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.

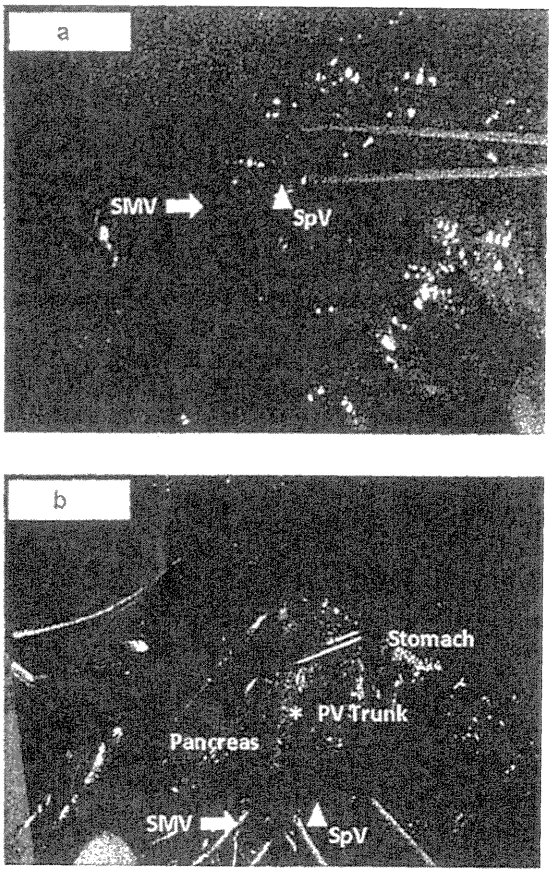

Fig. 2. (a) The SMV and the SpV were isolated and each taped with vessel loops. (b) The technical highlights showed the PV being drawn from superior to inferior border of the pancreas.

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.

Acknowledgments

We would like to thank Dr. Julian Tang from the Department of Education for Clinical Research, National Center for Child Health and Development, for proofreading and editing the manuscript. This work was supported in part by grants from the Scientific Research Fund of the Ministry of Education and by a Research Grant for Immunology, Allergy and Organ Transplant from the Ministry of Health, Labor and Welfare (24-8), Japan and Foundation for Growth Science, Japan.

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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Pediatr Transplantation 2014: 18: 860–867

Pediatric Transplantation
DOI: 10.1111/petr.12373

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

Shigeta T, Sakamoto S, Uchida H, Sasaki K, Hamano I, Kanazawa H, Fukuda A, Kawai T, Onodera M, Nakazawa A, Kasahara M. (2014) Basiliximab treatment for steroid-resistant rejection in pediatric patients following liver transplantation for acute liver failure. *Pediatr Transplant*, 18: 860–867. DOI: 10.1111/petr.12373.

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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Key words: acute liver failure – basiliximab – pediatric liver transplantation – steroid-resistant rejection

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Accepted for publication 5 September 2014

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.

Table 2. The demographics of the non-ACR, ACR, and SRR groups of pediatric ALF patients after LT

	non-ACR	ACR	SRR	p
n	16	17	7	
Age at LT (median)	17 days–12 yr 4 months (1 yr 3 months)	1 months–4 yr 4 months (9 months)	6 months–12 months (10 months)	0.427
BW at LT (kg [median])	2.6–32 (8.1)	3.5–17 (8.6)	7.6–9.6 (8)	0.669
Gender male/female	8/8	12/5	5/2	0.411
Living donor (n [%])	13 (76.5)	17 (100)	7 (100)	0.036
ABO-incompatible (n [%])	4 (25)	5 (29.4)	1 (14.3)	0.739
Etiologies (n [%])				
Unknown	11 (68.8)	14 (82.4)	7 (100)	0.215
Others	5 (31.3)	3 (17.7)	0 (0)	
EBV	2 (12.5)	1 (5.9)		
CMV	1 (6.3)			
HSV 1		1 (5.9)		
Echovirus 3		1 (5.9)		
MDS	1 (6.3)			
Hemochromatosis	1 (6.3)			

HSV, herpes simplex virus; MDS, mitochondria DNA depletion syndrome.

Table 3. The outcomes of basiliximab therapy in the SRR patients

Case	The interval between treatment and discharge (days)	ACR after basiliximab	CMV	EBV	Other infection	Outcome	Follow-up (months)	Present IS
1	18	+	+	–		Alive	27	TAC
2	90	+	+	–	Pneumocystis pneumonia	Alive	24	PSL, SRL
3	40	–	+	–		Alive	47	TAC
4	23	–	+	–		Alive	51	TAC
5	223	+	+	+		Graft loss, retransplant (POM 9), Alive	18	TAC, PSL, MMF, SRL
6	41	–	+	+		Alive	15	TAC, PSL
7	23	–	+	+		Alive	2	TAC, PSL, MMF

POM, postoperative months.

All patients survived, although one patient (Case 5) required retransplantation due to graft failure. The histopathological findings of his explanted graft revealed severe centrilobular rejection, including severe necrosis of zone 3, accompanied by suspected veno-occlusive disease. Veno-occlusive disease was not present in the other patients. Three patients (Cases 1, 3, and 4) could discontinue steroids after basiliximab therapy. Two patients (Cases 2 and 6) remained on prednisolone for more than two yr and one yr after basiliximab therapy, respectively. Two patients required low doses of prednisolone due to a slight increase of the values of liver function tests after basiliximab therapy at the end of the follow-up.

The changes in the results of liver function tests, such as the levels of total bilirubin, AST, and ALT after basiliximab therapy in all but one patient (Case 5) are shown in Fig. 1, which revealed a dramatic improvement of the liver function test after basiliximab therapy. The patients were discharged from the hospital with a median period from the date of basiliximab therapy to the resolution of ACR of 31.5 days (range: 18–90 days). In spite of improvement of the liver function, the duration of hospitalization was extended because the administration of ganciclovir was required in all but one patient (Case 5). Three patients (Cases 1, 2, and 5) experienced ACR at seven, 10, and 1.5 months after basiliximab therapy, respectively. All patients improved following treatment with single steroid pulse therapy. There was the same sentence in this paper.

Infectious complications after basiliximab therapy

Opportunistic infections were common, as evidenced by the CMV and EBV viremia and pneumocystis pneumonia. None of the patients developed CMV disease, although CMV-pp65 antigenemia was detected in all patients after basiliximab therapy. CMV immunoglobulin-G was

