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CASE REPORT

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

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

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

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

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

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Background

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

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

Case Report

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

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

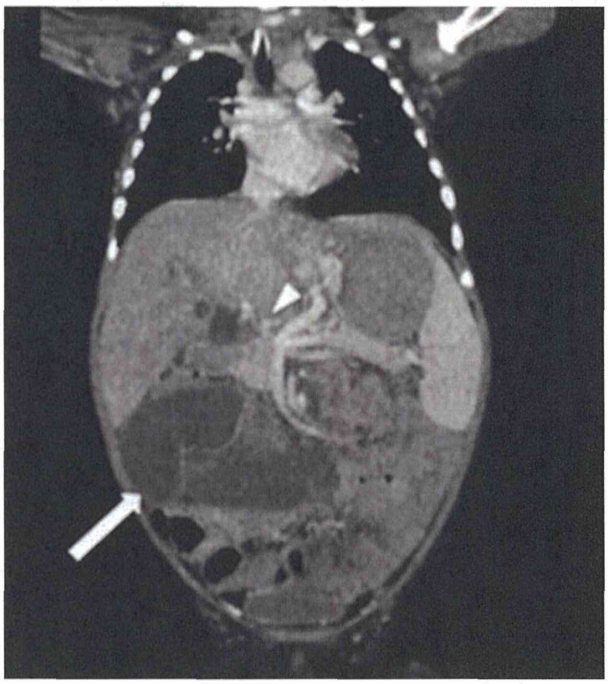

Figure 1. Enhanced abdominal computed tomography demonstrated dilated loops of intestine with wall thickening at the Roux-Limb (white arrow) and narrowing of the portal venous trunk (white arrowhead).

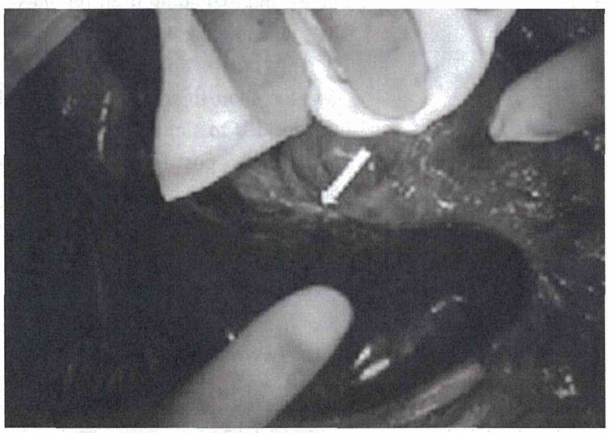

Figure 2. The operative findings at laparotomy revealed a dilated and ischemic afferent loop that was strangulated by a band (white arrow) extending from the mesentery to the transverse mesocolon.

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

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

Discussion

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

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

Conclusions

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

Conflicts of interest

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

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References:

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

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LIVER TRANSPLANTATION 21:362–368, 2015

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 21:362-368, 2015.* © 2015 AASLD.

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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.

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Potential conflict of interest: Nothing to report.

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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

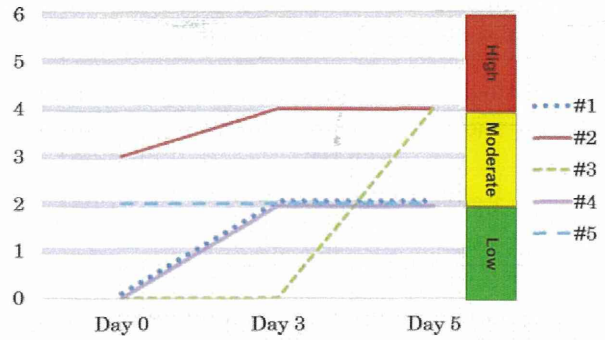

Figure 1. ALFED scores in 5 cases. The ALFED model is based on an overall risk score calculated for each case through the addition of the scores corresponding to their risk factors (hepatic encephalopathy > grade 2, PT-international normalized ratio > 5, arterial ammonia > 123 μmol/L, and serum bilirubin > 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.

