CT of the first donor and recipient pre-operatively. Because the left HA of the first donor was diverged from the right gastric artery, along with sufficient anastomosis length, the gastroduodenal artery was ligated and the common HA was transected in the first recipient. As well, the RHV, the common channel of the M + LHV, was transected and the PV was transected proximal to the bifurcation. Vascular plasty of the HV was conducted on the back table. RHV, MHV, LHV, and the left superficial vein were sutured together to create one orifice. The unified graft HV was anastomosed with that of the recipient to create one orifice using all of the HV. The PV was anastomosed with the branch patch in an end-to-end fashion. The graft liver was reperfused before microsurgical reconstruction of the HA. Roux-en-Y anastomosis was employed for biliary reconstruction.

Activated PC concentrate was administered during surgery and continued until post-operative day 7. The level of PC activity increased gradually and reached 64% on post-operative day 4. In spite of the discontinuation of activated PC concentrate, PC activity levels were adequately maintained at 80–90% with PT/aPTT constantly within the normal range (Fig. 2). The recipient received a standard immunosuppressive regimen consisting of low-dose steroids and tacrolimus. On post-operative day 20, she had an episode of severe acute cellular rejection and was treated with intravenous bolus steroids therapy. On post-operative day 26, she acquired an infection of cytomegalovirus and was treated with intravenous foscarnet sodium hydrate. On post-operative day 68, she was discharged. She did not develop any BCAA imbalances or symptoms of MSUD on a normal diet with full protein

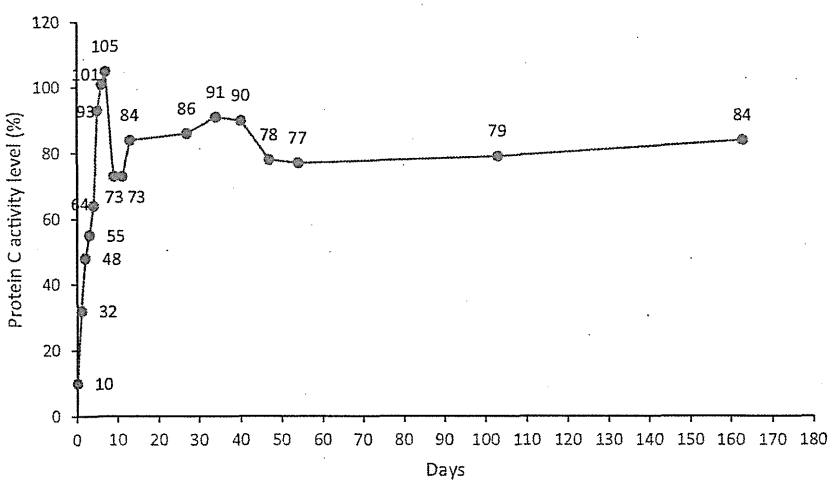

Fig. 2. Plasma PC activity levels with time after DLT.

Table 1. Amino acid levels before and after DLT

Amino acids	Normal range nmol/mL	DLT donor*		DLT recipient†	
		Before	After	Before	After
Leucine	80.9–154.3	154.6	137.9	114.4	125.3
Isoleucine	41.3–84.9	149.3	100.5	63.6	60.2
Valine	158–287.7	282.3	155.2	250.3	156.7
Alloisoleucine	ND	ND	ND	ND	ND

*Living-donor liver recipient with MSUD.
†Domino liver recipient with PC deficiency.

intake (Table 1). Six months after the DLT, PC activity was maintained at more than 80%. She remained symptom free.

All the patients and their parents gave informed consent. The study was approved by the Japanese Liver Transplantation Society and the ethical committee of each institute.

Discussion

This case highlighted two important clinical issues: PC deficiency can be successfully treated by living DLT with MSUD, and activated PC concentrate is useful and supported a safe perioperative period.

A total of 10 DLTs using livers from patients with MSUD have been reported (Table 2) (13–19). Domino livers were first considered as marginal grafts because the disease could manifest in the domino recipient (13). However, recipients of liver grafts from MSUD donors are not likely to develop protein intolerance because 60% of branched-chain ketoacid dehydrogenase activity occurs in the muscle (14). The patient and graft survival rates were 100%, and all the livers functioned normally. BCAA homeostasis was maintained with an unrestricted protein diet. MSUD livers maintained nearly normal levels of plasma amino acids and a favorable evolution with no disease development, demonstrating structurally normal liver parenchyma with hepatic function preserved. The literature reveals four previous reports of successful LTx for PC deficiency, but there have been no reports of DLT for PC deficiency (6–9). To the best our knowledge, this is the first case of LDLT for a pediatric recipient with MSUD, who in turn became a donor for a pediatric recipient with PC deficiency.

The use of whole liver for DLT can pose increased technical difficulty during operation (20). Livers obtained from patients with MSUD who had undergone LDLT inherently lack the retro-hepatic inferior vena cava and have multiple vessel and bile duct orifices. In this case, the MSUD patient and her living donor underwent 3D-CT before the operation to evaluate the anatomy of their HV, PV, and HA. We determined the cutting sites of the vessels based on the 3D-CT findings. The HV pedicle in the graft from the MSUD patient was short, and reconstruction was required in the second recipient. There were no surgical complications, and both recipients had good post-operative functional recovery.

Activated PC concentrate proved to be useful for perioperative management of PC deficiency. Prior to the transfer to our hospital, the patient had experienced thrombotic events and bleeding on many occasions while on FFP and dabigatran. While FFP and activated PC concentrate replacement may prevent thrombosis, they can cause fluid overload and pose a high risk of infection. Oral anticoagulant therapy, meanwhile, may risk fatal hemorrhage and often restricts normal childhood activities. Therefore, we decided to perform LTx for our patient.

Initiation of high-dose activated PC concentrate restored levels of PC in the perioperative period, allowing LTx to be performed safely in

Table 2. Outcome of DLT using MSUD

No.	LT for MSUD				DLT recipient				
	LTx	Age (yr)	Observation (m)	Outcome	Indication	Age (yr)	Observation (m)	Outcome	Study
1	DDLT	25	7	Alive	HCC and HCV	51	7	Alive	Khanna et al. (14)
2	DDLT	33	–	Alive	PSC	67	38	Alive	Gopasetty et al. (15)
3	DDLT	11	–	Alive	PFIC	24	25	Alive	Gopasetty et al. (15)
4	DDLT	18	–	Alive	Cystic fibrosis	20	18	Alive	Gopasetty et al. (15)
5	DDLT	23	–	Alive	Congenital hepatic fibrosis	22	20	Alive	Gopasetty et al. (15)
6	DDLT	22	–	Alive	PBC	52	5	Alive	Gopasetty et al. (15)
7	DDLT	5	–	Alive	Embryonal carcinoma	7	2	Alive	Gopasetty et al. (15)
8	LDLT	1	12	Alive	Biliary cirrhosis	2	12	Alive	Mohan et al. (16)
9	DDLT	24	30	Alive	Hemophilia A	52	30	Alive	Badell et al. (18)
10	LDLT	2	13	Alive	Biliary atresia	2	13	Alive	Feier et al. (19)
11	LDLT	1	6	Alive	PC deficiency	1	6	Alive	Present case

our patient. Notably, PC activity level reached 64% by post-operative day 4. Thereafter, PC activity steadily rose to adequate levels and the patient is doing well without any thrombotic events for over three months following the transplantation.

Based on the excellent results observed in this report, domino grafts from patients with MSUD could potentially be used in recipients who are low priority on the transplant waiting list and are likely to die without a transplant. Although elective DLT for PC deficiency remains controversial, reports of good outcome have established it as an acceptable therapeutic option. Furthermore, the continued success of DLT may help to mitigate existing ethical concerns. Nevertheless, prospective studies with long-term outcomes are needed to accurately determine the validity of DLT for PC deficiency.

Acknowledgment

The authors thank Professor George V. Mazariegos (Department of Pediatric Transplantation, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, USA), Professor Jean de Ville de Goyet (Department of Pediatric Surgery and Transplantation, Bambino Gesù Children's Hospital, Rome, Italy), and Professor João Seda Neto (Hepatology and Liver Transplantation, Hospital Sirio-Libanes, São Paulo, Brazil) for their advice on performing the project. Dr. Julian Tang of the Department of Education for Clinical Research, National Center for Child Health and Development, proofread and edited this manuscript.