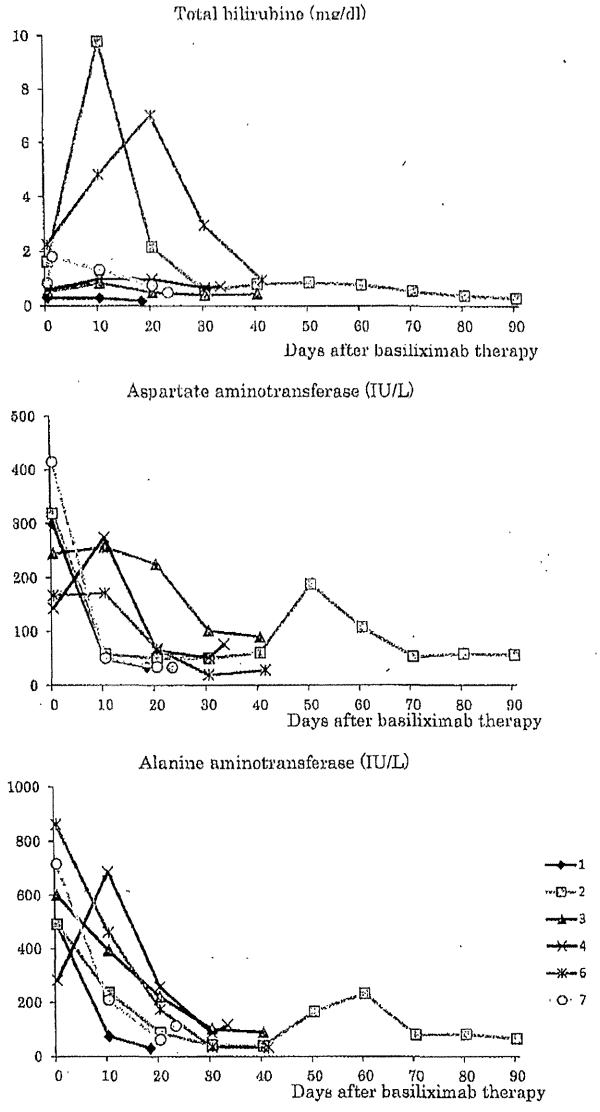

Fig. 1. Changes in liver function test parameters in six patients who exhibited improvements from the administration of basiliximab therapy to hospital discharge.

positive in all recipients before LT, and all but one (Case 5) had received livers from CMV-positive donors. The CMV-pp65 antigenemia in Cases 1 and 6 became positive within four days after basiliximab treatment; thus, the second dose of basiliximab was not administered. The CMV-pp65 antigenemia in Cases 2, 3, 4, and 7 became positive within two wk after basiliximab therapy, and in Case 5, it became positive after retransplantation. Although EBV-DNA was positive in three patients (Cases 5, 6, and 7) during the follow-up period, none of the patients developed post-transplant lymphoproliferative disease. One patient (Case 2) suffered from PCP nine months after basiliximab therapy and was successfully treated with the intravenous administration of trimethoprim–sulfamethoxazole.

The histopathological findings of the SRR patients

The histopathological findings are shown in Table 4. The histopathological grade of rejection on the Banff criteria was classified as moderate to severe rejection in all patients, and the venous endothelial inflammation scores for the central vein region were high. Centrilobular fibrosis was observed in six patients at the first rejection episode, with an interval from 11 to 44 days after LDLT. Centrilobular necrosis and hemorrhage were detected in the majority of patients. The RAI, including the presence of centrilobular perivenulitis, improved in three patients who received follow-up biopsies. No patient showed an anastomotic stricture of the hepatic vein by Doppler ultrasonography.

The changes in the proportions of $CD4^+CD25^+$ T cells and regulatory T cells before and after basiliximab therapy

The changes in the proportions of $CD4^+CD25^+$ T cells and Treg cells in the peripheral blood before and after basiliximab therapy are shown in Fig. 2. The proportions of $CD4^+CD25^+$ T cells in the patients before basiliximab therapy (mean ± standard deviation: 16.56 ± 6.49%) were significantly higher than those observed in the healthy control children (9.67 ± 2.17%, $p = 0.034$). The proportions of $CD4^+CD25^+$ T cells were significantly suppressed after basiliximab therapy compared to those measured in the patients before basiliximab therapy (16.56 ± 6.49% vs. 9.07 ± 3.96%, $p = 0.026$). There were no significant differences in the proportion of Treg cells in the peripheral blood measured before basiliximab therapy in the patients and those observed in the healthy control children (6.18 ± 3.01% vs. 6.91 ± 2.04%, $p = 0.853$). The proportions of Treg cells were also suppressed in the patients after basiliximab therapy compared to those observed in the patients before basiliximab therapy (2.71 ± 2.21% vs. 6.18 ± 3.01%, $p = 0.062$). The proportions of $CD4^+CD25^+$ T cells and Treg cells in the patients who developed graft loss after basiliximab therapy were 3.13% and 1.52%, respectively, which were the same as those observed in the other patients.

Discussion

Previous studies of LDLT for ALF in children, especially infants, have documented poor outcomes. Possible reasons for these results include

Table 4. The histopathological findings of the SRR patients

Case	POD of the 1st rejection	Centrilobular injury				RAI	
		Venulitis*	Fibrosis	Necrosis	Hemorrhage	Before the therapy	After the therapy
1	9	2	F0	–	–	6	–
2	23	3	F2	+	–	7	2
3	16	2	F2	+	+	5	0
4	14	2	F2	+	+	7	1
5	44	2	F2	+	+	6	–
6	11	2	F1	+	+	4	–
7	7	3	F2	+	+	7	–

*Venous Endothelial Inflammation Score according to the Banff schema.

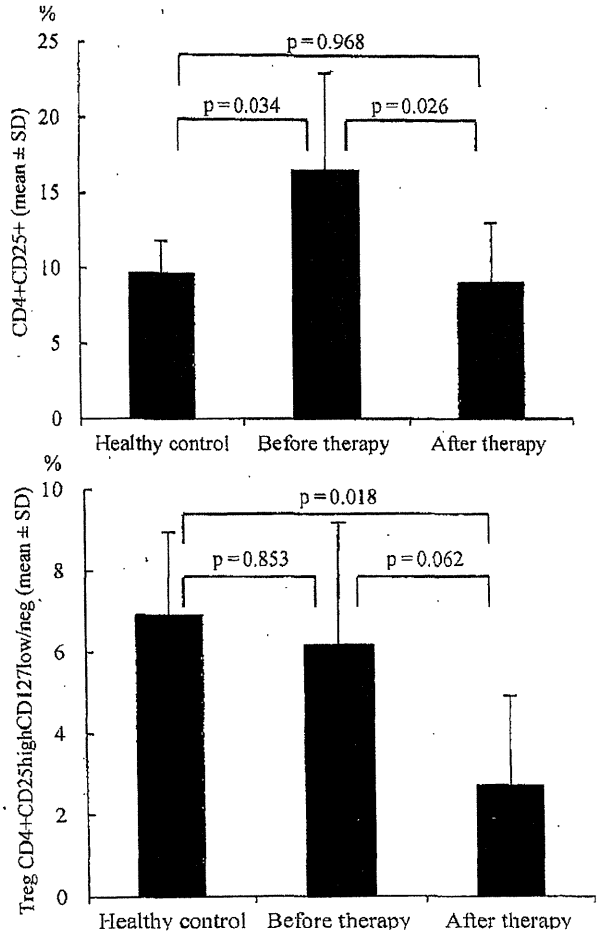

Fig. 2. Comparison of the levels of peripheral $CD4^+$ $CD25^+$ T cells and $CD4^+$ $CD25^{high}$ $CD127^{low/neg}$ regulatory T cells.

the age of the recipients, the cause of ALF, postoperative complications, for example, vascular complications, and the incidence or pattern of rejection (19–21). Among these reasons, technical difficulties in performing LDLT in infants are associated with problems of "large-for-size" grafts (33). The unknown etiology of ALF despite precise investigations before LDLT remains a big issue in this field (34). Patients classified with "cryptogenic hepatitis" may have possible causes of ALF that contraindicate the use of LT, such as mitochondrial respiratory chain disorders and familial hemophagocytic lymphohistiocytosis, although the amount of time for investigating the etiology may be limited owing to disease progression before LT (34).

