World Journal of Gastroenterology

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DOI: 10.3748/wjg.v20.i21.6638

World J Gastroenterol 2014 June 7; 20(21): 6638-6650
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

OBSERVATIONAL STUDY

Protocol liver biopsy is the only examination that can detect mid-term graft fibrosis after pediatric liver transplantation

Yukihiro Sanada, Koshi Matsumoto, Taizen Urahashi, Yoshiyuki Ihara, Taiichi Wakiya, Noriki Okada, Naoya Yamada, Yuta Hirata, Koichi Mizuta

Yukihiro Sanada, Taizen Urahashi, Yoshiyuki Ihara, Taiichi Wakiya, Noriki Okada, Naoya Yamada, Yuta Hirata, Koichi Mizuta, Department of Transplant Surgery, Jichi Medical University, Tochigi 329-0498, Japan
Koshi Matsumoto, Department of Clinical Pathology, Ebina General Hospital, Kanagawa Prefecture 243-0433, Japan
Author contributions: Sanada Y participated in making the research design, analyzing data, and writing the article; Urahashi T, Ihara Y, Wakiya T, Okada N, Yamada N and Hirata Y participated in the data collection, data analysis and writing of the discussion; Matsumoto K and Mizuta K participated in the research design, writing of the discussion and review of the article.
Correspondence to: Yukihiro Sanada, MD, PhD, Department of Transplant Surgery, Jichi Medical University, 3311-1 Yakushi-ji, Shimotsuke City, Tochigi 329-0498, Japan. yuki371@jichi.ac.jp
Telephone: +81-285-587069 Fax: +81-285-587069
Received: July 24, 2013 Revised: November 12, 2013
Accepted: November 28, 2013
Published online: June 7, 2014

Abstract

AIM: To assessed the clinical significance of protocol liver biopsy (PLB) in pediatric liver transplantation (LT).

METHODS: Between July 2008 and August 2012, 89 and 55 PLBs were performed in pediatric patients at two and five years after LT, respectively. We assessed the histopathological findings using the Metavir scoring system, including activity (A) and fibrosis (F), and we identified factors associated with scores of $\geqslant$ A1 and $\geqslant$ F1. Our results clarified the timing and effectiveness of PLB.

RESULTS: The incidences of scores of $\geqslant$ A1 and $\geqslant$ F1 were 24.7% and 24.7%, respectively, at two years after LT and 42.3% and 34.5%, respectively, at five years. Independent risk factors in a multivariate analysis of a score of $\geqslant$ A1 at two years included $\geqslant$ 2 h of cold ischemic time, no acute cellular rejection and an alanine amino transaminase (ALT) level of $\geqslant$ 20 IU/L (P = 0.028, P = 0.033 and P = 0.012, respectively); however, no risk factors were identified for a score of $\geqslant$ F1. Furthermore, no independent risk factors associated with scores of $\geqslant$ A1 and $\geqslant$ F1 at five years were identified using multivariate analysis. A ROC curve analysis of ALT at two years for a score of $\geqslant$ A1 demonstrated the recommended cutoff value for diagnosing $\geqslant$ A1 histology to be 20 IU/L. The incidence of scores of $\geqslant$ A2 or $\geqslant$ F2 at two years after LT was 3.4% (three cases), and all patients had an absolute score of $\geqslant$ A2. In contrast to that observed for PLBs at five years after LT, the incidence of scores of $\geqslant$ A2 or $\geqslant$ F2 was 20.0% (11 cases), and all patients had an absolute score of $\geqslant$ F2. In all cases, the dose of immunosuppressants was increased after the PLB, and all ten patients who underwent a follow-up liver biopsy improved to scores of $\leqslant$ A1 or F1.

CONCLUSION: PLB at two years after LT is an unnecessary examination, because the serum ALT level reflects portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect this finding.

