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# Romiplostim treatment allows for platelet transfusion-free liver transplantation in pediatric thrombocytopenic patient with primary sclerosing cholangitis

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Abstract: Thrombocytopenia is a major risk factor for cirrhotic liver disease. Patients with thrombocytopenia may have esophageal or gastric varices secondary to portal hypertension, leading to variceal bleeding which exposes the liver to further damage. Here, we present a female pediatric patient with PSC and CD, whose progressive thrombocytopenia was successfully controlled by romiplostim, a TPO receptor agonist. The patient developed bloody diarrhea at four yr of age, and was subsequently diagnosed with PSC and CD when seven yr old. While CD was well-controlled by immunomodulators, the patient's thrombocytopenia gradually progressed resulting in petechiae (platelet count of 11 × $10^9$/L) when she was 10 yr and four months old. She responded poorly to immunoglobulin and corticosteroids. Weekly subcutaneous injection of romiplostim was therefore initiated, and platelet counts were maintained over at 50 × $10^9$/L. She was able to undergo successful LDLT without platelet transfusion seven months after the initiation of romiplostim. Romiplostim was not required after LDLT with improved platelet counts. This case report suggests that romiplostim may be effective in the treatment of thrombocytopenic children with liver cirrhosis and portal hypertension, and in eliminating the need for platelet transfusion during the peri-transplant period.

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Key words: primary sclerosing cholangitis – thrombocytopenia – thrombopoietin – romiplostim – liver transplantation

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Thrombocytopenia is a risk factor for cirrhotic liver disease. Patients with thrombocytopenia may develop esophageal or gastric varices secondary to portal hypertension, leading to upper gastrointestinal bleeding that further damages the cirrhotic liver. Severe thrombocytopenia alone can be an indication for liver transplantation.

TPO, a cytokine produced mainly by the liver and kidney, stimulates megakaryopoiesis and platelet production (1). Romiplostim is a fusion protein that acts as a TPO receptor agonist. Although romiplostim has been increasingly used for treatment of chronic ITP in adults (2, 3), its use in children with thrombocytopenia has been sparse. In spite of that, the successful use of romiplostim in children suffering from chronic ITP was reported recently (4–7), suggesting that romiplostim may also be effective in the treatment of pediatric thrombocytopenia arising from chronic liver diseases. Here, we demonstrated the successful use of romiplostim in treating progressive thrombocytopenia in a 10-yr-old female patient with PSC and CD, as well as its role in eliminating the need for platelet transfusion in LDLT.

Abbreviations: ALT, alanine aminotransferase; AST, asparitc acid aminotransferase; CD, Crohn's disease; HNA, human neutrophil antigen; ITP, immune thrombocytopenic purpura; LDLT, living-donor liver transplantation; PELD, pediatric end-stage liver disease; PSC, primary sclerosing cholangitis; TPO, thrombopoietin.

**Case report**

The patient first developed bloody stool when she was four yr old, and was referred to our hospital for persistent bloody diarrhea, hepatosplenomegaly, growth retardation, and increased serum transaminases at the age of seven. Endoscopic and histologic evaluation led to diagnosis of PSC and CD. Her CD was well-controlled with the use of prednisolone followed by mesalazine and azathioprine. Leukocytopenia and thrombocytopenia, however, became evident, and the patient experienced frequent nose bleeding with platelet count of 30–50 $\times$ $10^9$/L (aged nine at that time). She was then listed as a candidate for brain-death liver transplantation. Subsequently, she was hospitalized due to a further decrease in platelet count (11 $\times$ $10^9$/L) when she was 10 yr and six months old.

At admission, her body weight and height was 28.3 kg (−0.7 s.d.) and 123.1 cm (−2.2 s.d.), respectively. On physical examination, her spleen was enlarged to 5 cm below the mid-left costal margin, and there were petechiae on the chest and extremities. Laboratory findings showed thrombocytopenia (14 $\times$ $10^9$/L), leukocytopenia (1.39 $\times$ $10^9$/L), normal level of serum albumin (3.8 g/dL), mild increase in serum transaminases (AST 60 IU/L, ALT 55 IU/L), γ-glutamyltransferase (61 IU/L), and total-bilirubin (1.38 mg/dL). While antibodies to neutrophil-specific antigens HNA-1a were present, antinuclear antibody and antiplatelet GPIIb-IIIa antibody were negative. Bone marrow aspiration demonstrated mild elevation of megakaryocytes and erythrocyte counts. There was no dysplasia or hypoplasia. The level of plasma TPO (0.80 fmol/mL) was comparable to controls (0.19–1.57 fmol/mL) (8).

Initial treatment with immunoglobulin (1 g/kg) was ineffective. Oral prednisolone (1 mg/kg/day) was then prescribed. Platelet count increased to 50–56 $\times$ $10^9$/L between one to two wk, but eventually dropped down to 10–20 $\times$ $10^9$/L in one month as the dose of prednisolone was tapered to 0.35 mg/kg/day.

After informed consent was obtained from the patient's parents, subcutaneous injection of romiplostim (2 μg/kg/wk) was initiated for severe thrombocytopenia which was difficult to control with the aforementioned conventional treatments. In the meantime, LDLT was considered for her significant cirrhosis with histologically F3 fibrosis and a PELD score of 19 (9). The dose of romiplostim was titrated to 4 μg/kg/wk by the fifth wk of treatment, which successfully brought the platelet count to over 50 $\times$ $10^9$/L. Improvement to her nose bleeding and petechiae were also observed. The patient eventually underwent LDLT (with her father as donor) when she was 11 yr and two months old. The histological study of the explanted liver revealed onion skin lesion with F3 fibrosis, which was consistent with PSC (Fig. 1). Platelet transfusion was not required throughout the perioperative period.

After the LDLT, the patient's platelet count was maintained at 41–198 $\times$ $10^9$/L without romiplostim injections. Plasma TPO level was comparable to that of pretreatment with romiplostim (0.75 fmol/mL and 0.65 fmol/mL after 10 and 14 days of LDLT, when platelet counts were 66 $\times$ $10^9$/L and 60 $\times$ $10^9$/L, respectively). The change in platelet counts, plasma TPO levels, and romiplostim dose are shown in Fig. 2. Post-surgery, the patient was put on tacrolimus

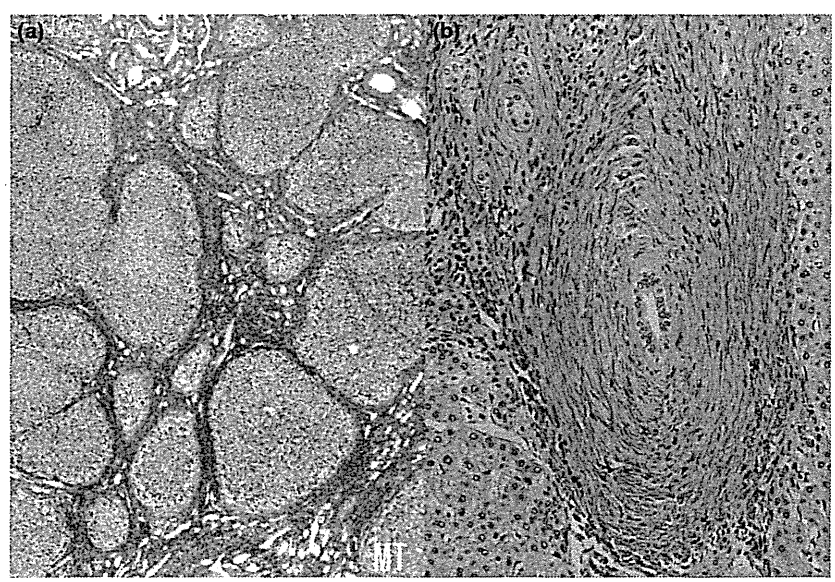


*Fig. 1.* Histopathologic findings of the explanted liver. (a) F3 fibrosis (Masson's trichrome stain, ×40). (b) Onion skin lesion (hematoxylin–eosin stain, ×200).