With respect to the incidence or pattern of rejection after LDLT for ALF in children, previous reports have demonstrated a high incidence of ACR that is often refractory to steroids, as well as common pathological features of centrilobular changes, such as central perivenulitis (20, 21, 35). Centrilobular changes, including perivenulitis and necrosis, are associated with late acute rejection during the long-term follow-up after pediatric LT (36). The presence of central perivenulitis is a significant risk factor for the development of centrilobular fibrosis and indicates a trend toward future chronic ductopenic rejection (37). In the current study, central fibrosis was detected in six patients at the first rejection episode, although the significance of centrilobular fibrosis in the long-term follow-up of pediatric LT was uncertain (38). Therefore, managing ACR with centrilobular changes, synonymously defined as SRR in the current study, is a key issue for improving the outcomes of LDLT for cryptogenic ALF in infants.

We demonstrated the effectiveness of basiliximab as rescue therapy for SRR in pediatric LT patients with ALF. The physiological mechanisms of basiliximab therapy acting on SRR are currently unexplained. Several possible pathways related to the effects of basiliximab on SRR have been described to date (39, 40). Cellular IL-2 production contributes to the development of steroid resistance. The multidrug resistance-1 gene product is P-glycoprotein 170, which

actively transports steroids and has been reported to be upregulated by IL-2 (39). Basiliximab has been also used to treat steroid-resistant ulcerative colitis. Creed et al. reported the effectiveness of basiliximab in cases of moderate to severe steroid-resistant ulcerative colitis and described the induction of apoptosis in large numbers of steroid-resistant T-cell clones in the bowel following basiliximab therapy (40). Basiliximab appears to restore the sensitivity of these cells to steroids, resulting in rapid cell depletion in the presence of steroids. The authors also demonstrated a marked synergistic effect of basiliximab in combination with steroids. We therefore consider that treatment with basiliximab as rescue therapy decreases the rate of steroid resistance.

We experienced a high incidence of infections, such as CMV and *Pneumocystis* pneumonia. The patients suffering from SRR had already become severely immunocompromised due to multiple cycles of steroid pulse therapy and the use of additional ISs before the introduction of basiliximab therapy. Aw et al. reported that five of seven pediatric recipients with SRR were successfully treated with basiliximab, and CMV-DNA became positive in two of their patients, but none of the patients developed CMV disease (16). In our series, CMV-pp65 antigenemia was positive in all patients after basiliximab therapy. Because ALF is considered to be a risk factor for CMV infection after LT, the correlation between basiliximab therapy and CMV infection is still unclear (30). One patient suffered from PCP nine months after basiliximab therapy. The patient did not receive PCP prophylaxis after basiliximab therapy. The correlation between basiliximab therapy and *Pneumocystis* Pneumonia is also uncertain. After the experience of PCP following basiliximab, we started PCP prophylaxis at least one yr for the patient with basiliximab therapy and/or with multiple ISs.

In this study, the levels of peripheral $CD4^+$ $CD25^+$ T cells were suppressed following the administration of basiliximab therapy. Treg cells, which play an important role in preventing graft rejection, were also suppressed after basiliximab therapy. Three patients experienced ACR after receiving basiliximab. However, there were no significant differences in the proportion of Treg cells between the patients with ACR (Cases 1, 2 and 7) and the other patients (Cases 4–6) (data not shown). The usefulness of monitoring the levels of peripheral $CD4^+$ $CD25^+$ T cells and Treg cells in the patients with SRR after LT was unclear, because we had no data for pediatric LT patients without SRR. Briem-Richter et al. reported that patients who undergo LDLT have significantly higher numbers of Treg cells and associated cytokine IL-4 serum concentrations than patients who undergo deceased donor LT (41). The rate of prior ACR episodes of 16% observed in the LDLT patients is lower than the 25% noted in the deceased donor LT patients, with no significant differences ($p = 0.75$). Further investigations should be conducted to determine the significance of Treg cells as an indicator of transplantation tolerance.

In conclusion, we herein demonstrated the effectiveness and safety of rescue therapy consisting of basiliximab for SRR. Basiliximab is a possible first-line rescue therapy for SRR after LT, and its use is recommended as soon as possible when the histopathological findings demonstrate centrilobular injuries at the first ACR episode to prevent SRR of pediatric LT patients with ALF.

Acknowledgments

This study was supported by a Grant from the National Center for Child Health and Development (24-8, 25-6).

Authors' contributions

T.S.: Participated in data acquisition, data analysis and interpretation, and drafting of the article; S.S. and M.K.: Participated in critical revision of the article for important intellectual content, approval of the article; H.U., K.S., I.H., H.K., A.F., T.K., M.O., and A.N.: Participated in data acquisition and analysis.

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Pediatr Transplantation 2015: 19: E1–E6

Pediatric Transplantation
DOI: 10.1111/petr.12376

Two-step transplantation for primary hyperoxaluria: A winning strategy to prevent progression of systemic oxalosis in early onset renal insufficiency cases

Sasaki K, Sakamoto S, Uchida H, Shigeta T, Matsunami M, Kanazawa H, Fukuda A, Nakazawa A, Sato M, Ito S, Horikawa R, Yokoi T, Azuma N, Kasahara M. (2015) Two-step transplantation for primary hyperoxaluria: A winning strategy to prevent progression of systemic oxalosis in early onset renal insufficiency cases. *Pediatr Transplant*, 19: E1–E6. DOI: 10.1111/petr.12376.

Abstract: Several transplant strategies for PH1 have been proposed, and LT is performed to correct the metabolic defects. The patients with PH1 often suffer from ESRD and require simultaneous LKT, which leads to a long wait due to the shortage of suitable organ donors. Five patients with PH1 underwent LDLT at our institute. Three of the five patients were under dialysis before LDLT, while the other two patients were categorized as CKD stage 3. An isolated LDLT was successfully performed in all but our first case, who had complicated postoperative courses and consequently died due to sepsis after retransplantation. The renal function of the patients with CKD stage 3 was preserved after LDLT. On the other hand, our second case with ESRD underwent successful LDKT six months after LDLT, and our infant case is waiting for the subsequent KT without any post-LDLT complications after the early establishment of PD. In conclusion, a two-step transplant strategy may be needed as a life-saving option for patients with PH1 and may be possible even in small infants with systemic oxalosis. While waiting for a subsequent KT, an early resumption of PD should be considered from the perspective of the long-term requirement of RRT.

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Key words: infantile oxalosis – living donor liver transplantation – primary hyperoxaluria type 1

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Accepted for publication 17 September 2014

PH1 is an autosomal recessive inherited disorder of glyoxylate metabolism, caused by a deficiency of the liver-specific peroxisomal AGT. Overproduction and urinary excretion of oxalate leads to urolithiasis and nephrocalcinosis, which may consequently result in renal failure. With advancing renal failure, the extrarenal deposition of oxalate in tissues such as the bones, retinas, and/or cardiovascular system occurs (1). Although the prognosis of PH1 has improved because of an increased awareness and earlier diagnosis, leading to earlier treatment, some patients have a rapidly progressing course, and other patients are diagnosed at advanced stages of renal failure (2). Among the various clinical phenotypes of PH1, the infantile form, which often exhibits a rapidly progressing course to ESRD and

Abbreviations: AGT, alanine-glyoxylate aminotransferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CKD, chronic kidney disease; ESRD, end-stage renal disease; GFR, glomerular filtration rate; GRWR, graft-to-recipient body weight ratio; HD, hemodialysis; KT, kidney transplantation; LDKT, living donor kidney transplantation; LDLT, living donor liver transplantation; LKT, liver and kidney transplantation; LL, left lobe; LLS, left lateral segment; LT, liver transplantation; MMF, mycophenolate mofetil; PD, peritoneal dialysis; PH1, primary hyperoxaluria type 1; RRT, renal replacement therapy.

systemic oxalosis (infantile oxalosis) generally before three yr of age, may be difficult to manage and shows poor outcomes (3, 4).