Conflict of interest

The authors of this manuscript have no conflict of interest.

References

1. Peters C, Casella JF, Marlar RA, Montgomery RR, Zinkham WH. Homozygous protein C deficiency: Observations on the nature of the molecular abnormality and the effectiveness of warfarin therapy. Pediatrics 1988; 81: 272–276.
2. Dreyfus M, Magny JF, Bridey F, et al. Treatment of homozygous protein C deficiency and neonatal purpura fulminans with a purified protein C concentrate. N Engl J Med 1991; 325: 1565–1568.
3. Ohga S, Ishiguro A, Takahashi Y, et al.; Japan Childhood Thrombophilia Study Group. Protein C deficiency as the major cause of thrombophilias in childhood. Pediatr Int 2013; 55: 267–271.
4. Branson HE, Katz J, Marble R, Griffin JH. Inherited protein C deficiency and coumarin-responsive chronic relapsing purpura fulminans in a newborn infant. Lancet 1983; 2: 1165–1168.
5. Hubbard AR. Standardization of protein C in plasma: Establishment of an international standard. Thromb Haemost 1988; 59: 464–467.
6. Casella JF, Lewis JH, Bontempo FA, Zitelli BJ, Markel H, Starzl TE. Successful treatment of homozygous protein C deficiency by hepatic transplantation. Lancet 1988: 1: 435–438.
7. Angelis M, Pegelow CH, Khan FA, Verzaro R, Tzakis AG. En bloc heterotopic auxiliary liver and bilateral renal transplant in a patient with homozygous protein C deficiency. J Pediatr 2001: 138: 120–122.
8. Florman SS, Fishbein TM, Schiano T, Letizia A, Fennelly E, DeSancho M. Multivisceral transplantation for portal hypertension and diffuse mesenteric thrombosis caused by protein C deficiency. Transplantation 2002: 74: 406–407.
9. Lee MJ, Kim KM, Kim JS, Kim YJ, Lee YJ, Ghim TT. Long-term survival of a child with homozygous protein C deficiency successfully treated with living donor liver transplantation. Pediatr Transplant 2009: 13: 251–254.
10. Wilczek HE, Larsson M, Yamamoto S, Ericzon BG. Domino liver transplantation. J Hepatobiliary Pancreat Surg 2008: 15: 139–148.
11. Kitchens WH. Domino liver transplantation: Indications, techniques, and outcomes. Transplant Rev 2011: 25: 167–177.
12. Popescu I, Dima SO. Domino liver transplantation: How far can we push the paradigm? Liver Transpl 2012: 18: 22–28.
13. Barshop BA, Khanna A. Domino hepatic transplantation in maple syrup urine disease. N Engl J Med 2005: 353: 2410–2411.
14. Khanna A, Hart M, Nyhan WL, Hassanein T, Panyard-Davis J, Barshop BA. Domino liver transplantation in maple syrup urine disease. Liver Transpl 2006: 12: 876–882.
15. Gopasetty M, Soltys K, Bond J, Sindhi R, Nayyar N, Mazariegos G. Domino liver transplantation from children and adults with maple syrup urine disease. Am J Transplant 2010: 10(Suppl 1): 68–69.
16. Mohan N, Karkra S, Kumaran V, et al. Twenty-two month liver recipient with maple syrup urine disease donates liver to another toddler: A report on the world's youngest domino pair. Liver Transpl 2010: 16(Suppl 1): S128.
17. Mazariegos GV, Morton DH, Sindhi R, et al. Liver transplantation for classical maple syrup urine disease: Long-term follow-up in 37 patients and comparative United Network for Organ Sharing experience. J Pediatr 2012: 160: 116–121.
18. Badell IR, Hanish SI, Hughes CB, et al. Domino liver transplantation in maple syrup urine disease: A case report and review of the literature. Transplant Proc 2013: 45: 806–809.
19. Feier FH, Miura IK, Fonseca EA, et al. Successful domino liver transplantation in maple syrup urine disease using a related living donor. Braz J Med Biol Res 2014: 47: 522–526.
20. Inomata Y, Zeledón ME, Asonuma K, et al. Whole-liver graft without the retrohepatic inferior vena cava for sequential (domino) living donor liver transplantation. Am J Transplant 2007: 7: 1629–1632.

Pediatr Transplantation 2015: 19: E142–E145

Pediatric Transplantation
DOI: 10.1111/petr.12543

A central approach to splenorenal shunt in pediatric living donor liver transplantation

Uchida H, Fukuda A, Masatoshi M, Sasaki K, Shigeta T, Kanazawa H, Nakazawa A, Miyazaki O, Nosaka S, Sakamoto S, Kasahara M. (2015) A central approach to splenorenal shunt in pediatric living donor liver transplantation. *Pediatr Transplant*, 19: E142–E145. DOI: 10.1111/petr.12543.

Abstract: The management of LSRS is a crucial problem to ensure a sufficient PV flow during pediatric LT. Although several techniques have been indicated to solve this problem, a more appropriate approach to LSRS is still needed in pediatric LT. We herein present a modified surgical approach to the ligation of LSRS via the left side of the IVC for a nine-month-old boy with severe portal hypertension and a history of Kasai portoenterostomy. LSRS was identified and exposed through the left side of the IVC and the dorsal surface of the pancreas from the superior side of the body of the pancreas. The post-operative course was uneventful with an excellent PV flow. The central approach for the ligation of LSRS is worth considering as an alternative procedure for a patient with collateral vessels and a history of multiple laparotomies.

Hajime Uchida[1], Akinari Fukuda[1], Matsunami Masatoshi[1], Kengo Sasaki[1], Takanobu Shigeta[1], Hiroyuki Kanazawa[1], Atsuko Nakazawa[2], Osamu Miyazaki[3], Shunsuke Nosaka[3], Seisuke Sakamoto[1] and Mureo Kasahara[1]

[1]Organ Transplantation Center, National Center for Child Health and Development, Tokyo, Japan, [2]Division of Clinical Pathology, National Center for Child Health and Development, Tokyo, Japan, [3]Division of Radiology, National Center for Child Health and Development, Tokyo, Japan

Key words: living donor liver transplantation – pediatric liver transplantation – portal vein flow – splenorenal shunt

Hajime Uchida, MD, National Center of Child Health and Development, Transplantation Center, 2-10-1 Okura, Setagaya, Tokyo 157-8535, Japan
Tel.: +81 3 3416 0181
Fax: +81 3 3416 2222
E-mail: uchida-h@ncchd.go.jp

Accepted for publication 20 May 2015

The management of the collateral vessels including an LSRS is crucial for avoiding the PV flow steal syndrome observed during LT. Although adequate and normalized PV pressure can lead to the spontaneous obliteration of the collateral vessels after deceased donor LT, an LSRS may continue to steal PV flow (1). In pediatric LT, selecting the appropriate technique for PV reconstruction and the ligation of the collateral vessels is essential to ensure a sufficient volume of PV flow (2, 3). Several techniques have been indicated to solve the LSRS problem in both living donor and deceased donor LT. Previously reported surgical techniques to solve the portal flow steal by an LSRS include direct ligation through the transverse mesentery, direct division with splenectomy, or ligation of left renal vein left to the IVC after the Kocher maneuver (4). Nevertheless, these techniques are all associated with some risks of surgical complications for patients with multiple collateral vessels and/or a potential risk for the development of renal insufficiency and/or a history of multiple laparotomies. In candidates for pediatric LT, most of the patients have a history of multiple laparotomies and/or congenital vascular malformation and/or the potential risk for the development of renal insufficiency, such as polycystic kidney disease and the history of renal transplantation. Appropriate strategies are essential for obtaining good outcomes and avoiding unnecessary complications. We herein review previous cases which required LSRS ligation during LT in our institution and present a modified surgical approach to the ligation of LSRS via the left side of IVC.

Abbreviations: BA, biliary atresia; BW, body weight; CT, computed tomography; IVC, inferior vena cava; LSRS, large splenorenal shunt; LT, liver transplantation; PV, portal vein; SRS, splenorenal shunt.