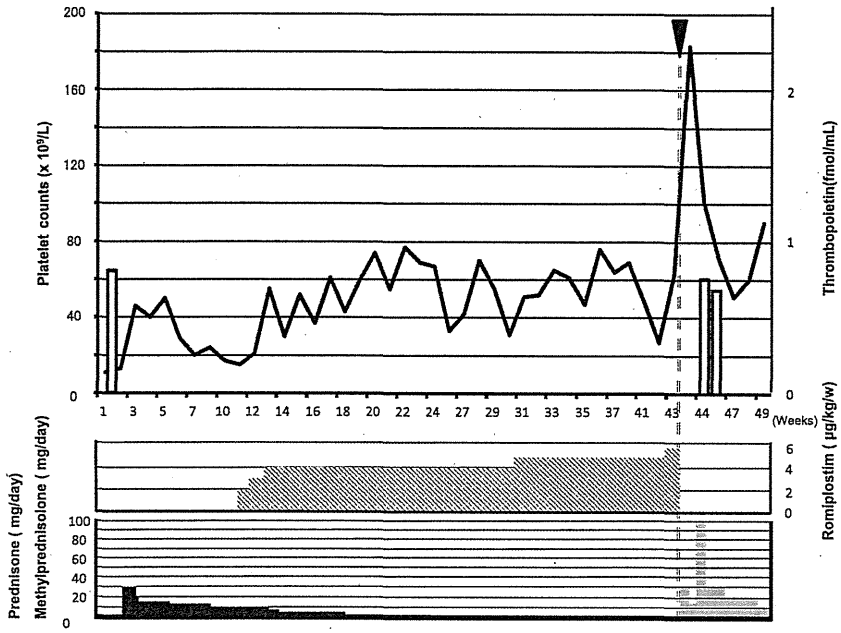


*Fig. 2.* Time course of the case: Platelet counts were increased after the initiation of romiplostim. Black arrow indicates the day of living-donor liver transplantation, while white bars indicate the levels of TPO. Diagonal striped bars, black bars, and gray bars indicate the dosage of romiplostim, prednisolone, and methylprednisolone, respectively.

and corticosteroids and recovered with no further problem reported.

## Discussion

We presented the case of a pediatric female patient with PSC and CD, whose thrombocytopenia was successfully treated with romiplostim. She was eventually able to undergo LDLT without platelet transfusion.

The clinical course of this patient suggests two important findings. First, romiplostim can be used as a therapeutic option for children with thrombocytopenia secondary to portal hypertension and hypersplenism. It can also bridge patients with thrombocytopenia to liver transplantation. The production of TPO is constitutive, and the plasma level of TPO is inversely regulated by circulating platelet count and megakaryocyte mass in the bone marrow (10). Decreased serum TPO levels in patients with liver cirrhosis as the degree of cirrhosis progresses have been reported before, while serum TPO levels remained normal in patients with chronic hepatitis (11). Impaired hepatic production of TPO may contribute to the development of thrombocytopenia in liver cirrhosis (11–13). In the present case, plasma TPO level before romiplostim administration was comparable to that of healthy children, even though thrombocytopenia is usually associated with increased TPO levels (8, 14). As LDLT was planned several months ahead (in view of her significant cirrhosis and PELD score), we decided to use romiplostim in the meantime to minimize potential complications arising from thrombocytopenia. The dose of romiplostim successfully maintained the patient's platelet count to >50 $\times$ $10^9$/L pretransplant. A likely reason for the wide range in platelet count post-surgery was the dynamic change of portal blood flow as a result of the healthy liver. Suppression of splenic function by immunosuppressive medication may have also contributed to the increased platelet numbers.

Second, romiplostim treatment can eliminate the need for platelet transfusion during the perioperative period. Patients with thrombocytopenia often require platelet transfusion during the peritransplant period. Potential adverse events or limitations of platelet transfusion include febrile non-hemolytic transfusion reaction, allergic reactions, infection, refractoriness to platelet transfusion, the need for hospitalization and its associated cost (15, 16). In our institution, 10 units of platelet transfusion, at a cost of US$745, are typically given for recipients whose platelet counts are less than 30 $\times$ $10^9$/L during liver transplantation. By comparison, romiplostim costs US$660 per vial. Although the cost of platelet transfusion varies by patients, weekly use of romiplostim may generally cost more than intermittent platelet transfusion. Nonetheless, the use of romiplostim could reduce various transfusion-related complications. Moussa et al. (17) reported pre-operative use of romiplostim in thrombocytopenic adult patients with chronic hepatitis C and liver cirrhosis; 33 of the 35 patients achieved platelet count of over 70 $\times$ $10^9$/L with romiplostim treatment and eventually

went through planned surgeries without platelet transfusion. Similarly, Afdhal et al. (18) reported that eltrombopag, an orally active TPO receptor agonist, reduces the need for platelet transfusion in chronic liver diseases and in patients who were undergoing elective invasive procedures.

Our treatment strategy suggested that romiplostim could be effective in improving thrombocytopenia, and potentially eliminating the need for platelet transfusion during the peritransplant period in children with significant portal hypertension and hypersplenism secondary to liver cirrhosis. To further investigate the efficacy and safety of romiplostim, we propose studies on a larger number of pediatric patients.

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### Conflict of interest

None.

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# Successful living donor liver transplantation for giant extensive venous malformation

Hatanaka M, Nakazawa A, Nakano N, Matsuoka K, Ikeda H, Hamano I, Sakamoto S, Kasahara M. Successful living donor liver transplantation for giant extensive venous malformation.

Abstract: We report our success in employing LDLT as a course of treatment for extensive hepatic VM. A 14-yr-old pediatric patient presented at our hospital with nosebleed, fatigability, orthopnea, and abdominal distension. He had a history of right hemicolectomy with primary anastomosis due to VM of the transverse colon at age seven. Coagulation abnormalities were apparent, characterized by high international normalized ratio of prothrombin time, decreased fibrinogen level, increased FDPs, and D-dimer. T2-weighted magnetic resonance imaging revealed numerous, variable-sized high signal intensity nodules. Abdominal ultrasonography and CT scan showed hepatomegaly with multiple hypo-echogenic lesions and arteriovenous shunting in the liver. Doppler ultrasound showed hypokinetic flow in the hypo-echogenic lesions of liver. Immediate LDLT was performed to avoid spontaneous rupture and DIC. The right lobe of the liver was implanted with temporary portocaval shunt to prevent intestinal congestion and bleeding. Pathologic examination of the explanted liver confirmed the presence of an extensive hepatic VM. The postoperative course was uneventful, and the patient remained symptom-free with normal liver function throughout the 12-month follow-up period.

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Key words: pediatric liver transplantation – living donor liver transplantation – Kasabach–Meritt syndrome – hepatic venous malformation

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VM are the most common form of benign neoplasm in the liver. VM affects boys and girls equally with a reported incidence of 1–2 per 10 000 births and a prevalence of 1%. Most VM (95%) are sporadic and can occur anywhere in the body, but are frequently seen in the head and neck (40%), extremities (40%), and trunk (20%) (1). VM of the colon and liver, although rare, have been previously reported (2, 3). Most VM are minor and require no treatment with close follow-up (4).

Mulliken et al. divided vascular anomalies into two distinct categories: vascular tumors (characterized by endothelial proliferation arising from preexisting vessels) and vascular malformation (congenital abnormalities in vasculogenesis with normal endothelium). Vascular tumors are characterized by rapid growth during infancy with abnormal endothelial proliferation, followed by gradual involution. In contrast, vascular malformation is represented by lesions composed of dysplastic vascular channel. Although not always apparent, vascular malformation is present at birth. It grows proportionally with the patient's age and does not spontaneously regress (4).

Depending on the type of vessel involved, the vascular malformation group was subdivided into high-flow (such as arteriovenous malformation and arteriovenous fistula) and low-flow lesions (such as venous and lymphatic malformations) (5). This classification was approved by the ISSVA, and it represents the current guidelines in the management of vascular lesions (5–7). Most VM of the liver are asymptomatic; however, some (especially large lesions) cause various complications, such as KMS (8–10).

Often times, VM are incidentally detected during abdominal imaging for other unrelated clinical conditions (1, 8–12). Accurate diagnosis of

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CT, computed tomography; DIC, disseminated intravascular coagulation; FDP, fibrin degradation product; ISSVA, International Society for the Study of Vascular Anomalies; KMS, Kasabach–Meritt syndrome; LDLT, living donor liver transplantation; LT, liver transplantation; SMA, smooth muscle actin; VM, venous malformations.

hepatic VM remains a controversy, and confusion exists with regard to their classification and treatment. Current treatment options of hepatic VM include surgical resection, sclerotherapy, and LT (12–14). Treatment outcome is dependent on correct diagnostic evaluation and treatment strategy. To date, there is no standard treatment for VM of the liver in children. Decisions concerning therapeutic approaches are considered on a case-by-case basis. For our pediatric patient, we performed a successful LDLT for extensive hepatic VM.