Transplantation strategies for PH1 have been proposed based on concomitant renal insufficiency (5). Simultaneous or sequential LKT is an appropriate therapeutic option for patients with ESRD, although most patients, especially children, have to wait to obtain two organs due to the shortage of suitable organ donors (6). While waiting for transplantation, the continuation of RRT is a crucial element of survival; however, there are some dilemmas that in which all forms of dialysis are inadequate to keep up with the high production of oxalate (7).

We herein review the previous cases of PH1 treated using LDLT at our institute, and present the detailed clinical courses of three cases with ESRD, especially the infantile case in which we performed early LT to avoid further deposition of oxalate and succeeded in early resumption of PD after LT.

A review of LDLT in our PH1 cases

Since November 2010, five children with PH1 underwent LDLT at our institute. These cases are summarized in Table 1. In our institute, we recommend preemptive LT for pyridoxine-resistant PH1 patients with CKD stage 3, isolated LT (if necessary, sequential KT) for CKD stage 4, and sequential or simultaneous LKT for CKD stage 5. Three patients were under dialysis at the time of LDLT, and we planned sequential LKT. The GFR of the other two patients were 58.9 and 47.8 mL/min/1.73 m^2, categorized as CKD stage 3. Because pyridoxine therapy could not prevent disease progression, we planned preemptive LT.

Case 1

A 17-yr-old male was suspected to have PH1 from his clinical history of recurrent nephrolithiasis when he was four yr old and started to take pyridoxine and citrate. He was diagnosed based on the low AGT catalytic activity of his liver. His renal function gradually declined, and PD had been introduced at the age of 13 yr. He had a past history of bilateral femoral neck fractures due to bone lesions related to PH1, and he was confined to a wheelchair. He received an LL graft from his mother. After LDLT, he suffered from portal vein thrombosis, which led to graft failure. He underwent retransplantation and received a whole liver graft from a deceased donor. However, he died due to sepsis, which might have

Table 1. The characteristics of donors and recipients

Case No.	Age at LDLT	BW at LDLT (kg)	Age at Dx/Sex	CKD Stage at Pre-Tx.	Genetic mutation	Mode of dialysis/Period	Pre-Tx eGFR (mL/min/1.73 m^2)	Donor/Age (yr)	Graft Type/GRWR (%)	AGT Activity of Graft Liver (%)	I/S	Complication	Current GFR (mL/min/1.73 m^2)	Outcome (F/U period)
1	17 yr	45.3	4 yr/M	Stage 5	N/A	PD/5 yr	N/A	Mother/46	LL/0.63	N/A	Tacrolimus Steroids	PVT	N/A	Died* (2 months)
2	15 yr	50.0	5 yr/F	Stage 5	N/A	HD/1 yr	N/A	Mother/39	LL/0.73	N/A	Tacrolimus Steroids	Massive ascites	N/A	Alive† (32 months)
3	8 months	7.1	4 months/F	Stage 5	p.Arg11X homozygous	PD/4 months	N/A	Mother/32	LLS/3.04	30.9	Tacrolimus Steroids	None	N/A	Alive (5 months)
4	10 yr	22.3	4 yr/F	Stage 3	p.Gln110 fs/ p.Lys12 fs	None	58.9	Mother/31	LLS/1.02	35.0	Tacrolimus Steroids MMF	None	67.8	Alive (20 months)
5	7 yr	23.5	7 yr/F	Stage 3	p.Tyr251Lys/ p.Ser275 fs	None	47.8	Mother/28	LLS/1.11	77.2	Tacrolimus Steroids MMF	None	54.0	Alive (12 months)

BW, body weight; CKD, chronic renal disease; Dx, diagnosis; F, female; F/U, follow-up; I/S, immunosuppressants; M, male; N/A, not assessed; Tx, transplantation.
*The patient underwent retransplantation due to graft failure eight days after LDLT.
†The patient underwent LDKT six months after LDLT.

been related to the requirement for continuous HD after retransplantation.

Case 2

A 15-yr-old female was suspected to have PH1 from her clinical history of recurrent nephrolithiasis when she was five yr old. She was diagnosed based on her urine and plasma oxalate level and her family history that her father had been diagnosed as PH1, although no conservative treatment was introduced. Her renal function deteriorated into ESRD, and HD was introduced at the age of 10 yr. She could conduct almost normal activities of daily living despite intermittent HD treatment before LDLT. She received an LL graft from her mother. She suffered from massive ascites for a few months after LDLT. She finally succeeded in undergoing LDKT by receiving a kidney graft from her grandmother six months after LDLT. Her hepatic and renal grafts are currently functioning well.

Case 3

A four-month-old female presented with ESRD, and PD was immediately introduced. Her plasma oxalate level was 167.2 μmol/L (reference range <1.8 μmol/L) and genetic testing confirmed a diagnosis of PH1. Medical management with pyridoxal phosphate was also initiated; however, it did not have much effect on her clinical course. Ophthalmoscopy revealed a rapid progression of oxalate deposition in the bilateral retinal epithelium (Fig. 1a,b). No other complications were found during the preoperative examinations, and her cardiac function was normal based on echocardiography. At the age of eight months, the patient underwent LDLT by receiving an LLS from her mother to decrease the risks associated with systemic oxalosis under PD, especially the rapid progression of retinal lesions. The previously inserted PD catheter was kept and protected during the operation. Intestinal manipulation was kept as a minimum, and anti-adhesive materials (Seprafilm; Genzyme Corporation, Cambridge, MA, USA) were used to prevent abdominal adhesions at the end of the operation. HD was performed for three days prior to LDLT to decrease the plasma oxalate level. Continuous HD had been performed based on the patient's cardiovascular condition and renal function for 12 days after LDLT, and thereafter, PD was successfully resumed. The postoperative clinical courses of the recipient and donor were uneventful, without any complications. After LDLT, the rapidly progressing oxalate deposition in retinal epithelium stopped, and the patient favorably gained weight to 8.8 kg at the age of 12 months without any neurodevelopmental delay. The patient continues to be managed under PD and renal anemia has been corrected by intermittent blood transfusion. KT will be performed when her body weight reaches 10 kg, although the plasma oxalate level has still been higher than the normal range after the LDLT (Figs. 2 and 3).