A review of cases required ligation of LSRS in LT

A total of 311 LTs were performed between November 2005 and December 2014 at the National Center for Child Health and Development, Tokyo, Japan, and seven patients (2.3%) required LSRS ligation during LT to obtain a sufficient PV flow (Table 1). The mean diameter of the LSRS was 10.6 mm (7–24.9 mm). For the ligation of LSRS, one of three types was selected

Table 1. Patients with the ligation of LSRS during LT

Patient	Age, sex (yr)	Indication for LT	BW (kg)	GRWR (%)	LSRS diameter (mm)	Number of previous laparotomy	Collateral vessels	Type of ligation	PV flow in POD7 (cm/s)	Vascular complication (follow-up period)
1	0.4, M	BA	6.9	3.23	7.3	2	Yes	Type 1	39.4	None (7 months)
2	0.5, F	BA	6.4	3.50	7.1	1	Yes	Type 1	36.7	None (3.5 yr)
3	2, F	BA	9.5	2.24	7	2	Yes	Type 1	29.4	None (7 months)
4	5, M	CHF	15	1.18	7.8	6	Yes	Type 2	35.9	None (2 months)
5	6, M	CAPV	16	1.96	9.9	4	Yes	Type 1	34.6	None (2 months)
6	18, M	CHF	32	1.29	24.9	0	Yes	Type 2	27.2	None (2.5 yr)
The presented case	0.6, M	BA	5.9	2.98	10.5	1	Yes	Type 3	19.6	None (8 months)

CAPV, congenital absence of the portal vein; CHF, congenital hepatic fibrosis; GRWR, graft recipient weight ratio; POD, post-operative day.

according to a history of laparotomy or according to a development of collateral vessels. The method of the ligation of the left renal vein is not performed in our institution. Type 1 ligation involves the ligation of LSRS through the transverse mesentery, type 2 involves the ligation of LSRS after the Kocher maneuver, and type 3 is the presented method.

Case

The patient was a nine-month-old male with BA. Kasai portoenterostomy was performed at five months of age. The patient was referred to our institute with an indication for LT due to recurrent cholangitis and a failure to thrive, with a Child-Pugh score of class C and a Pediatric End-Stage Liver Disease score of 15. The patient's height was 58.6 cm (−4.6 s.d.), and BW was 5.9 kg (−2.7 s.d.). Pre-operative CT revealed an attenuated PV trunk with developed collateral vessels, including the LSRS (Fig. 1). Collateral vessels were mainly present around the spleen and paracolic gutter. An abdominal Doppler ultrasound showed a decreased PV flow (9.3 cm/s). The patient underwent living donor LT with a left lateral segment graft from his father, who had an identical ABO blood type. The graft-to-recipient weight ratio was 2.82%. After the dissection of the PV trunk up to the confluence of the superior mesenteric vein and the splenic vein, the attenuated PV had to be replaced by an interposition graft of the inferior mesenteric vein from the donor. The left renal vein and LSRS were identified and exposed through the left side of IVC and dorsal surface of the pancreas from the superior side of the body of the pancreas (Fig. 2). The significant SRS was ligated to obtain sufficient front flow of PV. Doppler ultrasound showed a sufficient PV flow (46.1 cm/s) after reperfusion. The patient was discharged 35 days after transplantation. His post-operative course was uneventful, with an excellent PV flow ranging between 27.3 cm/s and 34.8 cm/s. CT scan showed patent PV and regression of the collateral vein, including the

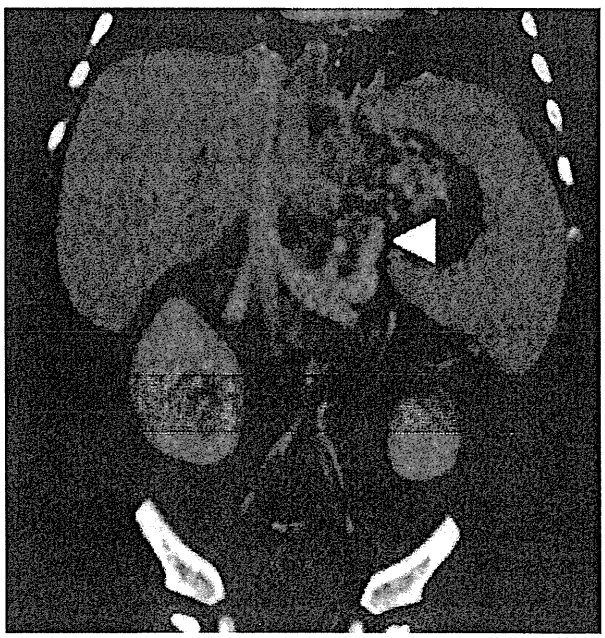

Fig. 1. Pre-operative CT angiography showed LSRS (arrowhead) in 3D reconstruction image.

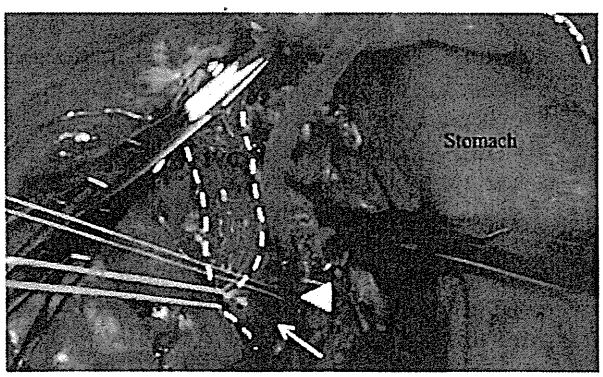

Fig. 2. The operative view, after removing the native liver, showed LSRS (arrowhead) draining into the left renal vein (arrow). IVC is surrounded by a dotted line.

Fig. 3. The post-operative 3D reconstruction image showed discontinued LSRS and no findings of PV and splenic vein thrombosis.

significant SRS on POD29 (Fig. 3). The patient had no complication with a good PV flow (29.3 cm/s) during eight months of follow-up.

Discussion

The management of LSRS is crucial to obtain adequate PV flow, which may influence graft and patient survival (5). Previous reports have described several techniques to prevent the PV flow steal syndrome by LSRS. However, each approach has surgical complication risks. Direct exposure of the LSRS through the transverse mesentery with the ligament of Treitz approach, as a conventional procedure, may easily cause bleeding in a patient with developed collateral vessels due to portal hypertension and history of previous laparotomies. Conversely, the direct division of SRS with splenectomy may also be associated with a high risk of bleeding and infection (6, 7). Furthermore, the Kocher maneuver for a patient with a history of Kasai portoenterostomy may lead to a high risk of gastrointestinal perforation, especially duodenal perforation with a poor prognosis (8). Recently, the ligation of the left renal vein left to IVC after the Kocher maneuver has been suggested to improve PV flow. Although this approach may be a relatively safe procedure to solve the LSRS problem, it could lead to slight renal insufficiency in a few patients (4). However, in pediatric LT, it is uncertain whether the ligation of the left renal vein is safe during the long-term post-operative course. Ligation of the renal vein may be critical for pediatric patients because they will need to receive immunosuppression therapy for a long time and some of them have congenital renal dysfunction, which also poses a potential risk for the development of renal insufficiency.

Compared to these techniques, our central approach technique has several advantages. First, it can prevent unexpected complications, such as duodenal perforation and pancreatic injury leading to a devastating post-operative course, because of the minimization of releasing operations. The approach to the left renal vein may be easy under a fixed operating view after removing the native liver. Second, direct ligation of LSRS can be achieved without the risk of bleeding from the collateral vessels in patients with portal hypertension. Basically, the collateral vessels are not developed in the area from confluence of IVC and the left renal vein to SRS. Therefore, our technique may be well suited for patients with a history of multiple laparotomies and portal hypertension.