Key words: Protocol liver biopsy; Graft fibrosis; Immunosuppression; Liver function test; Pediatric liver transplantation

Core tip: Few studies have investigated the impact of the timing and effectiveness of post-transplant protocol liver biopsy (PLB). We assessed the histopathological findings of these biopsies using the Metavir scoring system, and our results clarified the timing and effective-

ness of PLB. PLB at two years after pediatric liver transplantation is an unnecessary examination, because the serum alanine amino transaminase (ALT) level reflects portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect this finding.

Sanada Y, Matsumoto K, Urahashi T, Ihara Y, Wakiya T, Okada N, Yamada N, Hirata Y, Mizuta K. Protocol liver biopsy is the only examination that can detect mid-term graft fibrosis after pediatric liver transplantation. *World J Gastroenterol* 2014; 20(21): 6638-6650 Available from: URL: http://www.wjgnet.com/1007-9327/full/v20/i21/6638.htm DOI: http://dx.doi.org/10.3748/wjg.v20.i21.6638

INTRODUCTION

Liver transplantation (LT) is an established curative treatment for pediatric patients with end-stage liver disease or acute liver failure[1-3]. Graft fibrosis and/or chronic rejection can still occasionally lead to graft failure or even death despite improvements and innovations in immunosuppressive therapy, and the histopathological assessments performed after LT remain insufficient. It is therefore necessary to further improve the prognosis by maintaining the function of the liver graft using a minimum degree of immunosuppression to achieve an optimal balance between the effectiveness and side effects of individual immunosuppressants.

The development of liver graft fibrosis after pediatric LT has been reported to occur in 69%-97% of cases, including cases of mild fibrosis[4-8]. Graft dysfunction does not occur unless the fibrosis becomes advanced, and the occurrence of graft fibrosis or portal inflammation cannot be predicted using the standard liver function test (LFT) alone. Therefore, histopathological assessments using protocol liver biopsy (PLB) have recently been reported to be important[4-9]. However, the significance of mild to severe fibrosis is unclear, and the indications for the treatment of abnormal PLB findings are controversial. In addition, few studies have investigated the impact of the timing and effectiveness of PLB. This retrospective study assessed the clinical significance of the timing and effectiveness of PLB after pediatric living donor liver transplantation (LDLT).

MATERIALS AND METHODS

Patients

Between July 2008 and August 2012, 144 PLBs were performed in pediatric patients at two and five years after LDLT at the Department of Transplant Surgery, Jichi Medical University, Japan (Table 1). The observation period was between six and 55 mo.

Immunosuppressive therapy

Tacrolimus (Tac) and methylprednisolone (MP) were used as the standard postoperative immunosuppressive regimen. The target trough levels of Tac were 15-20 ng/mL during the first week, 8-12 ng/mL during the first month, 5-8 ng/mL during the first six months, 3-5 ng/mL during the first year and 2-4 ng/mL thereafter. MP was administered at an initial dose of 20 mg/kg intravenously on the morning of the operation and before graft reperfusion. The MP dose was thereafter decreased gradually to 3 mg/kg per day on postoperative day (POD) 1, 0.5 mg/kg per day on POD 7 and 0.25 mg/kg per day at one month after LDLT and was then discontinued within one year except in patients in whom immunosuppression could not be maintained at the lowest dose. Mycophenolate mofetil (MMF) was used when more potent immunosuppression was required, such as in ABO-incompatible recipients older than five years, patients with steroid-resistant acute rejection episodes and patients with liver dysfunction following the cessation of MP therapy.

Diagnosis of acute cellular rejection

All episodes of acute cellular rejection were diagnosed based on the histopathological findings of a liver biopsy. In all specimens, the diagnosis of acute cellular rejection was evaluated by highly experienced pathologists and graded into four classes according to the Banff scheme[10]. The degrees of portal infiltration by lymphocytes (P0-3), bile duct inflammation or damage (B0-3) and venous endothelial inflammation (V0-3) in the Banff scheme were evaluated. A liver biopsy was indicated when all liver function data (aspartate amino transferase, alanine amino transferase (ALT), gamma-glutamyl transpeptidase, and total bilirubin) were elevated compared with the previous data.