Discussion

The outcomes of organ transplantation in PH1 have improved over time, with recent LKT having been highly successful (8, 9). LKT is currently the most widely used procedure for the PH1 patients with severe renal insufficiency, and we also planned LKT for such patients (Case 1, 2, and 3). However, the number of the deceased donors is still low, and living donors remain the main source of donor organs in our country. The two-organ donation increases the burdens and the risks for living donors, and therefore, the timing of organ transplantation for PH1 patients used to be delayed, resulting in disappointing

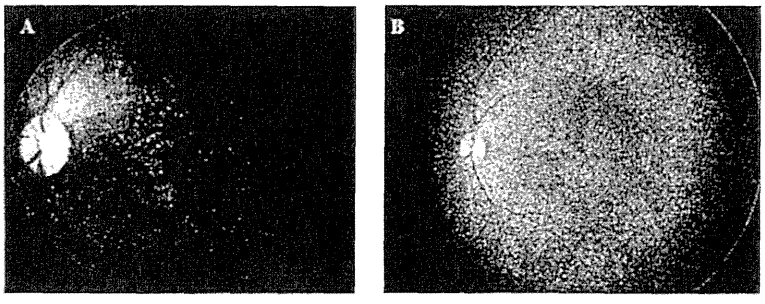

Fig. 1. The ophthalmoscopic findings of oxalate deposition in the retinal epithelium. The examination at the age of four months (no photo) showed normal findings; however, the findings at the age of five months (A) revealed multiple oxalate deposits in the retinal epithelium, and the intensity of oxalate deposition had increased at the age of seven months (B).

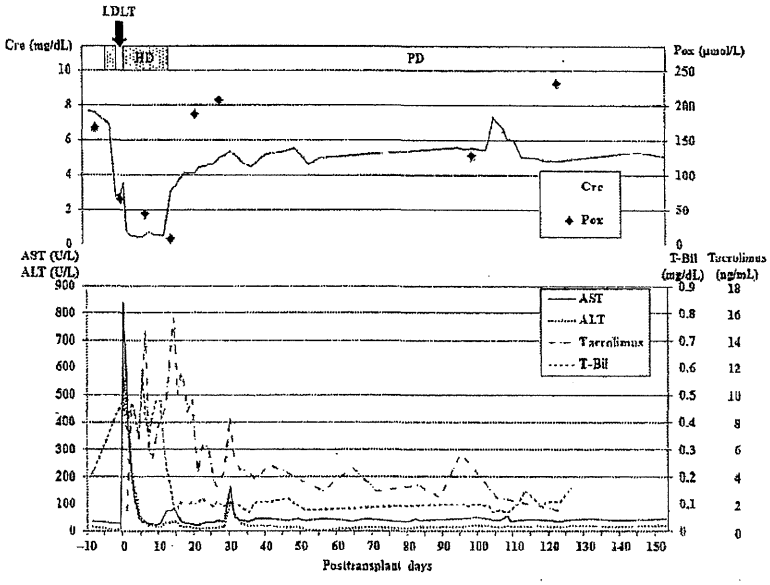

Fig. 2. The clinical course. The patient's postoperative clinical course was uneventful. The plasma oxalate level (Pox) was maintained at lower levels during the period of HD. Thereafter, it increased under PD. BW, body weight; Cre, creatinine; T-Bil, total bilirubin.

outcomes, as described in Case 1. We changed our policy to perform the organ transplantation before renal functions become severer. Preemptive LT may be an ideal transplantation strategy; however, it is difficult to decide the optimal timing because of heterogenous clinical courses of PH1 patients and the risks of the operation and the long-term immunosuppression. Previous literature reported that the threshold GFR below which degradation is unavoidable was estimated to be between 40 and 50 mL/min/1.73 m^2 (10), and therefore, we chose the pyridoxine-resistant PH1 patients with CKD stage 3 as the indication of preemptive LT (Case 4 and 5). For the PH1 patients with CKD stage 4, we recommend isolated LT and followed by sequential KT, if necessary. Even though the renal function deteriorates after LT, sequential living donor kidney donation is probably less aggressive for the donor than concurrent donation of two organs (11).

If a low body weight infant requires simultaneous LKT, it may be impossible to implant two adult organs into a very small abdominal cavity. Waiting for an infant with PH1 to be large enough for simultaneous LKT from an ideal size-matching pediatric deceased donor permits ongoing oxalate deposition throughout the waiting period, leading to the progression of complication and poor outcome, such as Case 1. The presented infant case revealed a rapid progression of retinal lesions, which could lead to vision impairment, and we decided to perform early LT (12). Sequential LKT may therefore be an appropriate therapeutic option for infants with systemic oxalosis, because an isolated LT, which is performed before the KT, can allow for earlier interruption of the oxalate accumulation (6).

When considering sequential LKT for a small patient, the use of RRT will be an important and challenging issue after LT. To the best of our knowledge, nine children with PH1 who were planned to undergo sequential LKT were previously reported (2, 6, 13–18) (Table 2). Although PD was tried after LT in two cases, both of these cases died before receiving sequential KT due to peritonitis and cytomegalovirus infection. PD is generally employed in low body weight pediatric patients, although this procedure may be difficult to perform after abdominal surgery. HD must be performed for patients with PH1 during the perioperative period for LT, although the long-term dependence on HD after LT is considered to be a

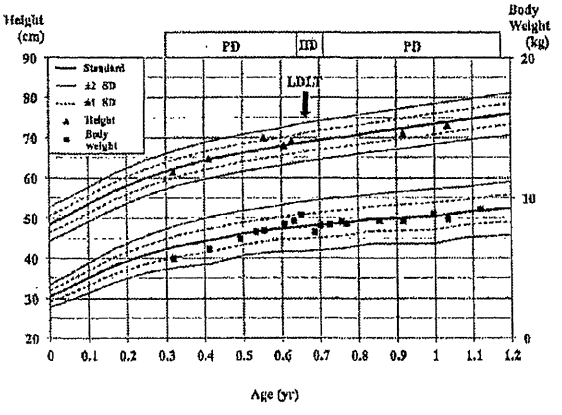

Fig. 3. The growth curve. An appropriate growth was obtained during the follow-up period after LDLT.

Table 2. A review of the previously published cases planned to undergo sequential liver-kidney transplantation for PH1

Case No.	Age (yr)/ Gender	Body Weight (kg)	Mode of dialysis Before LT	Donor Type (liver/kidney)	Interval between LT and KT	Mode of dialysis After LT	Outcome	Reference No.
1	1.3/F	6.2	PD	Father/Father	1.7 months	N/A	Alive	13
2	4.5/F	N/A	N/A	Living donor/Deceased donor	N/A	N/A	Alive	15
3	9/M	18.0	HD	Mother/Mother	4.7 months	N/A	Alive	14
4	0.9/F	N/A	N/A	N/A	9 months	HD	Alive	16
5	1.1/M	N/A	PD + HD	Deceased donor, Mother/Mother	8 months	N/A	Alive	6
6	1.4/M	9.1	PD	Father/Father	6 months	HD	Alive	17
7	3/M	N/A	HD	Deceased donor/Living donor	4 months	HD	Alive	18
8	2.3/M	N/A	PD	Living donor/Not performed	N/A	HD→PD	Died*	2
9	5/M	N/A	PD + HD	Deceased donor/Not performed	N/A	HD→PD	Died*	2
10†	17/M	45.3	PD	Mother, Deceased donor/ Not performed	N/A	HD	Died*	–
11†	15/F	50.0	HD	Mother/Grandmother	6 months	HD	Alive	–
12†	0.7/F	7.1	PD	Mother/Not performed	N/A	HD→PD	Alive	–

N/A, not assessed.
*All patients died due to infectious episodes.
†Our presented cases.

risk factor for various postoperative complications, such as catheter-related infections, especially in immunosuppressed patients, as was Case 1. Therefore, our strategy for RRT after LT prior to KT for a low body weight infant is an early resumption of PD, which can be achieved by performing meticulous surgical procedures, such as minimal intestinal manipulation and the use of anti-adhesive materials. Seprafilm has been widely used to reduce the incidence and severity of postoperative adhesion in pediatric abdominal surgery (19). In addition to these surgical techniques, postoperative management to keep the patency of the PD catheter using a heparin lock solution is important for the early resumption of PD.