## Patients and methods

A 14-yr-old male patient was admitted to our hospital with nosebleed, fatigability, orthopnea, and abdominal distension. He had a previous history of right hemicolectomy due to VM of the ascending colon seven year ago. At that time, multiple hemangiomas in the liver without hepatomegaly were pointed out by CT and abbreviation scan (Fig. 1a). Histology of the resected colon specimen revealed concomitant with cavernous hemangioma (VM). During the seven-year follow-up period, he had no specific symptoms related to hemangioma of the liver.

At admission, laboratory tests showed normal liver function: AST 20 U/L (normal range: 14–30 U/L), ALT 11 U/L (normal range: 9–36 U/L), γGTP 41 U/L (normal range: 9–48 U/L). Platelet count was slightly decreased (12 900 per cubic millimeter). However, coagulation abnormalities were evident: high international normalized ratio of prothrombin time of 1.27, decreased fibrinogen level of 75 mg/dL (normal range: 217–339 mg/dL), increased FDPs of 168.2 μg/mL (normal range: 0.0–5.0 μg/mL) and D-dimer level of 162.2 μg/mL (normal range: 0.0–1.0 μg/mL). T2-weighted magnetic resonance imaging revealed numerous, variable-sized high signal intensity nodules. Those findings were compatible with extensive hepatic VM. Abdominal CT scan revealed hepatomegaly with the liver extending into the pelvic cavity (Fig. 1b). The estimated liver weight by CT volumetry was 7142 mL (678% of the standard liver volume (15)). Hemangioma was not present in other organs. Doppler ultrasound showed hypokinetic flow in the liver's hypoechogenic lesions with mild intrahepatic arteriovenous shunting.

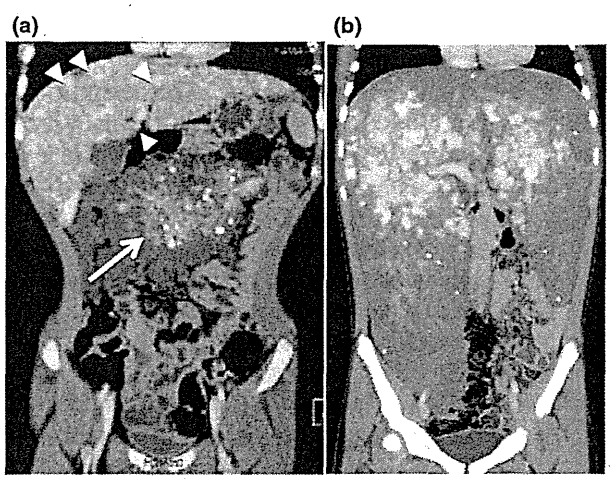


*Fig. 1.* (a) Enhanced abdominal CT at first visit (aged seven) shows a mass with calcification in the transverse colon (*arrow*) and multiple enhancing liver lesions (*arrowheads*). (b) Seven yr after (aged 14), the liver extends into the pelvic cavity. Whole liver volume was 7142 mL, as determined by CT volumetric analysis.

## Results

### LDLT

Prompt treatment modality was required in view of the potential risk of spontaneous rupture and DIC. As liver lesion was spread diffusely, surgical resection or sclerotherapy was deemed unsuitable. We decided LDLT to be the best treatment for the patient.

The patient's 38-yr-old mother with identical blood type acted as the donor. The liver graft, a right lobe that weighed 447 g (1.02% of graft-to-recipient weight ratio), was implanted. During the recipient hepatectomy, a temporary portocaval shunt was introduced to prevent intestinal congestion and massive bleeding. Significant reconstruction (with native left portal vein and donor ovarian vein interpositioning) was performed on the right inferior hepatic vein and segment 5 of the hepatic vein to avoid potential congestion in the graft liver. The explanted liver weighed 1449 g (Fig. 2). The operative procedure lasted 10 h and 54 min, and blood loss by patient was 266.5 mL/kg. During the transplantation, 24 units of red cell concentrates (3, 360 mL), 20 units of fresh frozen plasma (2400 mL), and 20 units of platelet were used as hemocomponents.

### Histological findings

Explanted liver showed diffuse lesion consisting of cavernous vascular spaces. The cavernous walls were composed of fibrous tissue containing

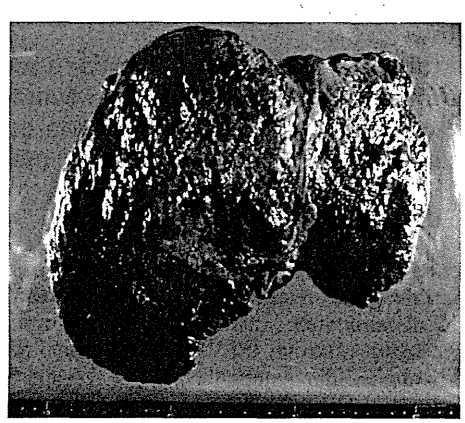

*Fig. 2.* Gross finding of the explanted liver.

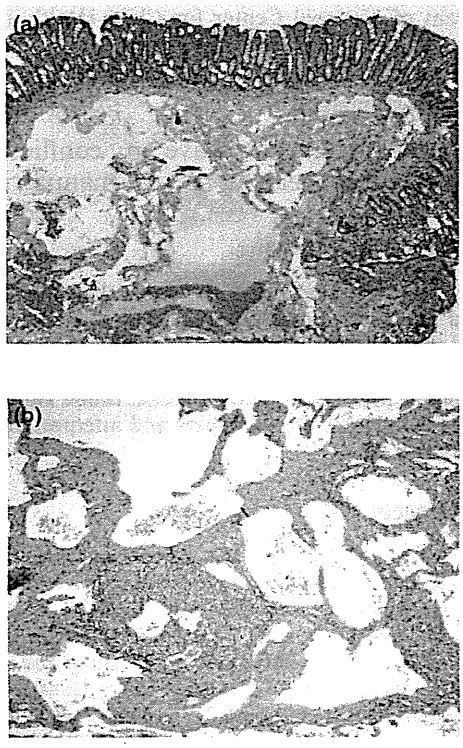


*Fig. 3.* Histological findings of the colon (a) and liver (b) (hematoxylin and eosin staining; original magnification a: ×40, b: ×100). Both show vascular malformation (cavernous hemangioma) with varying-sized vascular spaces lined by flattened endothelial cells. (b) Hepatic lesion contains trapped bile ducts and foci of parenchyma.

liver parenchyma and smooth muscle. Elastic lamina was unclear. Thrombosis with occasional formation of phleboliths was seen. Immunohistochemistry was positive for SMA, as well as for CD34 and CD31 in the thin, single-layered endothelium on the surface of the cavernous wall (but negative for D2-40, Prox1, and GLUT1). These findings indicated that the cavernous walls were derived from veins and displayed aberrant, irregular thickening.

### Outcome

According to ISSVA classification (7), the final pathological diagnosis was VM of the liver (Fig. 3). The postoperative course was uneventful. Coagulation abnormalities disappeared four months after LT. During the 12-month follow-up period, the patient reported no further complications and progressed with normal liver function.

## Discussion

Multiple, progressive, rapidly growing cutaneous hemangiomas may be associated with widespread visceral hemangiomas in the liver, lungs, gastrointestinal tract, brain and meanings. This presentation, referred to as diffuse neonatal hemangiomatosis, carries the risk of systemic complications, including high-output cardiac

Table 1. LT for giant VM

| Study | Age (yr), Gender | Patient(s) characteristics | Indication | Previous therapies | Outcome |
|---|---|---|---|---|---|
| Klompmarker et al. (20) | 27 yr Male | Male Size: NA Explant 15 kg | Hepatomegaly | Tranexamic acid, cryoprecipitate infusion | Normalization of liver function and coagulation |
| Chui et al. (19) | 33 yr, Female (a) 43 yr, Female (b) | Size: NA Explant 10 kg (a), 4 kg (b) | Dyspnea (a) Abdominal discomfort (b) | In second case: embolization (n = 1) | Normalization of liver function and coagulation |
| Longeville et al. (13) | 47 yr Male | Size: 25 cm Explant 3.6 kg | Bleeding after teeth extraction | Resection failed | Normalization of liver function and coagulation |
| Kumashiro* et al. (12) | 48 yr Female | Size: Na Explant 4.2 kg | Abdominal distention | Nafamostat mesilate, fresh frozen plasma | Normalization of liver function and coagulation |
| Ferraz et al. (17) | 25 yr Female | Size: 46 cm Explant 7.2 kg | Respiratory distress Abdominal distention | Embolization (n = 3) | Normalization of liver function and coagulation |
| Meguro* et al. (10) | 45 yr Female | Size: 15 cm, multiple lesions Explant 4.8 kg | Abdominal distention | Embolization (n = 2) | Normalization of liver function and coagulation |
| Vagefi et al. (11) | 32 yr Female | Size: 23 cm, systemic Hemangiomatosis | Abdominal discomfort | Interferon | Normalization of liver function and coagulation |
| Van Malenstein et al. (21) | 36 yr Female | Size: 24 cm, multiple lesions | Abdominal discomfort | Embolization (n = 5) | Complete resolution of complaints |
| The present case* (This study) | 14 yr Male | Size 30 cm, multiple lesions Explant 7 kg | Fatigability, orthopnea, abdominal distention | None | Normalization of liver function and coagulation |

NA, not available.
*LDLT.

failure, hemorrhage, and neurologic defects, accompanied by a high mortality rate. Infants who present with multiple cutaneous hemangiomas should be evaluated for the involvement of other organ systems with close follow-up (16). Our patient did not have a history of cutaneous hemangiomas or diffuse neonatal hemangiomatosis, but histopathology of explanted liver showed extensive VM (4).