PLB procedure and timing

We began to perform PLBs in pediatric patients at two and five years after LT in July 2008 because we experienced cases of normal LFTs coexisting with histopathological portal inflammation and fibrosis, including cases 4 and 5, which are discussed later. In those cases in which the dose of immunosuppressants was increased after the PLB, we generally performed a follow-up liver biopsy between six months and one year after the PLB. In addition to a PLB, an episode biopsy was performed when a recipient with a high serum level of ALT or hyaluronic acid was refractory to an increase in immunosuppressants.

The PLB necessitated an overnight stay at our hospital. A percutaneous transhepatic liver biopsy was performed under analgesia and sedation using ultrasonographically-guided 14 G Monopty (C.R.Bard, Inc. United States). Manual compressive hemostasis was conducted for 20 min, after which compressive bandage hemostasis was performed until the following day. Preventive cefoperazone and sulbactam were also administered on that day.

Table 1 Demographic characteristics of recipients and grafts undergoing protocol liver biopsy at two and five years after living donor liver transplantation

	PLB at two years after LDLT (n = 89)	PLB at five years after LDLT (n = 55)
Recipient characteristics at LDLT		
Gender	Male 37, female 52	Male 20, female 35
Age (mo)	22 (0-234)	19 (7-198)
Body weight (kg)	10.7 (2.6-58.5)	9.7 (5.9-64.9)
Original disease	BA 63, OTCD 9, AS 4, FHF 4, CEPS 3, graft failure 2, WD 1, PSC 1, CPS1D 1, LC 1	BA 43, OTCD 3, AS 2, WD 2, FHF 1, HB 1, CF 1, CEPS 1, graft failure 1
PELD or MELD	7.4 (-9.7-39.4)	8.6 (-8.9-39.4)
Operation time	13 h 25 min (7 h 33 min-30 h 28 min)	17h 19 min (11 h 11 min-30 h 28 min)
Cold ischemic time	2 h 17 min (36 min-8 h 6 min)	2 h 06 min (25 min-16 h 19 min)
Warm ischemic time	45 min (30 min-2 h 2 min)	1 h 00 min (30 min-4 h 27 min)
Blood loss volume (mL/kg)	77.0 (3.1-585.1)	45.5 (6.7-776.2)
Transfusion volume (mL/kg)	91.3 (0.0-597.7)	68.1 (0.0-670.7)
Donor and graft characteristics at LDLT		
Gender	Father; 45, mother; 44	Father; 30, mother; 25
Age (yr)	33 (23-57)	33 (23-53)
ABO compatibility	Identical; 55, compatible; 20, incompatible 14	Identical; 40, compatible; 8, incompatible 7
GV/SLV (%)	68.0 (33.0-120.9)	75.8 (35.7-121.2)
Graft type	Lateral segment; 57, left lobe; 23, S2 monosegment; 5, left lobe + caudate; 4	Lateral segment; 43, left lobe; 10, left lobe + caudate; 2
Recipient and graft characteristics at PLB		
Age (mo)	48 (24-259)	81 (68-257)
Body weight (kg)	15.6 (7.3-64.6)	21.4 (14.4-71.6)
Total bilirubin (mg/dL)	0.63 (0.25-3.25)	0.68 (0.26-2.55)
AST (IU/L)	30 (14-61)	27 (10-251)
ALT (IU/L)	17 (9-54)	17 (8-260)
γ-GTP (IU/L)	17 (6-440)	16 (9-510)
Hyaluronic acid (ng/mL)	21 (9-239)	17 (9-216)
IgG (mg/dL)	927 (440-2063)	1148 (475-2961)
GV/SLV (%)	90.6 (70.2-126.9)	93.0 (58.8-157.0)
Spleen volume (mL)	125 (0-892)	145 (0-692)
Trough of tacrolimus (ng/mL)	3.4 (0-10.1)	2.3 (0-15.5)