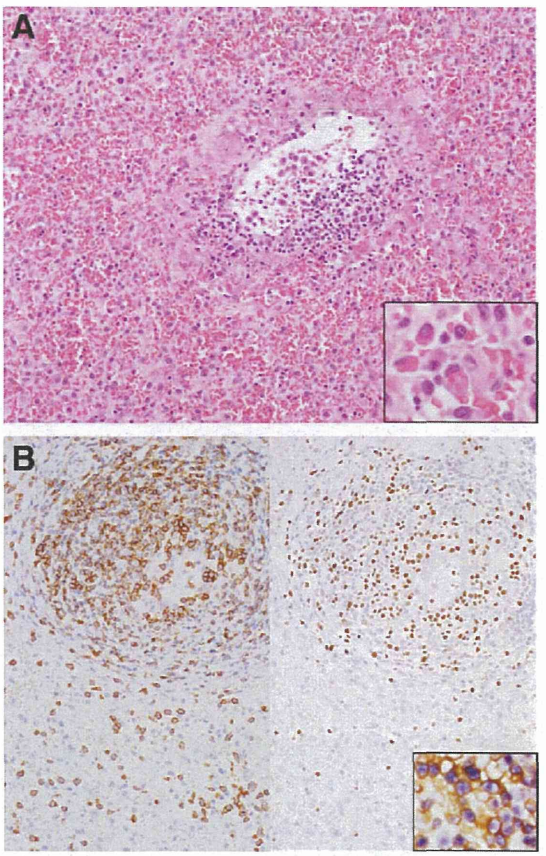

Figure 2. Photomicrograph of the explanted liver in case 2. (A) The explanted liver showed massive hepatocellular necrosis and extensive lymphohistiocytic infiltrate in the portal and lobular distribution. Hemophagocytosis was frequently seen (inset). (B) Infiltrating lymphocytes were predominantly CD8-positive 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

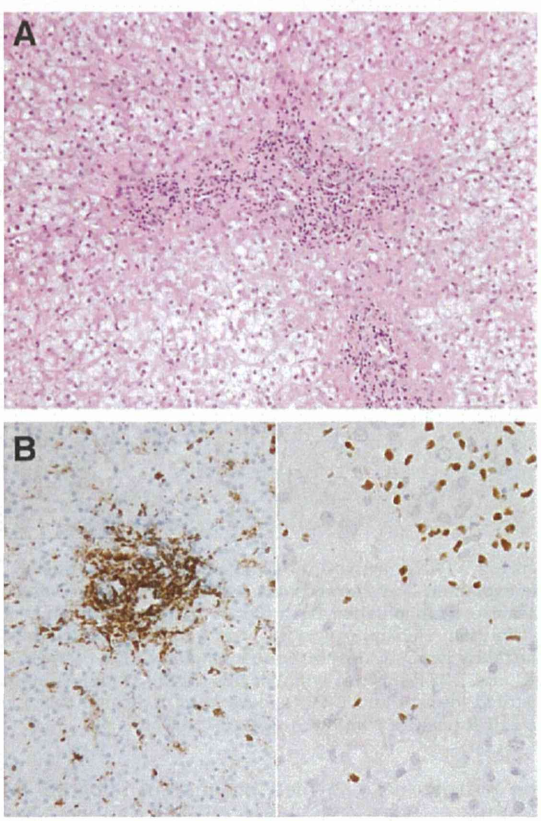

Figure 3. Photomicrograph of the liver biopsy in case 5. (A) Hepatocytes were swollen, but massive hepatocellular necrosis was not observed. (B) Atypical lymphoid infiltrate in the portal area was composed of CD8-positive T cells (left). In situ hybridization of EBERs revealed both portal and sinusoidal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

TABLE 3. EBV-Induced FHF Treated With LT

Age (Years)/Sex	Type of Transplant	Timing of Transplant	EBV Load (Plasma: Copies/g of DNA)	EBER-in situ hybridization (ISH) in Liver	Outcome After LT	Reference
1.6/female	Living donor	Day 15	5.1×10^4	+	Alive at 2 years 4 months	Present study
1.8/female	Cadaveric	Day 15	Positive for EBV DNA by PCR	+	Alive at 2 years	Feranchak et al.[2]
5/male	Living donor	Day 33	1.2×10^4	+	Died on day 93	Present study
8/male	Living donor	Not recorded	8.83×10^4	+	Alive at 8 days	Tohyama et al.[10]
9/female	Cadaveric	Day 11	3.4×10^5	+	Alive at 3 years 7 months	Present study
18/female	Cadaveric	Day 9	2.6×10^5	+	Alive at 1 year 0 months	Dumortier et al.[11]
19/male	Cadaveric	Day 10	Not recorded	Positive by PCR	Alive at 26 days	Park et al.[12]
44/female	Not recorded	Not recorded	Not recorded	Positive by PCR	Alive at 18 months	Mellinger et al.[3]

venulitis was not identified. 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 were negative for CD20 (Fig. 3B). Immunostaining of cytoplasmic CD3, CD8, and EBV by in situ hybridization revealed a sinusoidal distribution of the lymphocytes. In case 4, CD20 staining highlighted several large B cells with scattered CD4-positive T cells that were observed. T cell clonality studies revealed monoclonal proliferation in cases 4 and 5.

EBV DNA was detected with a real-time quantitative PCR assay in the CD8-positive T cell component in all 5 liver tissues. The pathological findings in all cases are summarized in Table 2.