Procedures such as radiation, arterial embolization, surgical resection, and LT are typically used for treatment of symptomatic giant hepatic hemangioma (9, 10, 12). In our case, radiation, arterial embolization, and surgical resection were deemed difficult due to preexisting thrombocytopenia and consumptive coagulopathy with extensive VM. It has been reported that the long-term outcomes from such treatment methods are less desirable than those from surgical resection and LT (13, 17, 18). In giant hemangioma/VM, the size of tumor may pose as a considerable risk factor for hepatectomy mainly due to massive intraoperative bleeding (19, 20). Precise preoperative management to decrease tumor size may increase the safety of surgery for extensive hepatic VM (1, 5).

Taking into consideration other literature (10–13, 17, 19, 20) together with our case, we found 10 cases of LT for giant hemangioma/VM with KMS/severe coagulopathy, of which three were male and seven were female (Table 1). Except for our patient, all patients were adults (25–48 yr of age) and received treatment before transplantation. Four patients underwent embolization, one for resection and another was treated with interferon. LDLT, which allows optional timing of transplantation, was indicated in three patients to ensure positive outcome (10, 12). One notable advantage of LDLT is the relatively higher availability of potential donor as compared to cadaveric transplantation, which has a significant mortality rate due to the limitation of available donors.

As our patient's hepatic VM was diffused across a large area, LDLT was determined to be the soundest and most effective treatment strategy. Our findings highlight the importance of LDLT as a treatment strategy for extensive hepatic VM and that periodical follow-up is necessary to ensure optimal timing of transplantation.

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#### Conflict of interest

None of the authors have any conflict of interest to declare concerning present manuscript.

#### Authors' contributions

Masahiro Hatanaka and Atsuko Nakazawa: Data analysis and interpretation, drafting of the article; Atsuko Nakazawa and Mureo Kasahara: Concept/design, critical revision of the article, approval of the article; Natsuko Nakano, Kentaro Matsuoka, Hitoshi Ikeda, Ikumi Hamano and Seisuke Sakamoto: Data analysis and interpretation, approval of the article.

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Pediatric Transplantation
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# Hepatic artery reconstruction preserving the pancreaticoduodenal arcade in pediatric liver transplantation with celiac axis compression syndrome: Report of a case

Uchida H, Sakamoto S, Matsunami M, Sasaki K, Shigeta T, Kanazawa H, Fukuda A, Nakazawa A, Miyazaki O, Nosaka S, Kasahara M. (2014) Hepatic artery reconstruction preserving the pancreaticoduodenal arcade in pediatric liver transplantation with celiac axis compression syndrome: Report of a case. *Pediatr Transplant*, 18: E232–E235. DOI: 10.1111/petr.12329.

Abstract: CACS is rare, although it has been reported to be a potential risk factor for hepatic artery thrombosis following LT. We herein present the case of a 14-yr-old male with stenosis of the origin of the celiac trunk. Preoperative CT and color ultrasonography showed narrowing of the proximal celiac artery. The patient underwent DDLT with standard arterial reconstruction without dividing the gastroduodenal artery. His postoperative course was uneventful, with an excellent hepatic artery flow on Doppler ultrasonography. Applying a meticulous preoperative evaluation and the appropriate surgical technique is crucial in patients with CACS.

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Key words: celiac artery compression syndrome – hepatic artery thrombosis – median arcuate ligament – pediatric liver transplantation

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CACS has been reported to be a potential risk factor for hepatic artery thrombosis after LT (1), with an incidence of 24% among the general population (2). This paper describes the management of hepatic artery reconstruction in a patient with CACS in which the pancreaticoduodenal artery arcade was preserved.

## Case report

The patient was a 14-yr-old male with type II citrullinemia. The diagnosis of type II citrullinemia was suspected at five days of age due to persistent hyperbilirubinemia, hyperammonemia, increased plasma citrulline/arginine levels, and a specific mutation with a relevant family history of an affected sister. Treatment with protein restriction and medication with sodium benzoate, phenyl acetate, and arginine were subsequently administered as the initial therapy. Despite the administration of medical treatment, several episodes of fulminant hyperammonemia and hepatic dysfunction followed at eight yr of age, requiring repeat hospitalization. The patient had been on the waiting list for DDLT beginning when he was 14 yr of age. Preoperative CT and color ultrasonography revealed stenosis of the origin of the celiac trunk (Fig. 1a,b). A diagnosis of CACS was suspected, given that the hepatic arterial blood supply ran primarily through the pancreaticoduodenal artery arcade via the superior mesenteric artery (Fig. 1c). The patient received a piggyback DDLT with a whole liver graft. Due to the sufficient front flow of the proper hepatic artery in the recipient, standard

Abbreviations: CACS, celiac axis compression syndrome; CT, computed tomography; DDLT, deceased donor liver transplantation; LT, liver transplantation; MAL, median arcuate ligament.

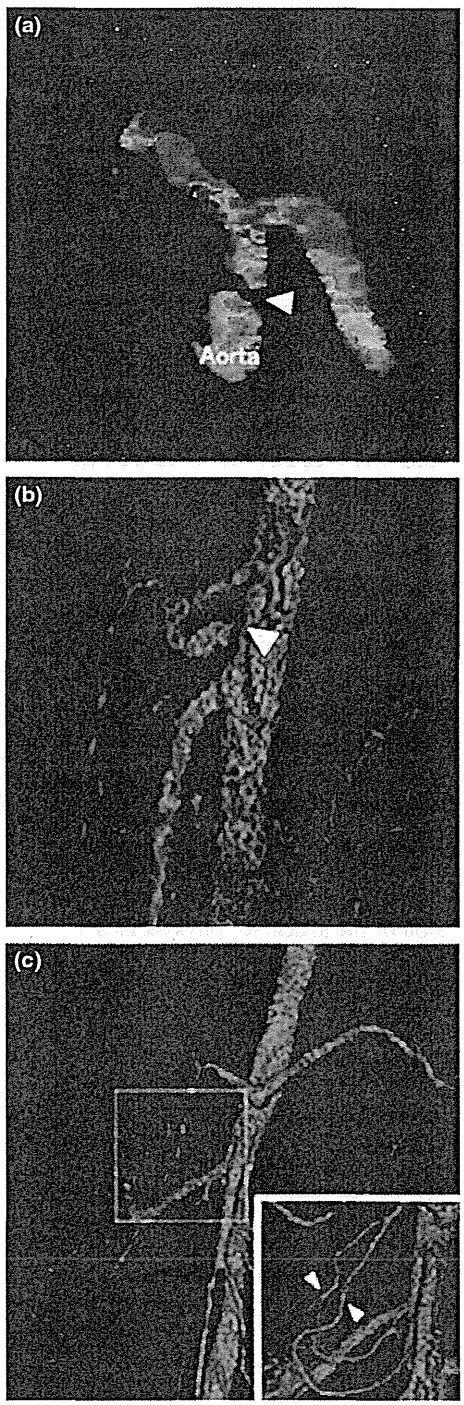


*Fig. 1.* (a) Color ultrasonography showing focal residual stenosis at the proximal celiac artery (arrowhead). (b) CT angiography showing the characteristic hooked narrowing of the proximal celiac artery on a 3D reconstruction image (arrowhead). (c) CT angiography showing the mesenteric hepatic collateral via the pancreaticoduodenal arcade (square box). A partial enlarged view of the lower right shows the collaterals (arrowhead).

reconstruction between the proper hepatic artery without diversion of the gastroduodenal artery and a common hepatic artery graft was accomplished using the microvascular technique (3). The vessel anastomosis was performed in an end-to-end fashion using interrupted sutures with 9-0 nylon. The intrahepatic arterial blood flow was 788 mL/min, with a resistance index of 0.56. The patient was discharged 17 days after DDLT. His postoperative course was uneventful, with an excellent hepatic artery flow on Doppler ultrasonography. Furthermore, he has been doing well without either protein restriction or any additional medication for the original liver disease.