PLB: Protocol liver biopsy; LDLT: Living donor liver transplantation; BA: Biliary atresia; OTCD: Ornithine transcarbamylase deficiency; AD: Alagille syndrome; FHF: Fulminant hepatic failure; CEPS: Congenital extrahepatic portsystemic shunt; WD: Wilson disease; PSC: Primary sclerosing cholangitis; CPS1D: Carbamoyl-phosphate synthase 1 deficiency; LC: Liver cirrhosis; HB: Hepatoblastoma; CF: Cystic fibrosis; PELD: Pediatric end-stage liver disease; MELD: Model for end-stage liver disease; GV/SLV: Ratio of graft volume to standard liver volume; AST: Aspartate amino transferase; ALT: Alanine amino transferase; IgG: Immunoglobulin G.

Assessment of the PLB findings

We assessed the histopathological features of the PLB samples using the Metavir scoring system[11], which grades the activity (A), *i.e.*, the amount of inflammation (specifically, the intensity of necro-inflammatory lesions), on a four-point scale from A0 to A3. Fibrosis (F) was graded on a five-point scale from F0 to F4.

Strategy of increasing the dose of immunosuppressants after LDLT

When the serum level of ALT or hyaluronic acid was found to be high in outpatients, we increased the dose of immunosuppressants if the suspected causes of the elevation of these levels was an immune response. When the serum level of ALT or hyaluronic acid was maintained at a normal level for a few months in the early period or for six months in the late period after LDLT, we gradually decreased the dose of immunosuppressants.

When the PLB score was ⩾ A2 or ⩾ F2, we increased the dose of immunosuppressants to provide the early treatment of portal inflammation or fibrosis. When the PLB grade was A0 and F0, we gradually decreased the dose of immunosuppressants.

Statistical analysis

The significance of the differences between two groups was evaluated using the chi-squared test. Associations between the recipient, donor or graft variables and abnormal histopathological findings were evaluated using univariate and backward selection multivariate Cox regression methods. A ROC curve analysis was performed to identify the cutoff value for the correlation between the ALT level and abnormal histopathological findings. All statistical analyses were performed using the StatView software package (SAS Institute, Cary, NC) and EZR (Saitama Medical Center, Jichi Medical University, Japan). Differences of $P < 0.05$ were considered to be significant.

RESULTS

Results of PLB at two years after LDLT

The incidence of scores of ⩾ A1 and ⩾ F1 at two years after LDLT was 24.7% and 24.7%, respectively. The ac-

Table 2 Risk factors for ⩾ A1 and ⩾ F1 of protocol liver biopsy at two years after living donor liver transplantation: univariate analysis