In pediatric LT, we proposed three types of techniques for the ligation of LSRS. Due to the aforementioned reasons, in our center, each technique is selected by depending on patients' case-by-case basis: type 1 for a patient with a history of multiple laparotomies or a history of duodenolysis; type 2 for a patient with several collateral vessels around Treitz ligament as a result of severe portal hypertension; and type 3 for a patient with a history of both multiple laparotomies and portal hypertension. All patients who underwent the ligation of LSRS at our institution could obtain sufficient PV flow without any vascular complications. Conversely, whether the ligation of LSRS is truly necessary is still debatable. Although controversial evidence exists, some transplant surgeon may consider that the PV steal syndrome is not observed when the liver parenchymal condition is normal and when the portal reconstruction is adequate with a low resistance to the flow even if the collateral vessels are not ligated (9, 10). Moreover, Cho et al. mentioned that a seemingly good intra-operative PV flow is not an accurate indicator for the closure of a SRS (11). However, in pediatric LT, the appropriate approach to LSRS is unknown and these hypotheses may not apply to pediatric patients. In pediatric patients, especially in infants, PV steal syndrome can cause irreversible PV thrombosis unless a sufficient PV flow is obtained (3). Therefore, a top priority during PV reconstruction may be the assurance of the PV flow for pediatric patients, instead of considering liver injuries due to a high flow of PV. Furthermore, the quality of

PV intima is also crucial for preventing PV thrombosis (12). Unfortunately, there have been no reports on an established method to evaluate the quality of PV during LT. Although the PV front flow and the degree of PV hypoplasia would be dependent on subjective judgment, PV anastomosis with the ligation of LSRS has been performed without any complications in our institution by the fixing operation team. Nevertheless, it is necessary to conduct further studies on a method for evaluating the quality of PV and the PV front flow in the future.

In conclusion, the central approach for the ligation of LSRS is worth considering as an alternative procedure to solve the PV flow problem due to LSRS.

Authors' contributions

H.U.: Participated in study design and writing of the paper; A.F.: Participated in study design and critical revision of the article for clinical content; M.M., K.S., H.K., T.S., and S.S.: Participated in the collection of the data; A.N.: Participated in critical revision of the article for physiological content; O.M. and S.N.: Participated in critical revision of the article for radiological content; and M.K.: Participated in study design and critical revision of the article for clinical content.

References

1. Castillo-Suescun F, Oniscu GC, Hidalgo E. Hemodynamic consequences of spontaneous splenorenal shunts in deceased donor liver transplantation. Liver Transpl 2011: 17: 891–895.
2. de Magnee C, Bourdeaux C, De Dobbeleer F, et al. Impact of pre-transplant liver hemodynamics and portal reconstruction techniques on post-transplant portal vein complications in pediatric liver transplantation: A retrospective analysis in 197 recipients. Ann Surg 2011: 254: 55–61.
3. Ueda M, Oike F, Kasahara M, et al. Portal vein complications in pediatric living donor liver transplantation using left-side grafts. Am J Transplant 2008: 8: 2097–2105.
4. Lee SG, Moon DB, Ahn CS, et al. Ligation of left renal vein for large spontaneous splenorenal shunt to prevent portal flow steal in adult living donor liver transplantation. Transpl Int 2007: 20: 45–50.
5. Nakano T, Lai CY, Goto S, et al. Significance of portosystemic shunt on graft survival in liver transplantation: A rat model. Transplant Proc 2008: 40: 2515–2516.
6. Troisi R, Hesse UJ, Decruyenaere J, et al. Functional, life-threatening disorders and splenectomy following liver transplantation. Clin Transplant 1999: 13: 380–388.
7. Settmacher U, Nussler NC, Glanemann M, et al. Venous complications after orthotopic liver transplantation. Clin Transplant 2000: 14: 235–241.
8. Dehghani SM, Nikeghbalian S, Kazemi K, et al. Outcome of bowel perforation after pediatric liver transplantation. Pediatr Transplant 2008: 12: 146–149.
9. Brems JJ, Hiatt JR, Klein AS, et al. Effect of a prior portasystemic shunt on subsequent liver transplantation. Ann Surg 1989: 209: 51–56.
10. Piscaglia F, Zironi G, Gaiani S, et al. Systemic and splanchnic hemodynamic changes after liver transplantation for cirrhosis: A long-term prospective study. Hepatology 1999: 30: 58–64.
11. Cho SY, Kim SH, Lee KW, Park SJ, Han SS, Kim YK. Ligation of left renal vein as a salvage procedure for splenorenal shunt after living donor liver transplantation: A case report. Transplant Proc 2009: 41: 4243–4245.
12. Gurevich M, Guy-Viterbo V, Janssen M, et al. Living donor liver transplantation in children: Surgical and immunological results in 250 recipients at Universite Catholique de Louvain. Ann Surg 2015: Jan 5. [Epub ahead of print]

Citation: Clinical and Translational Gastroenterology (2015) 6, e127; doi:10.1038/ctg.2015.55

www.nature.com/ctg

Development and Validation of a Novel Fibrosis Marker in Biliary Atresia during Infancy

Hirofumi Tomita, MD, PhD[1,2], Yasushi Fuchimoto, MD, PhD[3], Akihiro Fujino, MD, PhD[1], Ken Hoshino, MD, PhD[1], Yohei Yamada, MD[1], Yohei Masugi, MD, PhD[4], Michiie Sakamoto, MD, PhD[4], Mureo Kasahara, MD, PhD[5], Yutaka Kanamori, MD, PhD[3], Atsuko Nakazawa, MD, PhD[6], Fumiko Yoshida, MD[2], Seiya Akatsuka, MD, PhD[7], Miwako Nakano, MD, PhD[2] and Tatsuo Kuroda, MD, PhD[1]

OBJECTIVES: Most biliary atresia (BA) patients suffer from liver fibrosis and often require liver transplantation. The aim of this study was to develop and validate a novel fibrosis marker for BA patients aged <1 year—the infant BA liver fibrosis (iBALF) score—subsequent to the previously reported fibrosis marker for BA patients aged ≥1 year.

METHODS: From three institutions for pediatric surgery, BA patients and their native liver histology examinations performed at the age of <1 year were retrospectively identified and assigned to a development cohort (58 patients and 73 examinations) or validation cohort (92 patients and 117 examinations) according to their institutions. Histological fibrosis stages (F0–F4), blood test results, and clinical information at the time of liver histology examination were reviewed. The iBALF score was determined using multivariate ordered logistic regression analysis and was assessed for its associations with histological fibrosis stages.

RESULTS: The iBALF score equation was composed of natural logarithms, including serum total bilirubin level, blood platelet counts, and days of age. The score revealed a strong correlation with fibrosis stage ($r = 0.80$ and 0.73 in the development and validation cohorts, respectively; $P < 0.001$). The areas under the receiver-operating characteristic curves for diagnosing each fibrosis stage were 0.86–0.94 in the development cohort and 0.86–0.90 in the validation cohort ($P < 0.001$), indicating good diagnostic power. In addition, no patient with an iBALF score >6 (equivalent to F4) at the initial surgery survived with their native liver at 1 year of age ($n = 9$).

CONCLUSIONS: The iBALF score that was developed was a good noninvasive marker of native liver fibrosis for BA patients aged <1 year.

Clinical and Translational Gastroenterology (2015) **6**, e127; doi:10.1038/ctg.2015.55; published online 19 November 2015
Subject Category: Pediatrics

INTRODUCTION

Biliary atresia (BA) is a common cause of pediatric cholestasis due to obliterative cholangiopathy that develops in 1/5,000–1/19,000 newborns and is the most common indication for pediatric liver transplantation.[1] Because rapid progression of liver fibrosis is a prominent feature of BA patients, early diagnosis and timely surgical correction of cholestasis are needed.[1,2] In general, hepatoportoenterostomy is initially attempted to achieve initial bile drainage for most patients in whom the disease involves the bile duct at the porta hepatis (type 3 disease) and for whom a surgical anastomosis between the bile duct and the gastrointestinal tract cannot be created.[1] Although hepatoportoenterostomy can achieve initial bile drainage in 50–60% of cases, advanced liver fibrosis and possible progression of liver fibrosis after surgery lead to portal hypertension and cirrhosis.[1,2] Liver transplantation is performed secondarily when bile drainage is not achieved or when cirrhotic complications affect patients.[3] Thus, liver fibrosis is thought to be an important predictor of outcome for BA patients, for whom long-term survival with the native liver is only achieved in ~20%.[2,3]