## Discussion

CACS is a well-described anatomical entity caused by compression of the celiac axis by the MAL that was first described by Harijola in 1963 (4). Affected patients usually exhibit various clinical manifestations, including postprandial abdominal epigastric pain, nausea, occasional diarrhea, and weight loss (5); however, the present patient was asymptomatic. The incidence of CACS among transplant recipients has been reported to be 3.7–10.0% (1, 6, 7). The presence of CACS may predispose the patient to hepatic artery thrombosis, as it decreases common hepatic artery blood flow by more than 50% (1).

Recently, it has become possible to diagnose celiac axis compression with three-dimensional (3D) CT angiography and/or Doppler ultrasonography, especially in children (8). It has been documented that during expiration in the presence of celiac artery compression, the blood flow falls from optimal values of 400 mL/min to values <200 mL/min (1, 9). We do not measure the flow volume of the celiac artery routinely; however, we did note that there was a significant celiac arterial flow reduction from 35.7 to 16.6 cm/s in maximum velocity during expiration in the present case. We also did not perform any direct measurement of the pressure in the hepatic artery prior to/during the LT. However, such measurement would help to provide a definite diagnosis of the presence of CACS. Furthermore, aneurysms of the pancreaticoduodenal artery are occasionally found in the patient with CACS, and a proper diagnosis could facilitate the treatment for such a patient (10). Therefore, careful planning and follow-up are crucial in patients with CACS before LT, with strict assessment of the radiological findings.

Three types of hepatic artery reconstruction have been described in LT for CACS: release of the MAL, aortohepatic graft interposition, and standard reconstruction with preservation of the native gastroduodenal artery (7). Release of the MAL has been reported as the initial treatment in pediatric patients treated without LT, with a

reported successful rate of 67% (5). This unsatisfactory result may be explained by the relevant presence of atheromatic plaque in association with MAL, resulting in restenosis of the celiac artery (7). Moreover, the condition induced celiac trunk injury in 11.7% of LT cases (1). Meanwhile, Lindner et al. stated that the release of the compressing cuirass of the celiac plexus could be needed, in addition to the resection of MAL (11).

Hepatic artery reconstruction with aortohepatic graft interposition is performed with an iliac artery or prosthetic graft and the graft is implanted on the infrarenal aorta. However, aortohepatic graft reconstruction is associated with a relatively high incidence of thrombosis, with an incidence of 5.3–21.8% (12, 13). Normally, hepatic artery reconstruction is accomplished with 9-0 nylon interrupted sutures with a surgical microscope. The gastroduodenal artery of the recipient is ligated to the retrograde dissection of the hepatic axis down to the aorta until an adequate lumen of the gastro-hepatic artery and a normal arterial pressure are obtained. However, in the present case, we could obtain sufficient front flow of the proper hepatic artery without ligation of the gastroduodenal artery. Preserving the gastroduodenal arterial flow might be key for successful hepatic artery reconstruction in CACS cases. Tying the splenic artery to increase the hepatic arterial flow is not always performed, because it can reduce the portal venous flow/pressure and cause portal venous complications, especially in the pediatric patients with biliary atresia (14).

Percutaneous angioplasty with stenting has recently been reported as an alternative option for the treatment of MAL (15). Although it is a minimally invasive intervention, its long-term patency is not fully documented, especially in pediatric liver transplanted patients with immunosuppression. The efficiency and efficacy of the stenting in pediatric recipients should be discussed on case-by-case basis.

Between November 2005 and December 2013, 260 children underwent LT (including 11 cases of DDLT) at the National Center for Child Health and Development, Tokyo, Japan. Microvascular surgery for hepatic artery reconstruction has been employed since the beginning of the program, and no cases of hepatic artery thrombosis have been observed in our series. In addition to postoperative ultrasonography, intra-operative Doppler ultrasonography has been routinely used to evaluate the vascular patency at our center to make an early diagnosis of vascular and biliary complications. If the hepatic arterial flow is not sufficient with a low pulsatility index, we consider further dissection of the hepatic axis down to the aorta or splenic artery ligation, if the portal venous flow is sufficient. There have been some reports that the intra-operative assessment of the hepatic artery is important to reduce vascular complications (16, 17). Intra-operative Doppler ultrasonography might also prevent early hepatic artery complications (18).

In the present case, standard arterial reconstruction without division of the gastroduodenal artery was accomplished with the microsurgical technique. Lubrano et al. mentioned according to standard arterial reconstruction technique for patient with CACS and stated that the preserving gastroduodenal flow is feasible for LT cases performed in adults (7). The present case demonstrates that preserving the pancreaticoduodenal artery arcade may be appropriate for children, if the native proper hepatic arterial flow is sufficient, in the setting of hepatic artery reconstruction among CACS patients evaluated with a meticulous preoperative assessment and treated with the proper surgical technique.

### Authors' contributions

H.U.: Study design, writing of the paper; S.S.: Study design, critical revision of the article for clinical content; M.M., K.S., H.K., A.F., T.S.: Collection of the data; A.N.: Critical revision of the article for physiological content; O.M., S.N.: Critical revision of the article for radiological content; M.K.: Study design, critical revision of the article for clinical content.

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# Technical refinement in living-donor liver transplantation for hepatoblastoma with main portal vein tumor thrombosis – a pullout technique

Kanazawa H, Sakamoto S, Matsunami M, Sasaki K, Uchida H, Shigeta T, Fukuda A, Matsumoto K, Nakazawa A, Tanaka R, Kasahara M. (2014) Technical refinement in living-donor liver transplantation for hepatoblastoma with main portal vein tumor thrombosis – a pullout technique. *Pediatr Transplant*, 18: E266–E269. DOI: 10.1111/petr.12357.

Abstract: We present a case of a two-yr-old boy diagnosed with HBT with complete main PVTT. HBT was located in the bilateral lobe with PVTT involving the confluence of the SMV and the SpV. Cisplatin-based neoadjuvant chemotherapy was delivered; main tumor shrank and AFP levels decreased to below one hundredth. However, PVTT remained in the bilateral portal branches to the main trunk of PV. We describe the technical details of the portal venous tumor thrombectomy that was succeeded by a LDLT. The patient remained healthy 2.5 yr after LDLT, showing good patency of the PV with no evidence of recurrence of tumor.

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Key words: hepatoblastoma – liver transplantation – portal vein tumor thrombosis

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HBL is the most common malignant liver tumor in early childhood, accounting for 60–85% of all pediatric hepatic tumors (1). As it is a surgical tumor, the main form of treatment is surgical resection. Advances in imaging technology, systemic cisplatin-based neoadjuvant chemotherapy, and surgical resection have improved survival rates (2). Patients with HBL with resectable tumors have a disease-free survival rate of 80–90% (3). While more than 60% of lesions that appeared unresectable at initial imaging shrank with chemotherapy and eventually became resectable (2), some cases of HBL remained unresectable despite chemotherapy to control extrahepatic lesion. In such cases, whole liver resection is necessary, with liver transplantation being recognized as a valid therapeutic option to accomplish complete resection (4). HBL that invades the bilateral portal branches or the main portal trunk with tumor thrombosis is one of the most unresectable forms of tumor. Although macrovascular invasion posed as a

Abbreviations: FDG, $F^{18}$-fluoro-2-deoxy-D-glucose; HBL, hepatoblastoma; LDLT, living-donor liver transplantation; PET, positron emission tomography; POST-TEXT, post-treatment extent of disease; PRETEXT, pretreatment extent of disease; PV, portal vein.; PVTT, portal vein tumor thrombosis; SMV, superior mesenteric vein; SpV, splenic vein.

poor prognostic factor, the main aim is still to achieve complete resection for chemosensitive HBL through surgery (5).

Here, we report a pediatric patient with PVTT that remained in the SMV and the splenic venous junction (SMV-SpV junction) despite neoadjuvant chemotherapy. The patient eventually underwent complete surgical resection with PV thrombectomy and LDLT. We describe the details of the surgery of the PV thrombectomy that employed the use of a "pullout technique."