When LDLT is considered for patients with PH1, the heterozygous carriers of this disorder may not be living donor candidates due to their potential for decreased enzyme function. It has been reported that the use of heterozygous donors in patients with various autosomal recessive diseases shows no negative impact on either the donors or recipients (20), although the reference articles contributing this evidence did not include LDLT cases with PH1. The previous articles related to LT or LKT from living donors did not profoundly discuss this issue (2, 6, 13, 14, 21–24). On the other hand, there are several studies reporting cases of domino LT retrieving compound heterozygous organs with PH1 (25–29). Not surprisingly, all of those reported cases developed renal insufficiency after domino LT, and Farese et al. suggested that the compound heterozygous organs with PH1 should not be used for domino LT (27). We measured the AGT catalytic activity of the graft livers. We sampled living tissue before interrupting the blood flow, and the sample was immediately frozen. The values of the native and graft livers were described as the ratio to the healthy liver value. The values of graft livers were available for three cases, including 30.9% (Case 3), 35.0% (Case 4), and 77.2% (Case 5) (30), respectively. None of the donors showed any clinical symptoms or abnormal laboratory findings related to PH1. A previous report showed that the mean AGT activity in the PH1 patients was 3.3% and that of PH1 heterozygous carrier was intermediate at 30–50% (31). The interpretation of the relatively lower AGT catalytic activity of the graft livers in our series is unclear, and further evaluation of the impact of the use of organs from heterozygous carriers of PH1 will be necessary.

In conclusion, LT via a two-step transplant strategy, which can allow for the interruption of the oxalate accumulation, may be needed as a life-saving option for the patients with PH1, and may be possible even in low body weight infants. While waiting for a subsequent KT, an early resumption of PD should be considered from the perspective of the long-term requirement for RRT.

Acknowledgments

The authors thank Dr. Tatsuya Takayama at Jichi Medical University, Tochigi, Japan, and Dr. Masao Nagata at Hamamatsu University School of Medicine, Shizuoka, Japan, for their contributions measuring the plasma oxalate level and AGT catalytic activity.

Source of funding

This work was supported in part by grants from the Scientific Research Fund of the Ministry of

Education and by a Research Grant for Immunology, Allergy and Organ Transplant from the Ministry of Health, Labor and Welfare, Japan (21591403, 21-04, 21-042, 25-06).

Authors' contributions

K.S.: Study design, writing of the paper; S.S.: Study design, critical revision of the article for clinical content; H.U., T.S., M.M., H.K., A.F.: Collection of the data; A.N.: Critical revision of the article for laboratory content; M.S., S.I.: Critical revision of the article for nephrological content; R.H.: Critical revision of the article for clinical content; T. Y., N.A.: Critical revision of the article for ophthalmological content; M.K.: Study design, critical revision of the article for clinical content.

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ORIGINAL ARTICLE

Use of Serial Assessment of Disease Severity and Liver Biopsy for Indication for Liver Transplantation in Pediatric Epstein-Barr Virus-Induced Fulminant Hepatic Failure

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The decision to perform liver transplantation (LT) in patients with Epstein-Barr virus (EBV)–induced fulminant hepatic failure (FHF) relies on a precise assessment of laboratory and pathological findings. In this study, we analyzed clinical and laboratory data as well as the pathological features of the liver in order to evaluate the pathogenesis and the need for LT in 5 patients with EBV-induced FHF. According to the King's College criteria, the Acute Liver Failure Early Dynamic (ALFED) model, and the Japanese criteria (from the Acute Liver Failure Study Group of Japan), only 1 patient was considered to be a candidate for LT. However, explanted liver tissues in 3 cases exhibited massive hepatocellular necrosis together with diffuse CD8-positive T cell infiltration in both the portal area and the sinusoid. EBV was detected in the liver, plasma, and peripheral blood mononuclear cells (PBMNCs). In 2 cases indicated to be at moderate risk by the ALFED model, liver biopsy showed CD8-positive and EBV-encoded RNA signal–positive lymphocytic infiltration predominantly in the portal area, but massive hepatocellular necrosis was not observed. These patients were treated with immunosuppressants and etoposide under the diagnosis of EBV-induced hemophagocytic lymphohistiocytosis or systemic EBV-positive T cell lymphoproliferative disease of childhood. EBV DNA was detected at a high level in PBMNCs, although it was negative in plasma. On the basis of the pathological analysis of the explanted liver tissues, LT was proposed for the restoration of liver function and the removal of the EBV-infected lymphocytes concentrated in the liver. Detecting EBV DNA by a quantitative polymerase chain reaction in plasma and PBMNCs was informative. An accurate evaluation of the underlying pathogenesis is essential for developing a treatment strategy in patients with EBV-induced FHF. *Liver Transpl 000:000-000, 2014.* © 2014 AASLD.

Received July 9, 2014; accepted November 14, 2014.

In the majority of patients with infectious mononucleosis, asymptomatic, self-limited hepatic dysfunction, together with mild elevations of serum aminotransferase levels (2-3 times the upper limit of normal), is usually observed. In patients with Epstein-Barr virus (EBV)–induced fulminant hepatic failure (FHF), massive hepatocellular necrosis can happen.[1-3] For such cases, liver transplantation (LT) is imperative to prevent death.[2,3]

Abbreviations: ALF, acute liver failure; ALFED, Acute Liver Failure Early Dynamic; D-Bil, direct bilirubin; EBER, Epstein-Barr virus–encoded RNA signal; EBV, Epstein-Barr virus; EBV-T-LPD, systemic Epstein-Barr virus–positive T cell lymphoproliferative disease; FHF, fulminant hepatic failure; HLH, hemophagocytic lymphohistiocytosis; JALFSG, Acute Liver Failure Study Group of Japan; KCC, King's College criteria; LDLT, living donor liver transplantation; LT, liver transplantation; PBMNC, peripheral blood mononuclear cell; PCR, polymerase chain reaction; PT, prothrombin time; T-Bil, total bilirubin.

This work was supported in part by a grant from the National Center for Child Health and Development of Japan (24-4, 24-8).

Potential conflict of interest: Nothing to report.