Although assessment of liver fibrosis is considered to be useful in BA patients, liver histology examinations are generally performed only at the same time as surgical procedures; liver tissue is obtained via surgical wedge biopsy during laparotomy or total hepatectomy during liver transplant surgery; postsurgical liver biopsy examinations for monitoring fibrosis progression are not generally performed.[2] However, we have performed postsurgical liver biopsy examinations to more precisely evaluate native liver status and to determine the optimal timing for liver transplantation, mostly from living donors in Japan, in clinical practice. Because reliable, surrogate, noninvasive liver fibrosis markers in BA patients have been limited,[2] we previously developed a BA liver fibrosis (BALF) score using a retrospective analysis of postsurgical native liver histology examinations.[4] The BALF score was calculated using standard liver test results and age and is a potential liver fibrosis marker in BA patients aged ≥1 year; however, the score was unable to predict liver fibrosis in

[1]Department of Pediatric Surgery, Keio University School of Medicine, Tokyo, Japan; [2]Department of Pediatric Surgery, Saitama City Hospital, Midori-ku, Saitama-shi, Japan; [3]Department of Surgery, National Center for Child Health and Development, Okura Setagaya-ku Tokyo, Japan; [4]Department of Pathology, Keio University School of Medicine, Shinjuku-ku, Japan; [5]Department of Transplant Surgery, National Center for Child Health and Development, Okura Setagaya-ku Tokyo, Japan; [6]Department of Pathology, National Center for Child Health and Development, Okura Setagaya-ku Tokyo, Japan and [7]Department of Pathology, Saitama City Hospital, Midori-ku, Saitama-shi, Japan
Correspondence: Tatsuo Kuroda, MD, PhD, Department of Pediatric Surgery, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: kuroda-t@z8.keio.jp
Received 25 June 2015; accepted 12 October 2015

patients aged <1 year.[4] Because some patients require primary or early liver transplantation owing to rapid progression of liver fibrosis, we considered that an available, reliable and noninvasive liver fibrosis marker during infancy would be of great worth. In the current study, we developed a novel noninvasive fibrosis marker for BA patients aged <1 year, subsequent to the previously reported BALF score. This novel fibrosis marker was delineated as the infant BALF (iBALF) score and was validated in an independent population of BA patients.

METHODS

Study population and ethical considerations. The medical records of BA patients at three institutions for pediatric surgery were retrospectively reviewed, and 155 patients from whom native liver specimens had been obtained at <1 year of age between March 1993 and April 2014 were identified. The patients were assigned to either the development cohort ($n=60$) or the validation cohort ($n=95$), according to the participating institutions: the development cohort derived from Keio University Hospital and Saitama City Hospital, and the validation cohort derived from the National Center for Child Health and Development. We confirmed that the development and validation cohorts did not share the same patient. This study conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the ethical committees of all three participating institutions. All of the biopsies and surgeries were performed after obtaining written informed consent.

Liver tissue sampling and histology examinations. During the initial bile drainage surgery, wedge biopsy examinations were performed using surgical resection from the edge of the liver. Postsurgical liver histology examinations were performed in several patients from wedge biopsy specimens during re-laparotomy and from percutaneous liver biopsy specimens of ≥1.0 cm in length using an 18-gauge suction needle under ultrasonographic guidance. Explanted livers were obtained during liver transplant surgery and were histologically examined. Histological liver fibrosis stages were based on the documented findings by experienced pathologists at the time liver tissue samples were obtained; if needed, re-evaluation by an experienced pathologist participating in the current study was performed at each institution. For liver fibrosis grading, the Metavir scoring system[5] or the new Inuyama classification[6] was used with the following classifications: F0, no portal fibrosis; F1, portal fibrosis without septa; F2, portal fibrosis with rare septa; F3, numerous septa or lobular distortion without cirrhosis; and F4, cirrhosis.

Data collection and data exclusion. The patients' clinical information and blood test results were collected from the medical records in association with liver histology examinations. The collected clinical information included sex, disease type, history of surgical procedure, age at the time of surgery, age at tissue sampling, and method of tissue sampling. Patients who had a history of splenectomy or partial splenic embolization and those with BA splenic malformation syndrome were excluded. The disease type was determined according to the classification of the Japanese Biliary Atresia Society:[7] atresia at the level of the most proximal part of the common bile duct (type 1), hepatic duct (type 2), and porta hepatis (type 3). The collected blood test results included serum total bilirubin (TB), direct bilirubin, aspartate aminotransferase, alanine aminotransferase, γ-glutamyltransferase (GGT), albumin, and cholinesterase levels; prothrombin time-international normalized ratios; and platelet counts, which had been examined within a few days before liver tissue sampling. The impact of transfusion, cholangitis, and vitamin K deficiency on the blood test results was excluded to the greatest extent possible; if transfusion had been performed or cholangitis had occurred before liver tissue sampling, data preceding transfusion or cholangitis up to 1 month were used, whereas in cases of vitamin K deficiency at the time of initial surgery, data after correction of vitamin K deficiency were used. Cholangitis was defined as fever and serum TB elevation without any other apparent cause, and vitamin K deficiency was defined as coagulopathy that improved soon after vitamin K administration.

Development of the iBALF score. Development of the iBALF score was accomplished using a similar method to BALF score development.[4] To predict the histological fibrosis stage, ordered logistic regression analyses were performed, using the semiquantitative histological fibrosis grading as ordinal data (from F0 to F4) for the dependent variable; the logarithmic values of the collected blood test results and days of age at the time of corresponding histological examination served as the independent variables. To determine the iBALF score equation, significant independent variables and the regression coefficients from the multivariate analysis were used. The constant of the score equation was determined by bringing the cutoff values of the iBALF score for fibrosis prediction close to the previously reported BALF score cutoff values in patients aged ≥1 year (2.42 for ≥F2, 4.12 for ≥F3, and 5.64 for F4).[4]

Assessment of the iBALF score. After determination of the iBALF score equation from the development cohort, the scores were calculated from the development and validation cohort data; the values of the iBALF score were obtained along with the corresponding histological examination results. The diagnostic power of the iBALF score for predicting each fibrosis stage was assessed using a receiver-operating characteristic curve comparing the blood platelet counts and the aspartate aminotransferase-to-platelet ratio index (APRI), which has been the most widely investigated fibrosis marker in BA patients. The APRI was calculated using the following equation:[8]

APRI = (aspartate aminotransferase/upper normal limit/platelet counts (10^9/l)) × 100.

The upper normal limit of aspartate aminotransferase was determined according to the age-specific reference intervals for Japan.[9]

Assessment of the prognosis at 1 year of age. The prognosis of the patients who participated in the study from the initial surgery (initial bile drainage surgery or primary

liver transplantation) was assessed using serial data collection. The prognosis at 1 year of age was investigated as either death before liver transplantation, receiving liver transplantation before 1 year of age, or surviving with their native liver. Among the patients surviving with their native liver at 1 year of age, the earliest blood test results after reaching 1 year of age were collected from the medical records; if transfusion had been performed or cholangitis had occurred before the blood test was performed, the data at >1 month after transfusion or cholangitis were selected. The BALF score that had been developed to predict liver fibrosis stage in BA patients aged ≥ 1 year was then used to evaluate the status of the native liver. The BALF score was calculated using the following equation:[4]

BALF score = 7.196+1.438 × Log_e [TB (mg/dl)]+0.434 × Log_e [GGT (IU/l)] − 3.491 × Log_e [albumin (g/dl)]−0.670 × Log_e [age (years)].

Statistical analysis. The categorical and ordinal data are presented as frequencies and were statistically compared using the Fisher exact test. The continuous data are presented as medians (ranges) and were statistically compared using the Mann–Whitney *U*-test. Correlations between the ordinal and/or continuous data were assessed by the Spearman correlation coefficient (r). For logistic regression analyses, the P value of each independent variable was determined using the Wald χ^2-value (Wald), which was calculated by squaring the ratio of the regression coefficient divided by its standard error. For receiver-operating characteristic curve analyses, areas under the curve (AUCs) were calculated; an AUC of 1.0 indicates a test of perfect diagnostic power, whereas an AUC of 0.5 indicates no diagnostic power. Differences between AUCs were examined using the DeLong test. The cutoff values were determined as the points that showed high sensitivity and specificity in a balanced manner. P values <0.05 were considered statistically significant. Statistical analyses were performed using SPSS 22.0 software (IBM SPSS, Chicago, IL, USA) and R 3.1.0 software (The R Foundation for Statistical Computing Vienna, Austria; http://www.R-project.org/).