### Case

The patient was a 2.5-yr-old boy 12 kg in weight. The chief complaint was abdominal distension. A computed tomography (CT) showed multifocal liver tumors occupying the whole liver with a PVTT that extended to the SMV-SpV junction (Fig. 1a). PET with FDG study showed that

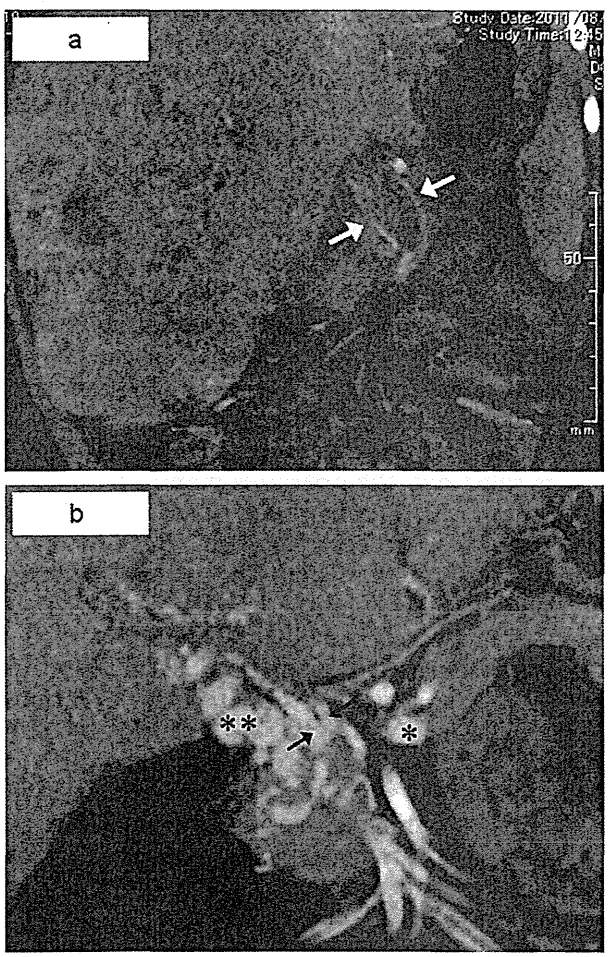


*Fig. 1.* (a) Large PV thrombosis (white arrow) in coronary view revealed by computed tomography. (b) Thin and long PV thrombosis (black arrow) from the bilateral portal branch to the confluence of the SMV and the SpV (asterisk). A collateral vein was present in the hepatoduodenal ligament (double asterisk).

huge liver tumor, PVTT, and the segment 6 of the right lung were positive. At the time, serum AFP level was markedly elevated at 580 000 ng/mL (normal range: <10 ng/mL). The case was assessed using the PRETEXT grouping system for HBL, as PRETEXT IV, C1, E0a, F1, H0, M1p, P2a, and V1 (6). Needle biopsy findings showed fetal and embryonic mixed-type HBL. Neoadjuvant chemotherapy by cisplatin–pirarubicin (tetrahydropyranyl–adriamycin) (CITA) was introduced in accordance with the protocol of the Japanese Study Group for Pediatric Liver Tumor protocol-2 (7). At the end of the third cycle of CITA, AFP levels decreased to 2373 ng/mL and the size of the tumor size was reduced. While a lung metastasis was no longer present after the fourth cycle of chemotherapy by ifosfamide, carboplatin, tetrahydropyranyl–doxorubicin, and etoposide (ITEC), AFP levels rose again to 6450 ng/mL. It was determined at that stage that the primary liver tumors could not be sufficiently controlled, prompting the need for transarterial chemoembolization. In addition, a systemic chemotherapy comprising cisplatin, vincristine, and fluorouracil (C5V) was delivered due to the suspicion that the patient might have impaired response to the previous rounds of chemotherapy (8). Subsequently, AFP level decreased to 2532 ng/mL. However, the PVTT, which assumed an atrophic shape and was negative in FDG–PET study, remained in the SMV-SpV junction. CT showed enlarged and tortuous collateral vessels had developed along the common bile duct in the hepatoduodenal ligament (Fig. 1b). The case was assessed again using POST-TEXT grouping system for HBL, as POST-TEXT III, C0, E0, F1, H0, M0, P2a, and V0. We scheduled an LDLT for total resection of HBL and removal of PVTT by either thrombectomy or total resection (including PV). The patient's 40-yr-old mother volunteered to have her left lateral segment, weighing 242 g, donated as a graft. The graft-to-recipient body weight ratio was 1.95%.

### Surgical procedures

Both collateral vessels and the PV were isolated and taped with vessel loops, and preserved until total hepatectomy. Intra-operative ultrasonography provided definitive imaging of the location of the PVTT which extended into the SMV-SpV junction; however, it was atrophic and appeared "floating." The SMV-SpV junction was exposed with meticulous dissection of the tributaries into the portal venous system. The SMV and the SpV were isolated and clamped at a distal site of the

junction (Fig. 2a). The PV was dissected from the posterior of the pancreas toward the liver and was easily pulled out through the dorsal side of the pancreas in a safe manner (Fig. 2b). The PVTT was completely thrombectomized by this "pullout" technique; intra-operative histology of the PVTT revealed no viable tumor cells within the excised specimens and the surgical margin of the PV. Subsequently, the PV was returned to its original position without using an interposed vein graft. PV anastomosis was accomplished at the confluence of the recipient PV and the left PV of the graft.

Pathological examination of the explanted liver showed a multicentric tumor with PVTT extending to the bilateral branches of the PV and the main trunk. About 35% of the whole tumor revealed necrotic changes, while viable tumor was composed of fetal and embryonic mixed-type HBL. The excised thrombus in the main portal trunk measured 22 ×2 × 2 mm with no viable tumor cells.

Tacrolimus and low-dose steroids were used for immunosuppression. The patient managed to wean off the steroids over three months (9).

After surgery, AFP levels decreased significantly. On postoperative day 13, the patient had mild acute cellular rejection, but was successfully treated with bolus administration of steroids. The patient was discharged 30 days after LDLT. Adjuvant chemotherapy with C5V commenced 40 days after LDLT. The patient remained in good health 2.5 yr after LDLT with normal AFP levels (5.6 ng/mL), good patency of the PV, and no evidence of recurrence of tumor.

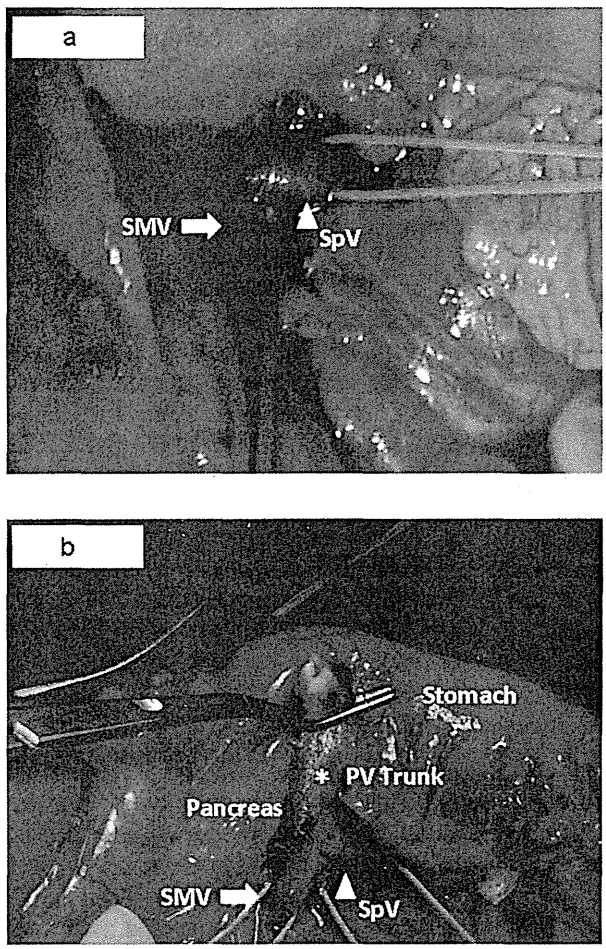


*Fig. 2.* (a) The SMV and the SpV were isolated and each taped with vessel loops. (b) The technical highlights showed the PV being drawn from superior to inferior border of the pancreas.