Variables	Incidence of ⩾ A1 (%)	*P* value	Incidence of ⩾ F1 (%)	*P* value
Recipient age at LDLT				
< 12 mo (*n* = 30) *vs* ⩾ 12 mo (*n* = 59)	26.7 *vs* 23.7	0.762	36.7 *vs* 18.6	0.062
Recipient body weight at LDLT				
< 10 kg (*n* = 43) *vs* ⩾ 10 kg (*n* = 46)	23.3 *vs* 26.1	0.757	27.9 *vs* 21.7	0.500
Original disease				
Cholestatic diseases (*n* = 69) *vs* others (*n* = 20)	33.3 *vs* 38.1	0.637	33.3 *vs* 38.1	0.637
PELD or MELD				
⩾ 20 (*n* = 22) *vs* < 20 (*n* = 67)	22.7 *vs* 25.4	0.803	31.8 *vs* 22.4	0.374
Donor age				
⩾ 35 yr (*n* = 39) *vs* < 35 yr (*n* = 50)	23.1 *vs* 26.0	0.751	25.6 *vs* 24.0	0.858
Gender combinations between donor and recipient				
Mismatch (*n* = 50) *vs* match (*n* = 39)	24.0 *vs* 25.6	0.858	22.0 *vs* 28.2	0.501
ABO compatibility				
Incompatible (*n* = 14) *vs* others (*n* = 75)	21.4 *vs* 25.3	0.755	14.3 *vs* 26.7	0.324
HLA-A				
Mismatch (*n* = 65) *vs* match (*n* = 24)	30.8 *vs* 8.3	0.029	27.7 *vs* 16.7	0.285
HLA-B				
Mismatch (*n* = 84) *vs* match (*n* = 5)	26.2 *vs* 0.0	0.187	25.0 *vs* 20.0	0.802
HLA-DRB[1]				
Mismatch (*n* = 76) *vs* match (*n* = 13)	26.3 *vs* 15.4	0.398	26.3 *vs* 15.4	0.398
Lymphocyte cross-matching				
⩾ 4 × (*n* = 7) *vs* negative (*n* = 82)	0.0 *vs* 26.8	0.114	28.6 *vs* 24.4	0.805
GV/SLV				
< 40 % (*n* = 6) *vs* ⩾ 40 % (*n* = 83)	33.3 *vs* 24.1	0.612	16.7 *vs* 25.3	0.636
Graft type				
Lateral segment graft (*n* = 57) *vs* others (*n* = 32)	21.1 *vs* 31.3	0.285	29.8 *vs* 15.6	0.136
Operation time				
⩾ 20 h (*n* = 12) *vs* < 20 h (*n* = 77)	16.7 *vs* 26.0	0.113	25.0 *vs* 24.7	0.975
Cold ischemic time				
⩾ 2 h (*n* = 49) *vs* < 2 h (*n* = 40)	32.7 *vs* 15.0	0.055	28.6 *vs* 20.0	0.351
Warm ischemic time				
⩾ 45 min (*n* = 45) *vs* < 45 min (*n* = 44)	20.0 *vs* 29.5	0.297	26.7 *vs* 22.7	0.666
Blood loss volume				
⩾ 100 mL/kg (*n* = 30) *vs* < 100 mL/kg (*n* = 59)	16.7 *vs* 28.8	0.209	26.7 *vs* 23.7	0.762
Transfusion volume				
⩾ 100 mL/kg (*n* = 41) *vs* < 100 mL/kg (*n* = 48)	22.0 *vs* 27.1	0.576	22.0 *vs* 27.1	0.576
Splenectomy				
Yes (*n* = 7) *vs* No (*n* = 82)	42.9 *vs* 23.2	0.247	28.6 *vs* 24.4	0.805
Portal vein complications				
Yes (*n* = 11) *vs* No (*n* = 78)	9.1 *vs* 26.9	0.199	27.3 *vs* 24.4	0.834
Hepatic arterial complications				
Yes (*n* = 6) *vs* No (*n* = 83)	16.7 *vs* 25.3	0.636	33.3 *vs* 24.1	0.509
Hepaticojejunostomic anastomotic stricture				
Yes (*n* = 14) *vs* No (*n* = 75)	21.4 *vs* 25.3	0.755	28.6 *vs* 24.0	0.716
Cytomegalovirus infection				
Yes (*n* = 29) *vs* No (*n* = 60)	31.0 *vs* 21.7	0.337	27.6 *vs* 23.3	0.663
Acute cellular rejection				
Yes (*n* = 29) *vs* No (*n* = 60)	10.3 *vs* 31.7	0.029	17.2 *vs* 28.3	0.255
Total bilirubin at PLB				
⩾ 0.7 mg/dL (*n* = 29) *vs* < 0.7 mg/dL (*n* = 60)	17.2 *vs* 28.3	0.255	24.1 *vs* 25.0	0.929
AST at PLB				
⩾ 30 IU/L (*n* = 49) *vs* < 30 IU/L (*n* = 40)	24.5 *vs* 25.0	0.956	34.7 *vs* 12.5	0.016
ALT at PLB				
⩾ 20 IU/L (*n* = 27) *vs* < 20 IU/L (*n* = 62)	40.7 *vs* 17.7	0.021	37.0 *vs* 19.4	0.075
γ-GTP at PLB				
⩾ 20 IU/L (*n* = 34) *vs* < 20 IU/L (*n* = 55)	32.4 *vs* 20.0	0.189	29.4 *vs* 21.8	0.420
Hyaluronic acid at PLB				
⩾ 20 ng/mL (*n* = 52) *vs* < 20 ng/mL (*n* = 37)	32.7 *vs* 13.5	0.039	23.1 *vs* 27.0	0.671
IgG at PLB				
⩾ 1200 mg/dL (*n* = 18) *vs* < 1200 mg/dL (*n* = 71)	27.8 *vs* 23.9	0.737	33.3 *vs* 22.5	0.343
ANA at PLB				
⩾ 20 × (*n* = 8) *vs* < 20 × (*n* = 81)	12.5 *vs* 25.7	0.401	12.5 *vs* 25.9	0.401
ASMA at PLB				
⩾ 20 × (*n* = 21) *vs* < 20 × (*n* = 68)	23.8 *vs* 25.0	0.913	28.6 *vs* 23.5	0.640

