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

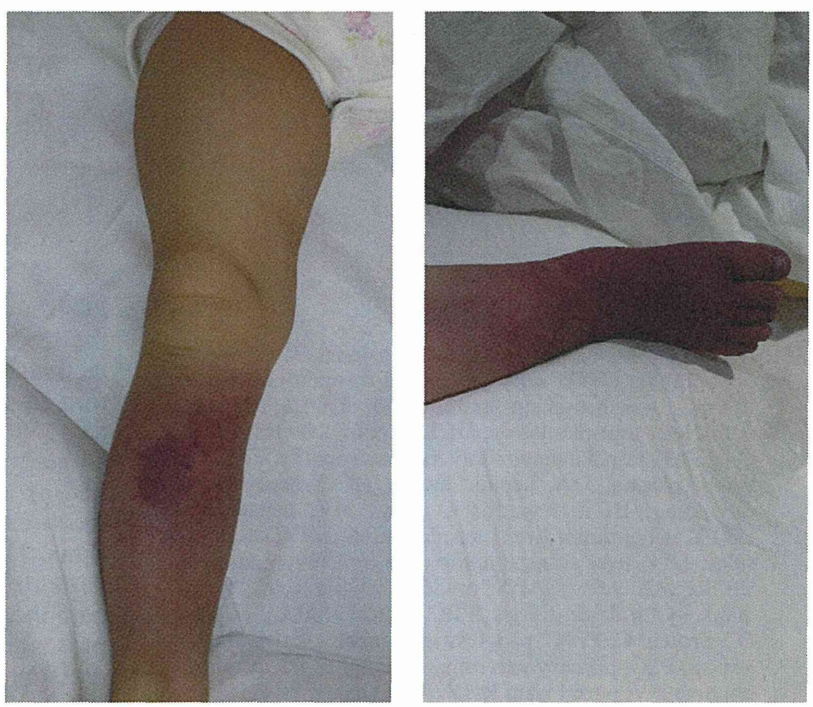

Fig. 1. Skin lesions were observed on the right foot and ankle arising from peripheral thrombotic events.

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

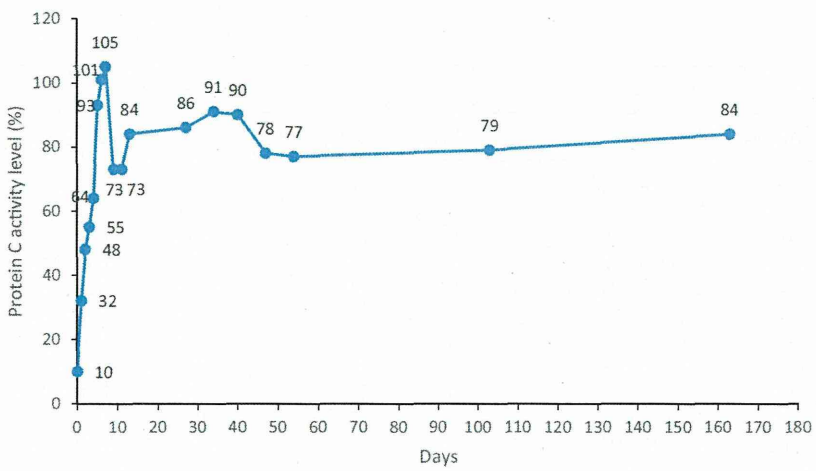

Fig. 2. Plasma PC activity levels with time after DLT.

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.

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Conflict of interest

The authors of this manuscript have no conflict of interest.

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

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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.

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

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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

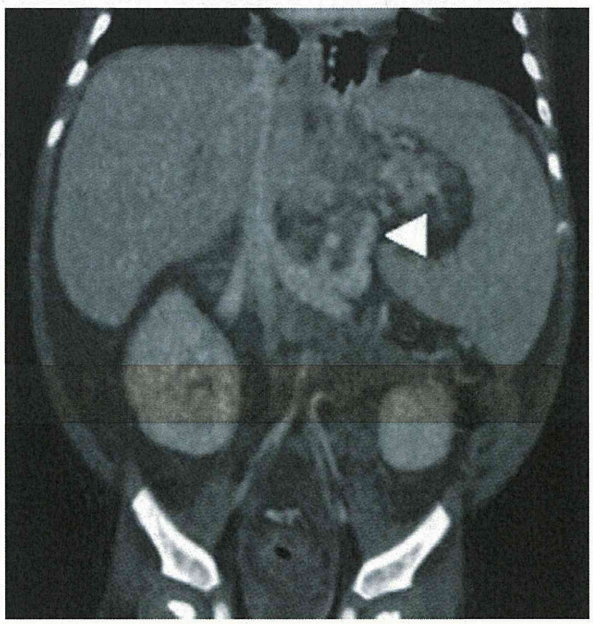

Fig. 1. Pre-operative CT angiography showed LSRS (arrowhead) in 3D reconstruction image.

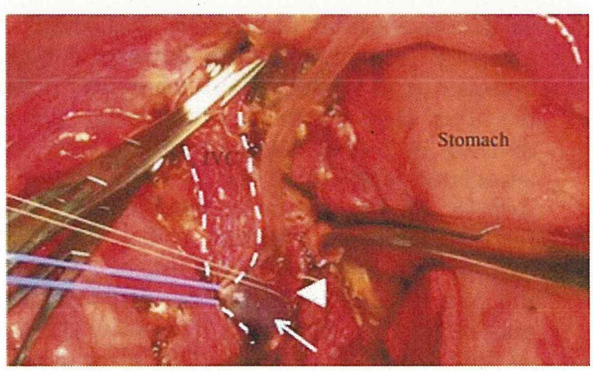

Fig. 2. The operative view, after removing the native liver, showed LSRS (arrowhead) draining into the left renal vein (arrow). IVC is surrounded by a dotted line.

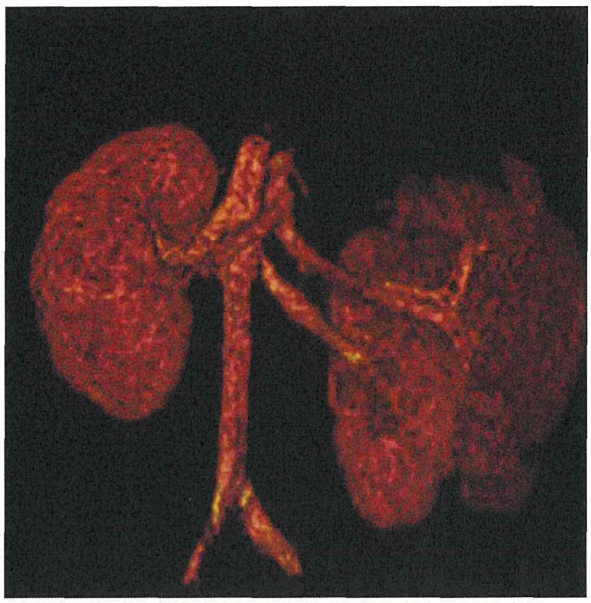

Fig. 3. The post-operative 3D reconstruction image showed discontinued LSRS and no findings of PV and splenic vein thrombosis.

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.

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

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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.

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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

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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