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DOI 10.1002/lt.24052
View this article online at wileyonlinelibrary.com.
LIVER TRANSPLANTATION.DOI 10.1002/lt. *Published on behalf of the American Association for the Study of Liver Diseases*

TABLE 1. Laboratory Data for the 5 Patients With EBV-Induced FHF on the Fifth Hospital Day

Case	Age/Sex	PT (%)	T-Bil (mg/dL)	D-Bil/T-Bil Ratio	Platelets (/μL)	Liver Atrophy	Plasma EBV DNA (Copies/μg of DNA)	Mononuclear Cell EBV DNA (Copies/μg of DNA)
1	1 year 7 months/female	38.8 (10.0)	1.86 (4.61)	0.67	6.5 (2.6)	Yes	5.1×10^4	5.2×10^2
2	5 years 0 months/male	30.2 (10.0)	11.91 (11.91)	0.31	2.4 (2.4)	No	2.9×10^3	4.4×10^5
3	9 years 3 months/female	29.1 (20.7)	3.14 (13.93)	0.51	4.9 (4.9)	Yes	3.4×10^5	1.5×10^2
4	2 years 3 months/female	37.7 (20.8)	2.85 (3.96)	0.49	12.5 (10.3)	Yes	Negative	3.8×10^4
5	5 years 8 months/male	49.8 (49.8)	1.44 (2.46)	0.52	20.5 (15.4)	Hepatomegaly	Negative	3.8×10^5

NOTE: The peak (worst) values are indicated in parentheses. Liver atrophy was evaluated by ultrasonography on the fifth hospital day and was compared to observations at admission.

Although liver grafts are readily available in Japan because of a willingness of parents to be donors, living donor liver transplantation (LDLT) should be prevented whenever possible because it affects the quality of life of both the donor and the recipient. A number of prognostic models have been used to predict the outcome of acute liver failure (ALF) or FHF when a consideration for LT is present.[4-6] The most widely applied models are the King's College criteria (KCC) and the Model for End-Stage Liver Disease/Pediatric End-Stage Liver Disease; however, the need for a better prognostic model remains because each of those models has its own limitation in terms of either sensitivity or specificity. Kumar et al.[5] developed a new prognostic model, known as the Acute Liver Failure Early Dynamic (ALFED) model, that is based on whether the levels of predictive variables remain persistently high or elevated during the first 3 days of hospitalization. The authors found that early changes in the prognostic markers predicted outcomes that were better than the static baseline levels. The ALFED model was superior to the KCC and the Model for End-Stage Liver Disease, even when the 3-day serial values were taken into consideration.[5]

For patients with EBV-induced FHF, however, the use of a prognostic model may not be sufficient to perform a strict and accurate assessment. Liver biopsy is useful in making a definite decision for LT. Here, we analyzed clinical and laboratory data as well as pathological features of the liver to evaluate the pathogenesis of FHF and the necessity for LT in patients with EBV-induced FHF.

PATIENTS AND METHODS

Among 44 pediatric FHF patients transferred to our hospital for consideration for LT between January 2006 and April 2014, 5 had EBV-induced FHF. All patients were previously healthy and immunocompetent. No patients had any family history of liver disease, metabolic disease, immunodeficiency, or hemophagocytic lymphohistiocytosis (HLH). Clinical data were collected from the patients' medical records.

Quantification of EBV DNA in plasma, peripheral blood mononuclear cells (PBMNCs), and liver lymphocytes was carried out with a real-time quantitative polymerase chain reaction (PCR) assay with the TaqMan system (Applied Biosystems, Warrington, United Kingdom) as described elsewhere.[7] Amplification of EBV DNA was performed with the ABI-Prism 7500 real-time PCR system (Applied Biosystems) with EBV-specific primers derived from conserved sequences in the BALF5 gene encoding the EBV DNA polymerase. A peripheral blood EBV DNA load > 102 copies/μg of DNA was considered a significant elevation because >95% of healthy EBV carriers had an EBV DNA load below this level.[4] EBV-infected cells were examined in each component of liver lymphocytes (ie, CD19-positive, CD4-positive, CD8-positive, and CD56-positive components) with the IMag cell separation

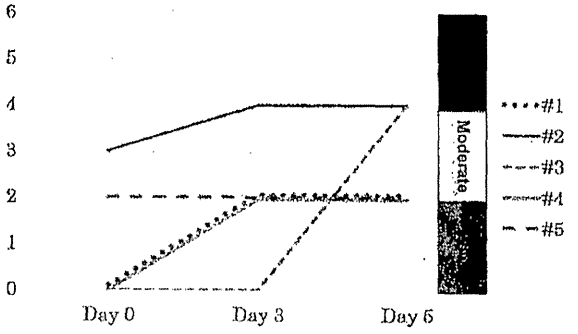

Figure 1. ALFED scores in 5 cases. The ALFED model is based on an overall risk score calculated for each case through the addition of the scores corresponding to their risk factors (hepatic encephalopathy > grade 2, PT-international normalized ratio > 5, arterial ammonia > 123 μmol/L, and serum bilirubin > 15 mg/dL). Originally, the cases were stratified into 3 risk categories on the basis of the risk factors at admission (day 0) and their dynamicity over 3 days (day 3) as follows: 0 to 1, low risk; 2 to 3, moderate risk; and 4 to 6, high risk. On day 3, case 2 was stratified into the high-risk category, whereas cases 1, 4, and 5 remained at moderate risk. Case 3, who had a score of 0 on day 3, attained a score of 4 on day 5 and was stratified into the high-risk category.

system (BD Pharmingen, San Jose, CA) according to the protocol supplied by the manufacturer.[7]

Pathological analysis was performed on either the explanted liver or the biopsied liver tissues. For immunohistochemistry, antibodies against cytoplasmic CD3, CD4, CD8, CD20, CD56, granzyme B, and perforin were used (Nichirei Biosciences, Tokyo, Japan). The EBV genome was detected by in situ hybridization with Epstein-Barr virus–encoded RNA signals (EBERs; EBER peptide nucleic acid probe, Dako, Japan). Double staining for CD20 and EBERs as well as CD45RO and EBERs was performed to detect the phenotype of the EBV-infected cells. Immunoglobulin/T cell receptor clonality assays were performed with BIOMED-2 with formalin-fixed, paraffin-embedded liver tissues.[8]

The study was conducted with the approval of the ethics committee of the National Center for Child Health and Development (no. 466).

RESULTS

Clinical Findings

All 5 patients were treated with high-dose dexamethasone, plasma exchange, and continuous hemodiafiltration. Cases 1 and 2 were additionally treated with cyclosporine A for hemophagocytic syndrome. Case 2 was diagnosed with HLH according to the HLH2004 criteria,[9] but case 1 did not fulfill the criteria. Three patients (cases 1, 2, and 3) underwent LT; cases 1 and 2 underwent LDLT 15 and 33 days after the initial presentation, respectively, whereas case 3 received urgent deceased donor LT 11 days after the initial presentation. Cases 1 and 3 remained healthy 2 years 4 months and 3 years 7 months after LT, respectively.