RESULTS

Patient characteristics. We excluded two and three patients with BA splenic malformation syndrome from the development and validation cohorts, respectively. No patient had a history of splenectomy or partial splenic embolization before data collection. One histology examination using percutaneous needle biopsy obtained after the initial surgery from a development cohort patient was inappropriate for evaluation and was excluded from the study. After exclusions, the development cohort included 58 patients and 73 liver histology examinations, and the validation cohort included 92 patients and 117 liver histology examinations. The timing of the patients' participation and tissue sampling in the development and validation cohorts is summarized in Figure 1. Patient characteristics according to the development and validation cohorts are shown in Table 1. Significant differences between the development and validation cohorts were found in the frequencies of disease type ($P=0.02$) and initial bile drainage surgical procedure ($P=0.03$): the validation cohort included more patients with type 3 disease requiring hepatoportoenterostomy. Significant differences regarding liver transplantation before 1 year of age were also found: the validation cohort included fewer patients received primary liver transplantation, and more patients received liver transplantation after bile drainage surgery than in the development cohort ($P<0.001$). Days of age at the time of liver transplantation were significantly lower in the validation cohort than in the development cohort ($P=0.009$).

Liver histology and blood test results. In the development cohort, 10 (13.7%) histology examinations showed a liver fibrosis stage of F1, whereas 19 (26.0%) showed a stage of F2, 20 (27.4%) showed a stage of F3, and 24 (32.9%) showed a stage of F4. In the validation cohort, eight (6.8%) histology examinations showed a stage of F1, 23 (19.7%) showed a stage of F2, 27 (23.1%) showed a stage of F3, and 59 (50.4%) showed a stage of F4. Liver histology examinations and the corresponding blood test results from the development and validation cohorts according to the biopsy examination or liver transplantation are presented in Table 2. At the time of biopsy examinations, serum direct bilirubin levels were significantly lower and serum albumin levels were significantly higher in the development cohort than in the validation cohort ($P=0.03$ and $P<0.001$, respectively), because the development cohort involved a greater number of needle biopsy examinations, which were performed for patients with a better surgical response than the validation cohort ($P=0.002$). At the time of liver transplantation, blood test results were significantly worse in the development cohort than in the validation cohort, indicating different timing of liver transplant surgery between the cohorts.

Determination of the iBALF score equation. The results of the ordered logistic regression analyses in the development cohort are shown in Table 3. In the univariate analyses, natural logarithms of the blood platelet counts provided the highest significance (Wald = 31.461, $P<0.001$). In the multivariate analysis, the second significant independent variable was identified as natural logarithms of the serum TB levels using a forward selection method. As the third independent variable, natural logarithms of the prothrombin time-international normalized ratios and days of age were significant; we selected the days of age, because the distribution of the iBALF score approached the distribution of the previously reported BALF score. Finally, natural logarithms of the serum TB levels, blood platelet counts, and days of age at examination were selected as significant independent variables. The iBALF score equation was determined as:

iBALF score = 8+1.185 × Log_e [TB (mg/dl)] − 1.882 × Log_e [platelet count (10^9/l)]+1.093 × Log_e [age (days)].

iBALF scores according to the liver fibrosis stages. Figure 2 shows the boxplots for the iBALF score and APRI vs. the histological fibrosis stages in the development and validation cohorts. The iBALF score was more strongly correlated with the histological fibrosis stage than the APRI in both cohorts ($r=0.80$ and 0.73 in the development and validation cohorts, respectively; $P<0.001$). Between the

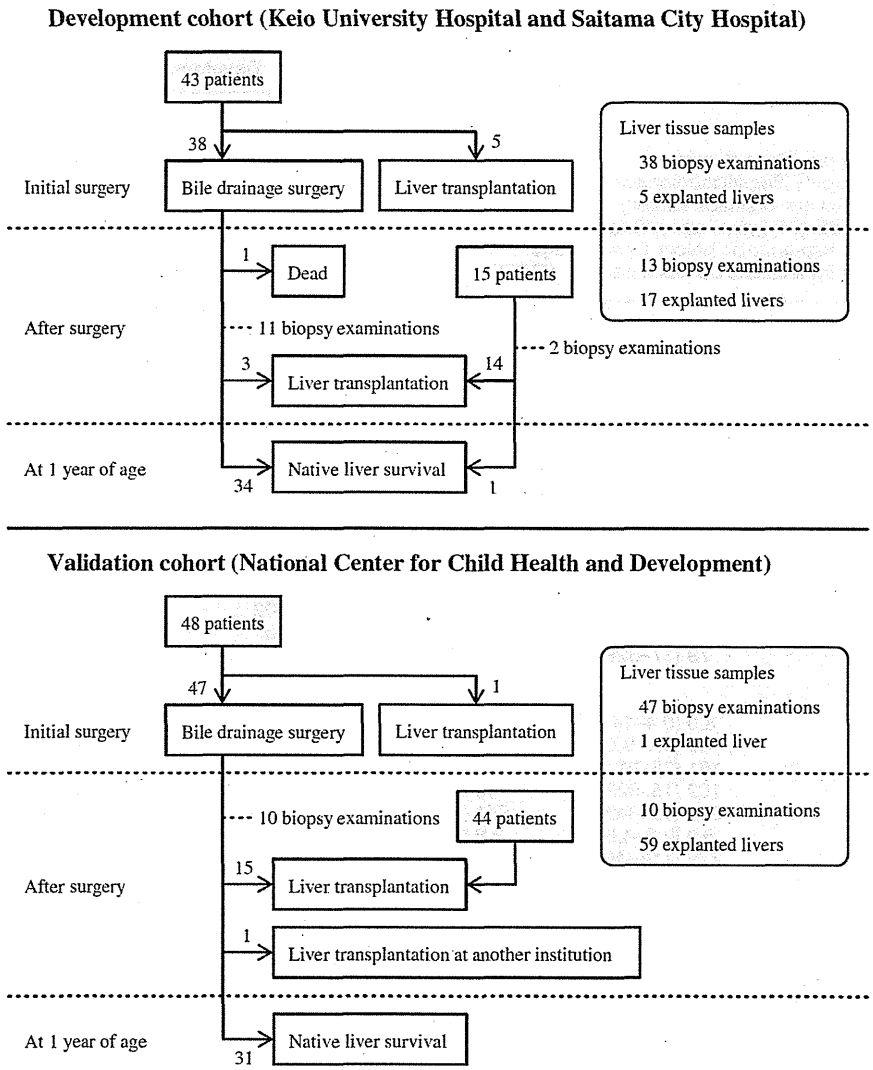

Figure 1 Timing of the patients' participation and tissue sampling in the development and validation cohorts. The number was counted after excluding two and three patients with biliary atresia splenic malformation syndrome from the development and validation cohorts, respectively.

cohorts, the iBALF score in the histology examinations displaying F4 showed a significant difference ($P=0.006$); the median iBALF score values were 8.08 (range, 4.75–10.71) in the development cohort and 6.84 (range, 2.88–9.69) in the validation cohort. No significant difference was found in the other histological fibrosis stage groups.

Diagnostic power of the iBALF score. Figure 3 shows the receiver-operating characteristic curves of the iBALF score for diagnosing each fibrosis stage, compared with the APRI. In the development cohort, the AUCs of the iBALF score were 0.84 for a fibrosis stage ≥F2, 0.91 for ≥F3, and 0.96 for F4 ($P<0.001$). In the validation cohort, the AUCs of the iBALF score were 0.86 for ≥F2, 0.90 for ≥F3, and 0.89 for F4 ($P<0.001$); the diagnostic power for F4 fibrosis appeared to be worse than in the development cohort. The AUCs of the iBALF score were significantly greater than those of the APRI in diagnosing ≥F2 ($P=0.03$) and F4 ($P=0.01$) in the development cohort, indicating more favorable diagnostic power than the APRI; no significant difference was found in diagnosing ≥F3 in the development cohort and in diagnosing ≥F2, ≥F3, and F4 in the validation cohort.