## Discussion

The guidelines for early consultation with a transplant surgeon to perform primary liver transplantation to treat unresectable HBL include: (1) HBL having characteristics of multifocal PRETEXT IV without extrahepatic lesion, (3) unifocal centrally located PRETEXT II and III involving the three main hilar structures, or (2) all three of the main hepatic veins and POST-TEXT III with macroscopic vascular invasion (5). In our case, the HBL invaded bilateral portal branches with tumor thrombosis in the main portal trunk. While neoadjuvant chemotherapy succeeded in shrinking the size of the PVTT, it persisted in the main portal trunk and extended into the SMV-SpV junction. In the event that chemotherapy is effective, PVTT can become atrophic and mostly replace blood clots. When the risk of residual malignant cells in the PVTT is ruled out, the operative management of PVTT in patients undergoing liver transplantation would be dependent on the extent of PVT and the method of portal reconstruction according Yerdel's classification (Grade 1–4) (10). It has been reported previously that no special technique is required for the reconstruction of the PV in Grade 1 cases. PVT classified as Grade 1 is defined as minimally or partially thrombosed PV, in which the thrombus is mild or at most confined to <50% of the vessel lumen, with or without minimal extension into the SMV. Hence, only thrombectomy with cramping PV on the SMV-SpV junction is usually required. In our case, the PVTT decreased in size after neoadjuvant chemotherapy. It was classified as Grade 1 as it was confined to <50% of the vessel lumen.

Although neoadjuvant chemotherapy has been reported to reduce AFP level and allowed negative FDG–PET uptake into PVTT, our patient required complete thrombectomy due to the

potential risk of residual malignancy in the PVT. However, we decided against using total replacement with interposition vein graft as a first-line procedure because the PVTT went through a dramatic atrophic change after chemotherapy. Instead, the PV was clamped at the SMV-SpV junction. There were two reasons to our judgment. There was a risk of residual malignancy due to the cramp nipping at the edge of the PVTT. Hence, the SMV and the SpV were clamped separately at a distal site from the junction. We adopted a "pullout" technique to achieve good operative and visual field for complete thrombectomy. Even in such a case, a set of the procedures were performed safely in good operative field acquired by pullout techniques. In the event that malignant cells were present on the margin of the PV through the intra-operative rapid diagnosis, portal reconstruction using interposition vein graft may be performed as a second-line procedure.

The other reason was that our patient developed collateral vessels, which resulted in cavernous transformation in the hepatoduodenal ligament. We had to retain the collateral vessels in order to avoid intestinal congestion until PVTT was resected and the native liver removed. There was a high risk of bleeding when approaching the PVTT from the side of hepatoduodenal ligament that contained the distended collateral vessels.

In view of the factors, the "pullout" technique was very useful in resecting the PVTT completely. Furthermore, this technique can be combined with a portal reconstruction using interposition vein graft when the PV is sclerotic and had undergone atrophic change in recipients; for example, in patients with biliary atresia, direct anastomosis of PV cannot be performed due to an insufficient front flow because of some collateral vessels derived from the main PV. We have applied the "pullout" technique in such cases and achieved positive outcome (11).

In conclusion, our patient underwent LDLT for HBL with PVTT that extended to the SMV-SpV junction. The "pullout" technique allowed a good operative field to perform complete thrombectomy safely.

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### Authors' contributions

H. Kanazawa: Participated in research design and writing of the paper, conducted research, provided reagents of analytic tools, and analyzed the data; S. Sakamoto, A. Fukuda, and M. Kasahara: Participated in research design and conducted research; K. Sasaki, H. Uchida, M. Matsunami, T. Shigeta, R. Tanaka, K. Matsumoto, and A. Nakazawa: Conducted research.

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# Basiliximab treatment for steroid-resistant rejection in pediatric patients following liver transplantation for acute liver failure

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Abstract: An IL-2 receptor antagonist, basiliximab, decreases the frequency of ACR in liver transplant (LT) recipients as induction therapy. The aim of this study was to evaluate the effectiveness of basiliximab against SRR as rescue therapy in pediatric LT patients with ALF. Forty pediatric ALF patients underwent LT between November 2005 and July 2013. Among them, seven patients suffering from SRR were enrolled in this study. The median age at LT was 10 months (6–12 months). SRR was defined as the occurrence of refractory rejection after more than two courses of steroid pulse therapy. Basiliximab was administered to all patients. The withdrawal of steroids without deterioration of the liver function was achieved in six patients treated with basiliximab therapy without patient mortality, although one patient developed graft loss and required retransplantation for veno-occlusive disease. The pathological examinations of liver biopsies in the patients suffering from SRR revealed severe centrilobular injuries, particularly fibrosis within one month after LT. We demonstrated the effectiveness and safety of rescue therapy consisting of basiliximab for SRR in pediatric LT recipients with ALF.

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Calcineurin inhibitors, such as TAC and cyclosporine, combined with steroids are standard ISs, and their use has reduced the frequency of ACR following LT (1). ACR frequently occurs in pediatric LT patients, at a previously reported rate of 41–55% (2, 3). Steroid therapy remains the mainstay of the initial management of acute rejection, coupled with an increase in baseline immunosuppression (4). Although steroid pulse therapy is the first therapeutic option for treating ACR, SRR occurs in 8.1–34% of LT recipients, which results in graft and patient loss (3, 5, 6). The administration of additional ISs, such as MMF, SRL, and antithymocyte globulin, has previously been reported as rescue therapy for SRR (7–9). The efficacy of antithymocyte globulin in treating SRR is largely attributed to its ability to deplete T cells (4). The successful use of antilymphocyte therapy for pediatric patients suffering from SRR and late ACR with cholestasis was reported (10). Although antithymocyte globulin is effective for SRR, sepsis remains a significant complication (7). While SRR was previously an immediate indication for potent antilymphocyte preparations, this is now effectively treated with chimeric or humanized IL-2 receptor monoclonal antibodies (4).

Basiliximab (Simulect™; Novartis Pharma, Basel, Switzerland), a chimeric monoclonal antibody that blocks the α-subunit (CD25) of the IL-2 receptor in association with activated T

Abbreviations: ACR, acute cellular rejection; ALF, acute liver failure; ALT, alanine aminotransferase; AST, asparate aminotransferase; BW, body weight; CMV, cytomegalovirus; EBV, Epstein–Barr virus; IL, interleukin; IS, immunosuppressant; LDLT, living donor liver transplantation; LT, liver transplantation; MMF, mycophenolate mofetil; mPSL, methylprednisolone; PCP, *Pneumocystis* pneumonia; POD, postoperative day; RAI, rejection activity index; SRL, sirolimus; SRR, steroid-resistant rejection; TAC, tacrolimus; Treg, $CD4^+$ $CD25^{high}$ $CD127^{low/neg}$ regulatory T.

helper cells, is usually used as induction therapy in LT (11). The use of basiliximab contributes to reducing the incidence of ACR and allows the dose of calcineurin inhibitors and corticosteroids to be decreased following pediatric LT, with a low incidence of side effects (4, 12–14). However, basiliximab did not affect the long-term patient or graft survival after LT (15). Few studies have reported the use of basiliximab for SRR as rescue therapy (16, 17).

ALF is a rare and fatal disease with a high mortality rate in children (18). The outcomes of LT in patients with cryptogenic ALF are poor in comparison with patients with cholestatic disease, particularly in infants (19–22). The outcomes of pediatric LT for ALF have improved recently, and the patient and graft survival rates at one yr were 71–93.4% and 64–88%, respectively (23–26). The main cause of death was reported to be sepsis, and the incidence of ACR was 36.7–39.4%. On the other hand, certain data also suggest that the patient survival rate among infants with ALF following LT was only 40% at one yr due to the high incidence of SRR (19–22). It has been reported that SRR accounts for 50% of the deaths in children who have undergone LT for ALF. Moreover, it has been speculated that the long-lasting unknown origin of ALF may cause an accelerated immune response in children even after successful LT (19). Therefore, reducing the frequency of SRR is key for improving the short- and long-term survival among pediatric patients with ALF.

The aim of this study was to evaluate the effectiveness of basiliximab against SRR as rescue therapy in pediatric patients with ALF who have undergone LT.

## Patients and methods

Forty pediatric ALF patients underwent LT between November 2005 and July 2013 at the National Center for Child Health and Development. During the same period, 195 pediatric patients with non-ALF liver diseases underwent LT. The original diseases in the non-ALF group consisted of biliary atresia in 106, metabolic disease in 45, congenital hepatic fibrosis/Caroli disease in 14, and other diseases in 30 cases. The median age at LT in the ALF group and non-ALF group was 10 months (17 days–12 yr four months) and 16 months (two months–17 yr 11 months) and the median BW was 8.2 kg (2.6–32 kg) and 9.4 kg (3.7–63.8 kg), respectively. Among them, seven patients in ALF group who were diagnosed with SRR were enrolled in this study.