DISCUSSION

EBV infection is an extremely rare cause of ALF and accounted for only 0.21% of ALF cases in a cohort of 1887 adult patients.[3] To date, there have been 25 reported cases of EBV-induced FHF or severe ALF, and they have presented with a mortality rate of 68% (17 of 25).[2,3,10-12] Among the 8 survivors, emergency LT was performed in 5.[2,3,10-12] Characteristics of the 8 EBV-induced FHF patients who received LT, including the 3 patients in the present study, are summarized in Table 3. All examined cases were positive for EBERs in liver tissues, which showed extremely high levels of EBV DNA (10^4-10^5) in plasma. Currently, specific pathological findings for EBV-induced FHF are not yet available in the literature.

Notably, all but 1 patient survived and remained healthy after emergency LT. The patient who died at 93 days after LT (case 2) underwent surgery 33 days after the initial presentation. The timing of LT in this case was clearly much later than that in the other cases (9-15 days from the initial presentation).

We re-evaluated each of our patients with the KCC and the ALFED model. According to the KCC and the ALFED model, only 1 patient (case 2) was regarded as a candidate for LT. Previously, the KCC were found to be of limited usefulness for patients with FHF in Japan. The predictive accuracy of the criteria, as adopted for the patients seen between 1993 and 1995, was found to be only 55% for the assessment conducted at the onset of hepatic encephalopathy and 53% for the assessment conducted on day 5 after the onset of encephalopathy.[13] Hence, a new set of criteria for use in Japan was established by the ALFSG in 1996 and updated in 2011.[6] When the prognosis of patients with a total score of 5 or more was judged to be death, the predictive accuracy was 0.80, with sensitivity, specificity, positive predictive, and negative predictive values greater than 0.70 even in the validation cohort.

According to the ALFED model, case 3 was at low risk on day 3 but was at high risk on day 5. The ALFED assessment on day 3 seems to be in line with the JALFSG criteria on day 5 in Japan where LDLT is

predominant because the waiting time for a deceased donor from listing to LT is 2 to 3 days in most countries. Hence, strategies for patients with a moderate risk in the ALFED model should be determined on a case-by-case basis. In case 1, severe coagulopathy and a low platelet count persisted on day 5, and LDLT was performed without liver biopsy. On the other hand, liver biopsy was critical for the determination of the treatment strategy for cases 4 and 5. Notably, case 5 was diagnosed with EBV-T-LPD by liver biopsy, which is a contraindication for LT.

A primary EBV infection often causes infectious mononucleosis, whereby B cells are infected with EBV and polyclonal B cell expansion is accompanied by oligoclonal or monoclonal proliferation of CD8-positive cytotoxic T cells.[14] EBV also triggers secondary HLH. In EBV-induced HLH, the targets of EBV infection are CD8-positive T cells and oligoclonally or monoclonally proliferated T cells, which induce hypercytokinemia and, in turn, lead to hemophagocytosis and dysfunction of various organs.[15,16] EBV-T-LPD may develop in association with acute or chronic EBV infections and EBV-induced HLH. However, differentiating reactive T cell lymphocytosis from EBV-T-LPD can be a diagnostic challenge, especially in EBV-induced HLH, because monoclonality analysis alone is unable to support the diagnosis. Upon the initiation of steroid or immunosuppressive treatment for EBV-induced HLH, it can also be difficult to predict if the patient with EBV-induced FHF will require LT, conservative treatment for HLH, or aggressive chemotherapy. Hence, liver biopsy is important not only for the evaluation of EBV-T-LPD in EBV-induced HLH but also for the assessment of potential massive or submassive hepatocellular necrosis, although there are limitations of biopsy in terms of the sampling artifact. In cases 4 and 5, the liver biopsies showed CD8-positive T cell proliferation without massive necrosis, which suggested that LT was not necessary. In these 2 patients, a high level of EBV DNA was detected in PBMNCs but not in the plasma, possibly because of EBV-infected T cells not yet lysed by cytotoxic T cells. On the other hand, in the 3 patients who received LT, high levels of EBV DNA were detected in both PBMNCs and the plasma.

Patients with EBV-induced FHF usually have severe coagulopathy, which makes biopsy difficult because of bleeding. As such, many physicians tend to avoid liver biopsy. To that end, we opted for open liver biopsy rather than percutaneous and transjugular liver biopsy because of the limitation of the substantial sampling artifact regarding the extent of hepatic necrosis. We agree that plural or serial transjugular liver biopsy would be helpful as an alternative.[17] However, transjugular liver biopsy is not covered by the national insurance system in Japan. Donaldson et al. reported that the percentage of necrosis appeared to have a significant discriminatory prognostic value, and significantly greater hepatocellular necrosis was seen in nonsurvivors versus survivors regardless of the etiology of FHF. Despite that, there were a few survivors who had liver necrosis greater than 70% in that study. Because acute hepatocellular injury and early rapid regeneration are investigated in some cases, massive necrosis does not necessarily mean a poor prognosis. In such cases, a model such as the ALFED model is necessary to decide the treatment strategy.