Cutoff value and diagnostic accuracy of the iBALF score. The cutoff values and diagnostic accuracies of the iBALF score for predicting histological fibrosis stages are shown in Table 4. The cutoff values of the development cohort were 3.00 for a fibrosis stage ≥F2, 3.99 for ≥F3, and 5.75 for F4, which were brought close to the previously reported cutoff values of the BALF score by adjusting the constant of the iBALF score equation. The diagnostic accuracies of the iBALF score for each fibrosis stage were acceptable: 78.1–93.2% in the development cohort and 80.3–82.9% in the validation cohort. The validation cohort appeared to have lower diagnostic accuracy for F4 diagnosis than the development cohort (82.0% vs. 93.2%, respectively).

Table 1 *Patient characteristics of the development and validation cohorts*

	Development cohort	Validation cohort	*P*-value
Number of patients	58	92	
Sex (male/female)	25/33	28/64	0.12
Disease type (type 1/type 2/type 3/unknown)	9/2/45/2	6/0/85/1	0.02
Initial bile drainage surgery (hepaticoenterostomy/hepatoportoenterostomy/none)	3/50/5	2/89/1	0.03
Days of age at the initial bile drainage surgery	74 (17–151) ($n=53$)	73 (27–195) ($n=91$)	0.28
Liver transplantation before 1 year of age (primary/after bile drainage surgery/none)	5/17/36	1/60/31	<0.001
Days of age at liver transplantation before 1 year of age	290 (179–356) ($n=22$)	233 (126–346) ($n=61$)	0.009
Number of histology examinations per each patient (1/2/3/4)	46/10/1/1	69/21/2/0	0.59

The categorical and ordinal data are presented as the number of patients and were statistically compared using the Fisher exact test. The continuous data are presented as medians (ranges) and were statistically compared using the Mann–Whitney *U*-test.

Table 2 Comparisons of the liver histology examinations and corresponding blood test results between the development and validation cohorts according to the biopsy examination or liver transplantation

	Biopsy examination			Liver transplantation		
	Development cohort	Validation cohort	*P*-value	Development cohort	Validation cohort	*P*-value
Number of examinations	51	57		22	60	
Wedge/needle	41/10	56/1	0.002			
Fibrosis stage (F1/F2/F3/F4)	10/19/18/4	8/23/19/7	0.78	0/0/2/20	0/0/8/52	0.72
Days of age	79 (17–328)	77 (27–345)	0.96	290 (179–356)	232 (126–346)	0.01
Blood test results						
TB (mg/dl)	8.0 (0.4–14.5)	8.3 (0.6–25.8)	0.06	20.6 (5.5–47.7)	12.1 (1.2–33.9)	<0.001
DB (mg/dl)	4.9 (0.1–9.5)	5.6 (0.3–17.6)	0.03	14.5 (3.2–34.4)	8.7 (0.6–22.1)	0.001
AST (IU/l)	161 (35–917)	150 (44–473)	0.77	269 (55–560)	162 (61–659)	0.007
ALT (IU/l)	109 (15–922)	110 (24–447)	0.98	127 (30–240)	110 (29–426)	0.44
GGT (IU/l)	582 (62–3434)	741 (36–2610)	0.15	124 (50–1010)	253 (20–1452)	0.28
Albumin (g/dl)	3.9 (2.3–4.8)	3.6 (2.6–4.3)	<0.001	3.2 (2.2–4.1)	3.0 (1.9–4.2)	0.72
ChE (IU/l)	279 (116–461)	270 (128–395)	0.86	140 (53–334)	143 (57–367)	0.73
PT-INR	1.03 (0.84–1.48)	1.00 (0.81–1.91)	0.19	1.41 (0.95–2.54)	1.28 (0.95–2.18)	0.047
Platelet count ($\times 10^9$/l)	448 (172–1092)	444 (111–982)	0.93	118 (48–276)	196 (34–760)	0.02

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ChE, cholinesterase; DB, direct bilirubin; GGT, γ-glutamyltransferase; PT-INR, prothrombin time-international normalized ratio; TB, total bilirubin. The categorical and ordinal data are presented as the number of examinations and were statistically compared using the Fisher exact test. The continuous data are presented as medians (ranges) and were statistically compared using the Mann–Whitney *U*-test.

Prognosis at 1 year of age according to the iBALF score at the initial surgery. Figure 4 shows the relationships between the iBALF score at the initial surgery and outcomes. The outcomes are presented as the need for liver transplantation before 1 year of age or as the BALF score at 1 year of age as a noninvasive liver fibrosis marker. None of the nine patients with an iBALF score >6 survived with their native liver: five patients in the development cohort underwent liver transplantation as the initial surgery, and four patients in the validation cohort required liver transplantation before 1 year of age. Among the patients who survived with their native liver at 1 year of age, the correlations between the iBALF score at the initial surgery and the BALF score at 1 year of age were not significant in the development ($n=34$, $r=0.19$, $P=0.29$) or validation ($n=31$, $r=0.04$, $P=0.81$) cohorts.

DISCUSSION

The BALF score was the first noninvasive fibrosis marker developed specifically for postsurgical BA patients aged ≥1 year; herein, the iBALF score was additionally developed for BA patients aged <1 year. Although the BALF score calculated for patients aged <1 year was previously reported to show apparently high values regardless of the liver fibrosis stages,[4] the iBALF score showed strong correlations with the histological liver fibrosis stages and good diagnostic powers for each fibrosis stage in the development and validation cohorts. The differences between the BALF and iBALF scores in patients aged <1 year were mainly derived from serum GGT level (included in the BALF score) and age (included in both scores), both of which had reverse coefficients in the logistic regression analyses for predicting liver fibrosis stages. Serum GGT elevation was reported to be associated with advanced fibrosis in patients aged ≥1 year,[4] but the current study indicated that serum GGT elevation was associated with less-advanced fibrosis in patients aged <1 year. The effects of age on liver fibrosis progression were positive in patients aged <1 year and negative in patients aged ≥1 year.[4] Although different equations were needed, we adjusted the iBALF score to have similar values for each fibrosis stage as the previously reported BALF score values in patients aged ≥1 year, this will aid in more easily understanding the iBALF scores in comparison with BALF scores, regardless of the age of the child. We suggest that the iBALF and BALF scores can monitor liver fibrosis in a similar manner before and after 1 year of age, respectively.

Table 3 Ordered logistic regression analyses for predicting liver fibrosis stages in the development cohort

Variable	Coefficient (95% confidence interval)	Standard error	Wald	*P*-value
Univariate analysis				
Log_e (platelet count ($\times 10^9$/l))	−2.859 (−3.858 to −1.860)	0.510	31.461	<0.001
Log_e (age (days))	1.812 (1.119–2.506)	0.354	26.213	<0.001
Log_e (TB (mg/dl))	1.517 (0.891–2.142)	0.319	22.565	<0.001
Log_e (albumin (g/dl))	−7.950 (−11.270 to −4.631)	1.694	22.038	<0.001
Log_e (PT-INR)	7.126 (4.125–10.127)	1.531	21.662	<0.001
Log_e (ChE (IU/l))	−2.841 (−4.078 to −1.604)	0.631	20.272	<0.001
Log_e (DB (mg/dl))	1.269 (0.706–1.832)	0.287	19.534	<0.001
Log_e (GGT (IU/l))	−0.926 (−1.398 to −0.454)	0.241	14.772	<0.001
Log_e (AST (IU/l))	0.924 (0.235–1.612)	0.351	6.920	0.009
Log_e (ALT (IU/l))	0.278 (−0.312–0.868)	0.301	0.852	0.36
Multivariate analysis				
Log_e (TB (mg/dl))	1.185 (0.574–1.796)	0.312	14.452	<0.001
Log_e (platelet count ($\times 10^9$/l))	−1.882 (−3.052 to −0.712)	0.597	9.935	0.002
Log_e (age (days))	1.093 (0.232–1.955)	0.439	6.190	0.01

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ChE, cholinesterase; DB, direct bilirubin; GGT, γ-glutamyltransferase; PT-INR, prothrombin time-international normalized ratio; TB, total bilirubin.