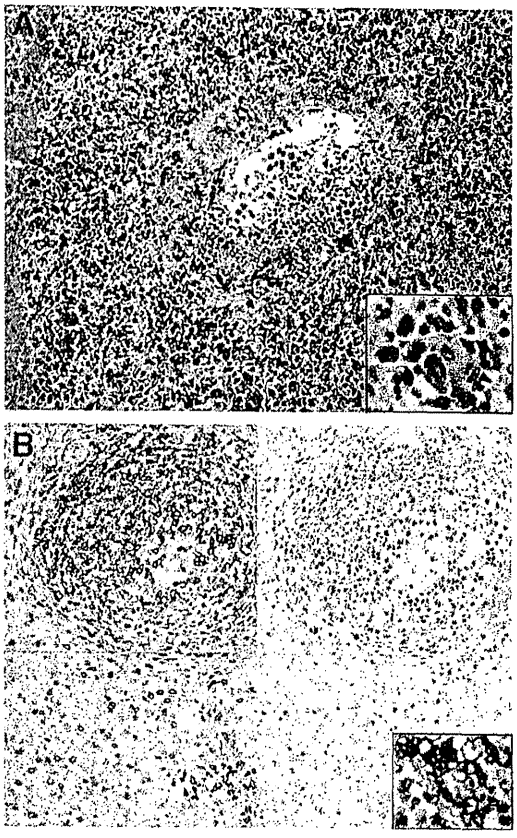

Figure 2. Photomicrograph of the explanted liver in case 2. (A) The explanted liver showed massive hepatocellular necrosis and extensive lymphohistiocytic infiltrate in the portal and lobular distribution. Hemophagocytosis was frequently seen (inset). (B) Infiltrating lymphocytes were predominantly CD8-positive T cells (left) and EBER-positive lymphocytes (right). Double staining for CD45RO (brown) and EBERs (dark blue) indicated that most of the EBER-positive lymphocytes were T cells (inset).

One patient (case 2) died because of allograft failure resulting from veno-occlusive disease 93 days after LT. An early determination of the indications for LT was primarily based on the guidelines proposed by the Acute Liver Failure Study Group of Japan (JALFSG) in 2011.[6] Five variables, namely, the prothrombin time (PT) percentage, serum total bilirubin (T-Bil), direct bilirubin (D-Bil)/T-Bil ratio, platelet count, and liver atrophy, were used to calculate the score at the time of development of hepatic encephalopathy higher than grade 2. The decision for LT was based on a serial assessment of the severity of FHF according to the JALFSG score during the first 5 days of commencing artificial liver support. The JALFSG score in cases 1, 2, and 3 remained high and increased during the first 5 days of hospitalization: the scores on day 0 (on admission) and day 5 were 2 and 4 for case 1, 5 and 7 for case 2, and 4 and 5 for case 3, respectively.

For case 4, for which liver biopsy on day 20 after the initial presentation showed hepatocyte ballooning without massive necrosis, the diagnosis was EBV-

TABLE 2. Pathological Features of the Liver in the 5 Patients With EBV-Induced FHF

Case	Massive Necrosis	Hemophagocytosis	Infiltrated Lymphocytes	EBV-Infected Cells
1	+	+	T (CD8 ≫ CD4) ≫ B	CD8+ T
2	+	+	T (CD8)	CD8+ T
3	+	−	T (CD8)	CD8+ T
4	−	−	T (CD8 ≫ CD4) ≫ B	CD8+ T
5	−	−	T (CD8)	CD8+ T

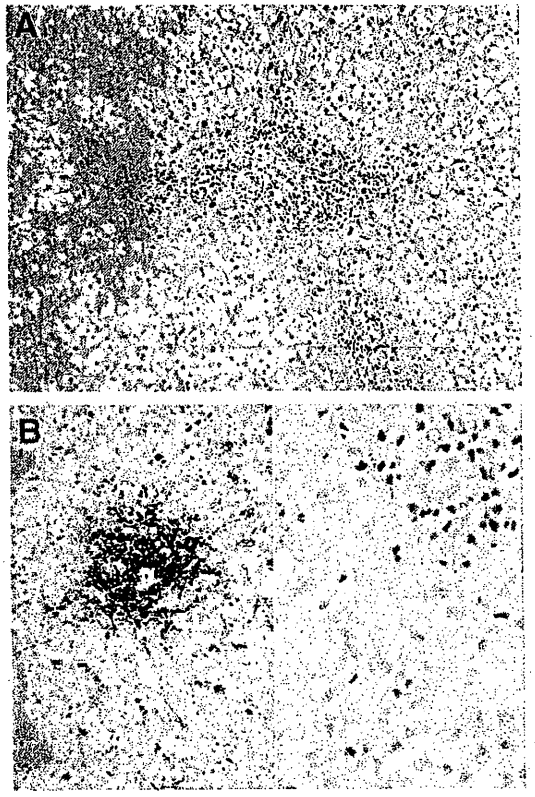

Figure 3. Photomicrograph of the liver biopsy in case 5. (A) Hepatocytes were swollen, but massive hepatocellular necrosis was not observed. (B) Atypical lymphoid infiltrate in the portal area was composed of CD8-positive T cells (left). In situ hybridization of EBERs revealed both portal and sinusoidal distributions of the EBV-infected lymphocytes (right).

induced HLH according to the HLH2004 criteria. The patient was treated with prednisolone, cyclosporine A, and etoposide. She remained healthy at 4 months. One patient (case 5), who was diagnosed with systemic Epstein-Barr virus–positive T cell lymphoproliferative disease of childhood (EBV-T-LPD), by liver wedge biopsy on day 12 after the initial presentation, was treated with prednisolone, cyclosporine A, and etoposide. Subsequently, he received high-dose combined chemotherapy and allogeneic bone marrow transplantation. He remained healthy for 2 years 3 months with no abnormality in familial HLH-associated genes (perforin, syntaxin11, Munc13-4, and Munc18-2) or SH2D1A (X-linked lymphoproliferative disorder-associated gene). Laboratory data for all 5 patients are summarized in Table 1. The JALFSG scores in cases 4 and 5 were low during the first 5 days of hospitalization; scores on days 0 and 5 were 1 and 2 for case 4 and 1 and 1 for case 5, respectively.

The ALFED scores for the 5 patients from day 0 to day 5 are shown in Fig. 1. Case 2 was a high risk on day 3 and was considered to be a candidate for LT. Case 3 was a low risk on day 3 but became a high risk (score 4) on day 5. Cases 1, 4, and 5 remained at moderate risk from day 3 to day 5. Only case 2 fulfilled the KCC, whereas the others never fulfilled it at any time of the clinical course (from admission to day 5).

Pathological Findings

Explanted Liver

All 3 explanted livers were atrophic, and the weight was 412 g in case 1, 358 g in case 2, and 600 g in case 3. The cut surface showed a dark red color. In cases 2 and 3, massive hepatocellular necrosis was diffused in distribution, and the percentage of hepatocellular necrosis was more than 90%. In case 1, hepatocyte ballooning was marked, whereas hepatocellular necrosis was patchy in distribution. All explanted livers showed extensive lymphohistiocytic infiltrate in both the portal and lobular distributions (Fig. 2A). Ductular reaction and cholestasis were present. Central venulitis characterized by lymphocytic infiltration was evident. Numerous macrophages were seen in the parenchyma; hemophagocytosis was seen in cases 1 and 2 (Fig. 2A, inset). Liver lymphocytes were positive for cytoplasmic CD3, CD8, granzyme B, perforin, and EBERs, but they were negative for CD4, CD20, and CD56. EBER-positive lymphocytes were positive for CD45RO but negative for CD20 (Fig. 2B). In case 1, CD20 staining highlighted several large B cells, with scattered CD4-positive T cells observed. T cell clonality studies revealed monoclonal proliferation in cases 2 and 3, and T cell clonality was not evaluated in case 1.

Liver Biopsy

In cases 4 and 5, although hepatocytes were swollen, massive hepatocellular necrosis was not observed. Atypical lymphoid infiltrate in the portal area was composed of small to medium lymphocytes with hyperchromatic, irregular nuclei (Fig. 3A). Central