In conclusion, an early and precise assessment of the pathogenesis of EBV-induced FHF is critical to determine the next course of treatment. We found that a serial assessment of the severity of FHF based on the ALFED score during the first 5 days of commencement of artificial liver support helped to determine the necessity for LT. Liver biopsy should be mandatory to assess the pathogenesis of EBV-induced FHF.

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REFERENCES

1. Papatheodoridis GV, Delladetsima JK, Kavallierou L, Kapranos N, Tassopoulos NC. Fulminant hepatitis due to Epstein-Barr virus infection. J Hepatol 1995;23:348-350.
2. Feranchak AP, Tyson RW, Narkewicz MR, Karrer FM, Sokol RJ. Fulminant Epstein-Barr viral hepatitis: orthotopic liver transplantation and review of the literature. Liver Transpl Surg 1998;4:469-476.
3. Mellinger JL, Rossaro L, Naugler WE, Nadig SN, Appelman H, Lee WM, Fontana RJ. Epstein-Barr virus (EBV) related acute liver failure: a case series from the US Acute Liver Failure Study Group. Dig Dis Sci 2014; 59:1630-1637.
4. O'Grady JG, Alexander GJ, Thick M, Potter D, Calne RY, Williams R. Outcome of orthotopic liver transplantation in the aetiological and clinical variants of acute liver failure. Q J Med 1988;68:817-824.
5. Kumar R, Shalimar, Sharma H, Goyal R, Kumar A, Khanal S, et al. Prospective derivation and validation of early dynamic model for predicting outcome in patients with acute liver failure. Gut 2012;61:1068-1075.
6. Naiki T, Nakayama N, Mochida S, Oketani M, Takikawa Y, Suzuki K, et al. Novel scoring system as a useful model to predict the outcome of patients with acute liver failure: Application to indication criteria for liver transplantation. Hepatol Res 2012;42:68-75.
7. Imadome K, Fukuda A, Kawano F, Imai Y, Ichikawa S, Mochizuki M, et al. Effective control of Epstein-Barr virus infection following pediatric liver transplantation by monitoring of viral DNA load and lymphocyte surface markers. Pediatr Transplant 2012;16:748-757.
8. Langerak AW, Groenen PJ, Brüggemann M, Beldjord K, Bellan C, Bonello L, et al. EuroClonality/BIOMED-2 guidelines for interpretation and reporting of Ig/TCR clonality testing in suspected lymphoproliferations. Leukemia 2012;26:2159-2171.
9. Henter JI, Horne A, Arico M, Egeler RM, Filipovich AH, Imashuku S, et al. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124–131.
10. Tohyama T, Takada Y, Watanabe J, Hatano M, Kushihata F, Inoue H, et al. ABO-incompatible living

donor liver transplantation saved a child with fulminant hepatic failure caused by primary EBV infection [abstract]. Liver Transpl 2011;17(suppl 1):S146.

11. Dumortier J, Mekki Y, Rimmelé T, Ber C, Maillard E, Fafi-Kremer S, et al. EBV-induced fulminant hepatic failure: favorable outcome after liver transplantation [in French]. Gastroenterol Clin Biol 2007;31:725-728.
12. Park HW, Lee SG, Hwang S, Kim KH, Park YH. Epstein-Barr virus associated fulminant hepatitis requiring liver transplantation (case report) [abstract]. HPB 2001;13 (Suppl.3):187.
13. Sugihara J, Naito T, Ishiki Y, Murakami N, Naiki T, Koshino Y, et al. A multicenter study for liver transplantation in Japanese Acute Hepatic Failure Study Group (1996) [in Japanese]. Acta Hepatol Jpn 2001;42:543-557.
14. Callan MF, Steven N, Krausa P, Wilson JD, Moss PA, Gillespie GN, et al. Large clonal expansions of CD8+ T cells in acute infectious mononucleosis. Nat Med 1996;2: 906-911.
15. Kawaguchi H, Miyashita T, Herbst H, Niedobitek G, Asada M, Tsuchida M, et al. Epstein-Barr virus–infected T lymphocytes in Epstein-Barr virus–associated hemophagocytic syndrome. J Clin Invest 1993;92:1444-1450.
16. Okano M, Gross TG. Acute or chronic life-threatening disease associated with Epstein-Barr virus infection. Am J Med Sci 2012;343:483-489.
17. Donaldson BW, Gopinath R, Wanless IR, Phillips MJ, Cameron R, Roberts EA, et al. The role of transjugular liver biopsy in fulminant liver failure: Relation to other prognostic indicators. Hepatology 1993;18:1370-6374.