### Basic immunosuppression protocol at our hospital and the diagnosis of ACR

Basic immunosuppressive treatment after LT consisted of TAC and low-dose steroids. Briefly, the trough level of TAC was maintained between 10 and 12 ng/mL for the first month and between 8 and 10 ng/mL for the subsequent three months. mPSL was administered at a dose of 1 mg/kg from POD 1–3, 0.5 mg/kg from POD 4–6 and 0.3 mg/kg on POD 7. Prednisolone was given orally at a dose of 0.3 mg/kg from POD 8–28 and 0.1 mg/kg after POD 29. In the patients with ALF, which has previously been reported to be associated with a higher incidence of ACR, the treatment with prednisolone was continued for longer than six months after LT until the liver function became stable (20).

The indication for a liver biopsy was based on the presence of increased AST (normal range: 24–50 IU/L) and ALT (normal range: 9–34 IU/L) levels more than three times the upper limit of the normal range or an increase of more than 50% over the previous record. The histological diagnosis and grading of ACR were determined according to the Banff schema (27, 28). The grade of centrilobular fibrosis was classified as follows: F0: absence of fibrosis, F1: fibrous central vein expansion, F2: central vein fibrosis with incomplete septa, F3: C-C bridging fibrosis, and F4: cirrhosis (29). The presence of veno-occlusive disease was assessed by liver biopsy in all patients. The patency of hepatic vessels, including outflow blockage of the hepatic vein, was routinely investigated after LT using Doppler ultrasonography.

### Management of ACR

All rejection episodes were treated with a color steroid bolus injection, generally given at a dose of 10 mg/kg of mPSL for three days. SRR was defined as the occurrence of refractory rejection after two cycles of steroid pulse therapy. The use of basiliximab and antithymoglobulin for liver transplant patients is not still covered by the universal health insurance system in Japan because they are currently unauthorized drugs. In this study, basiliximab treatment was indicated for patients suffering from SRR, because infections remain a significant complication of antithymoglobulin therapy (7). Basiliximab, which was administered at a dose of 10 mg twice on days 0 and 4, was adopted as rescue therapy in the SRR patients.

### Characteristics of the SRR group before basiliximab therapy

Characteristics of the SRR group are presented in Table 1. The age and BW in SRR group were 6–12 months (median 10 months) and 7.6–9.6 kg (median 8 kg), respectively. Multiple cycles of steroid pulse therapy, ranging from two to 17 cycles, were performed. Cases 3 and 4 underwent more than 10 cycles of steroid pulse therapy because they frequently suffered from ACR before the introduction of basiliximab therapy for SRR at our hospital. Additional ISs, such as MMF and SRL, were indicated in five patients (MMF in two, SRL in one, and both of them in two patients). Basiliximab was administered beginning 18–762 days after LT. A second dose of basiliximab was given to all but three patients, who did not receive the subsequent dose due to CMV infection (Cases 1 and 6) and graft failure (Case 5).

### Management of opportunistic infections after LT

The treatment for CMV infection was preemptively performed at our institute, which was described elsewhere (30). Briefly, CMV-pp65 antigenemia was monitored weekly for

Table 1. Characteristics of the recipients with SRR

| Case | Age at LT | No. of steroid pulse treatments | IS used for the treatment of SRR | Age at basiliximab therapy | Timing of basiliximab after LT | Dose of basiliximab |
|---|---|---|---|---|---|---|
| 1 | 6 months | 3 | TAC, mPSL | 2 yr 2 months | POD 44 | 10 mg |
| 2 | 7 months | 3 | TAC, mPSL, SRL | 2 yr 10 months | POD 29/38 | 10 mg/10 mg |
| 3 | 9 months | 12 | TAC, mPSL, MMF, SRL | 2 yr 2 m | POD 762/771 | 10 mg/10 mg |
| 4 | 10 months | 17 | TAC, mPSL, MMF, SRL | 2 yr 10 months | POD 524/528 | 10 mg/10 mg |
| 5 | 10 months | 5 | TAC, mPSL, MMF | 1 yr 7 months | POD 262 | 10 mg |
| 6 | 10 months | 3 | TAC, mPSL | 10 months | POD 18 | 10 mg |
| 7 | 12 months | 2 | TAC, mPSL, MMF | 12 months | POD 42, 46 | 10 mg/10 mg |

the first three months after LDLT, during treatment for ACR or when the patients presented with symptoms and laboratory data suspected to indicate CMV infection. If the presence of more than five CMV antigen-positive cells/50 000 white blood cells was revealed, intravenous ganciclovir (5 mg/kg/dose, every 12 h) was initiated for the first two wk, followed by a maintenance dose of intravenous ganciclovir (5 mg/kg/dose, every 24 h) until the CMV-pp65 antigenemia became negative.

The EBV management protocol after LDLT at our institute was described elsewhere (31). Briefly, the EBV viral loads in the peripheral blood were detected using a real-time quantitative polymerase chain reaction method and were monitored once per week for the first two months after LDLT, followed by every 1–3 months. If the symptoms and laboratory data suggested EBV infection or there were high values of EBV-PCR (more than $10^2$ copies/μg DNA), immunosuppression was withdrawn.

Preventive therapy for PCP was administered according to the prophylactic use of trimethoprim–sulfamethoxazole (0.05 mg/kg/day) for the first three months after LDLT.

### Monitoring of the $CD4^+$ $CD25^+$ T cells and regulatory T calls before and after basiliximab therapy

Monitoring of peripheral $CD4^+$ $CD25^+$ T cells and Treg cells was performed in the patients receiving basiliximab therapy for SRR (n = 6, Cases 1, 2, 4–7). Peripheral blood samples obtained from the patients and healthy controls were subject to Ficoll–Hypaque density gradient centrifugation to isolate the peripheral blood mononuclear cells. Peripheral blood lymphocyte subsets were determined with a FACSAria IIIu instrument (Becton Dickinson, Mountain View, CA, USA) using anti-human CD4, CD127, and CD25 monoclonal antibodies conjugated with fluorescein isothiocyanate, phycoerythrin, or allophycocyanin (BioLegend, San Diego, CA, USA), respectively. The FlowJo software program (TreeStar Inc., Ashland, OR, USA) was used for all aspects of the data analyses. The levels of $CD4^+$ $CD25^+$ T cells were monitored to assess the effects of basiliximab, and the levels of Treg cells were monitored because human Treg cells have been demonstrated to have great potential for use in therapeutic interventions to prevent graft rejection (32). Blood samples were obtained before and one wk after the administration of basiliximab therapy. The data of healthy children of similar age were collected as healthy controls (n = 7; male: 4, female: 3, five months–three yr three months, median: one yr seven months). Informed consent was obtained from all patients, healthy children, and their parents.

### Statistic analysis

The statistical analysis was performed using the Kruskal–Wallis test for the analysis of the median age and BW, and the Tukey test for the analysis of the flow cytometry data. Proportions were compared using the chi-square test. p-Values of $<0.05$ were considered to be significant. Patient survival and graft survival were evaluated according to the Kaplan–Meier method and compared using the log-rank test. The software program SPSS version 18.0 (SPSS, Inc., Chicago, IL, USA) was used for the statistical analysis.

## Results

### Comparison of the characteristics of the ALF group and non-ALF group

Biopsy-proven ACR developed in 24 (60%) and 75 patients (38.5%), respectively, in the ALF and non-ALF groups (p = 0.014). Moreover, SRR was revealed in seven (17.5%) and five patients (2.6%) in the ALF group and non-ALF group, respectively (p = 0.001). The overall survival of the 40 patients with ALF at one and five yr was lower than that of the non-ALF group (84.8% and 84.8% vs. 92.1% and 91.4%, respectively), but the differences were not significant (p = 0.23).

### Comparison of the characteristics of the non-ALF group, ALF group, and SRR group among the ALF patients

The median age at LT in the non-ACR group was higher than that in the other group, but the difference was not significant (p = 0.427). All of the cases in the ACR and SRR groups received grafts from live donors rather than deceased donors (p = 0.036). The etiology of ALF was unknown in all patients in the SRR group (p = 0.215) (Table 2).

### The outcomes of ALF patients suffering from SRR

No patients have died as a result of SRR or chronic rejection thus far. The basiliximab treatment outcomes for SRR are shown in Table 3.