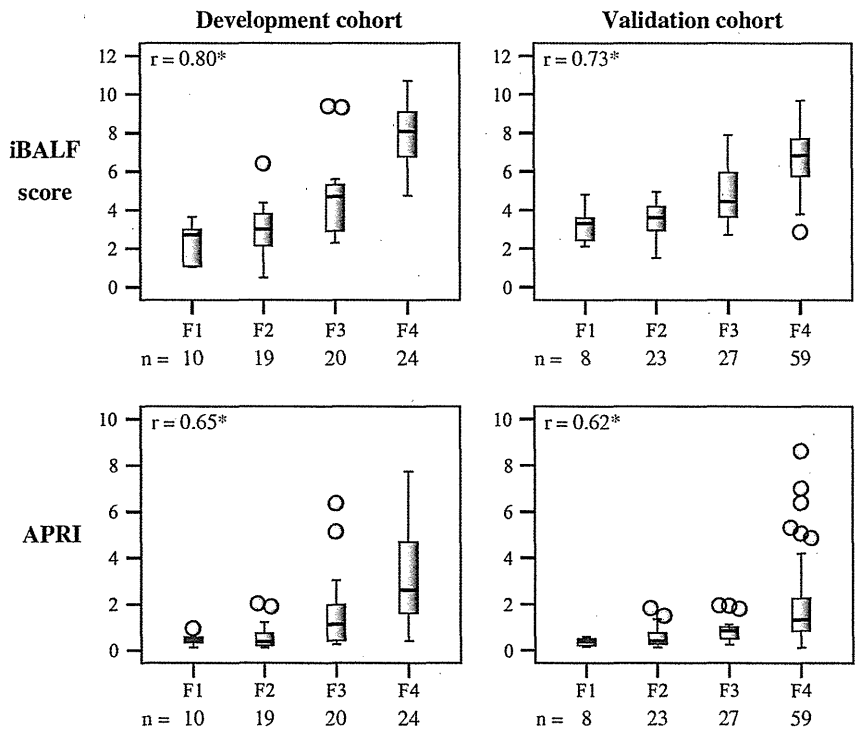

Figure 2 Values of the infant biliary atresia liver fibrosis (iBALF) score and aspartate aminotransferase-to-platelet ratio index (APRI) according to the histological fibrosis stages. Boxplots show the median values with the interquartile ranges, and error bars indicate the smallest and the largest values within 1.5 box-lengths of the upper and the lower quartiles. Circles represent the individual points for outliers. Correlations between the markers and the fibrosis stages were evaluated using the Spearman correlation coefficient (*r*); **P*<0.001.

For infants with BA at presentation, two types of surgical procedure could be chosen—bile drainage surgery or liver transplantation. There were two reports regarding effects on outcomes after liver transplantation comparing early failure of hepatoportoenterostomy, which was defined as the need for liver transplantation within the first year of life, and primary liver transplantation. Alexopoulos *et al.*[10] described that early failure of hepatoportoenterostomy adversely affected patient and graft survival rates. Neto *et al.*[11] reported that early failure of hepatoportoenterostomy had no effect on patient and graft survival, that late failure of hepatoportoenterostomy had a protective effect compared with primary liver transplantation, and that previous hepatoportoenterostomy increased biliary complications and bowel perforations after liver transplantation. Thus, it is important to know which patients can benefit from bile drainage surgery at presentation. In this study, we attempted to reveal the association between the iBALF score at the initial surgery and prognosis using the BALF score at

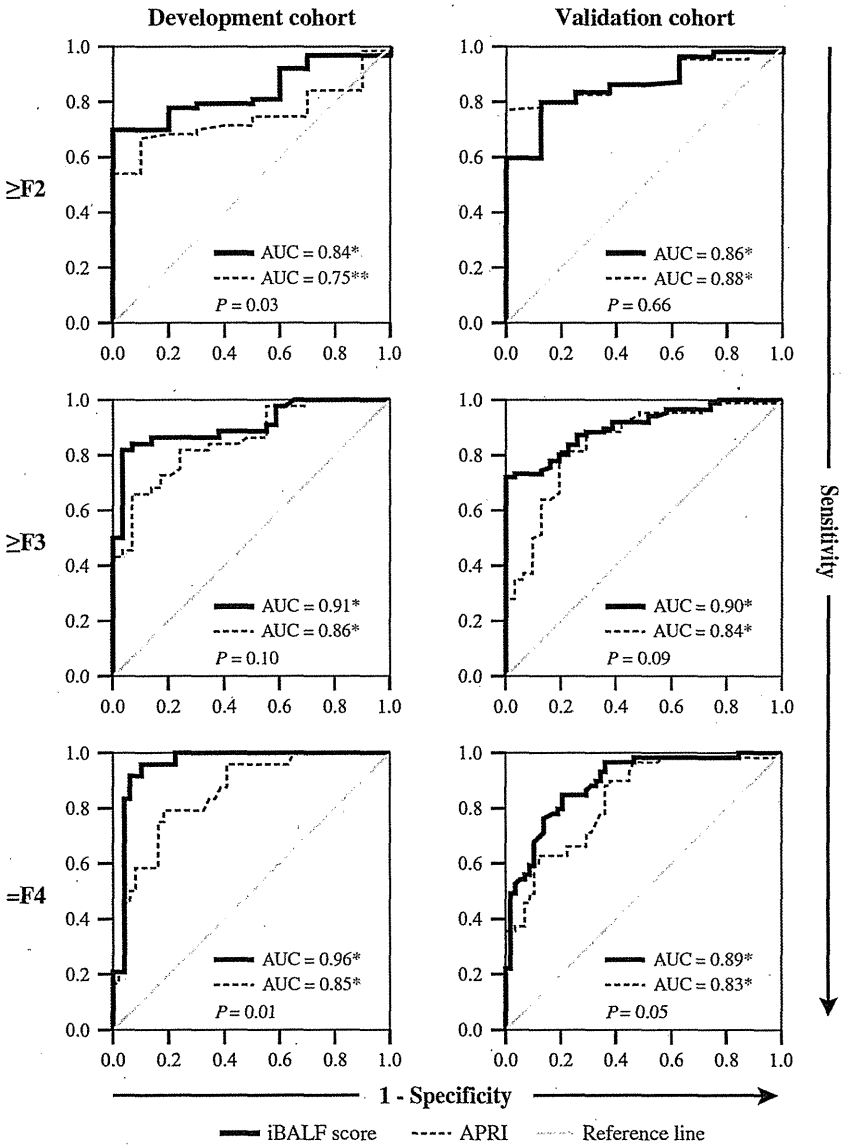

Figure 3 Receiver-operating characteristic curves of two fibrosis markers for diagnosing each fibrosis stage. Evaluated noninvasive markers included the infant biliary atresia liver fibrosis (iBALF) score (thick lines) and the aspartate aminotransferase-to-platelet ratio index (APRI, dashed lines). Gray lines indicate the reference lines. The diagnostic power of each marker was assessed by calculating the area under the curve (AUC); *$P<0.001$, **$P=0.01$. The P values in the panels represent the differences between AUCs of the iBALF score and the APRI using the DeLong test.

1 year of age. The results (Figure 4) suggest that BA patients with an iBALF score >6 at presentation might require liver transplantation rather than bile drainage surgery. However, the number of these severely affected patients was small in both cohorts. Except for these severely affected patients, the iBALF score at the initial surgery did not seem to be associated with native liver survival at 1 year of age. There was no correlation between the iBALF score at the initial surgery and the BALF score at 1 year of age among the patients with native liver survival, suggesting that liver fibrosis at the initial surgery had a limited effect on liver fibrosis progression or remission. We previously reported similar data on the actual fibrosis stages in 15 patients aged ≥2 years who underwent serial histological examinations at the time of initial surgery and after surgery and who were included in the development cohort of the current study: seven of these 15 patients showed remission of fibrosis, five showed the same fibrosis stage, and three showed progression of fibrosis.[4] We believe that effective postsurgical antifibrotic therapy for BA patients is needed and that noninvasive fibrosis monitoring would be highly valuable in clinical practice and study.

In addition to our previous report, several other studies have proposed noninvasive markers to assess liver fibrosis in BA patients. The APRI, which was originally developed to predict cirrhosis in hepatitis C patients,[8] has been widely investigated in BA patients. Kim *et al.*[12] described that the correlation coefficient between the APRI and Metavir fibrosis score from 35 patients at the time of hepatoportoenterostomy was