Trough of tacrolimus at PLB				
⩾ 3.0 ng/mL (n = 54) *vs* < 3.0 ng/mL (n = 32)[1]	25.9 *vs* 25.0	0.924	24.1 *vs* 25.0	0.924

[1]Three cases which were used a cyclosporine were removed. LDLT: Living donor liver transplantation; PELD: Pediatric end-stage liver disease; MELD: Model for end-stage liver disease; GV/SLV: Ratio of graft volume to standard liver volume; PLB: Protocol liver biopsy; AST: Aspartate amino transferase; ALT: Alanine amino transferase; IgG: Immunoglobulin G; ANA: Antinuclear antibody; ASMA: Antismooth nuclear antibody.

Table 3 Risk factors for ⩾ A1 and ⩾ F1 of protocol liver biopsy at two and five years after living donor liver transplantation: multivariate analysis

Variables	OR	95%CI	*P* value
Risk factors for ⩾ A1 of PLB at two years after LDLT			
HLA-A mismatch			
Mismatch *vs* match	0.46	0.145-1.479	0.194
Cold ischemic time			
⩾ 2 h *vs* < 2 h	4.15	1.164-14.789	0.028
Acute cellular rejection			
Yes *vs* No	0.20	0.046-0.878	0.033
ALT			
⩾ 20 IU/L *vs* < 20 IU/L	4.64	1.409-15.306	0.012
Hyaluronic acid			
⩾ 20 ng/mL *vs* < 20 ng/mL	3.30	0.982-11.076	0.054
Risk factors for ⩾ F1 of PLB at two years after LDLT			
Recipient age			
< 1 yr *vs* ⩾ 1 yr	1.54	0.506-4.706	0.446
AST			
⩾ 30 IU/L *vs* < 30 IU/L	2.68	0.775-9.238	0.120
ALT			
⩾ 20 IU/L *vs* < 20 IU/L	1.86	0.646-5.335	0.251
Risk factors for ⩾A1 of PLB at five years after LDLT			
Cold ischemic time			
⩾ 2 h *vs* < 2 h	2.94	0.778-11.140	0.112
Acute cellular rejection			
Yes *vs* No	2.26	0.728-7.035	0.158
Risk factor for ⩾ F1 of PLB at five years after LDLT			
Acute cellular rejection			
Yes *vs* No	2.75	0.876-8.637	0.083

PLB: Protocol liver biopsy; LDLT: Living donor liver transplantation; ALT: Alanine amino transferase; AST: Aspartate amino transferase.

tivity score was A0 in 67 patients, A1 in 19 patients and A2 in three patients, and the fibrosis score was F0 in 67 patients, F1 in 21 patients and F2 in one patient.

The impact of various recipient and graft variables on scores of ⩾ A1 and ⩾ F1 was assessed, and the results are summarized in Table 2. A univariate analysis revealed the following variables to be risk factors for a score of ⩾ A1 at two years after LDLT: HLA-A mismatch, no acute cellular rejection, ALT level of ⩾ 20 IU/L, and hyaluronic acid level of ⩾ 20 ng/mL (P = 0.029, P = 0.029, P = 0.021 and P = 0.039, respectively). The only variable with P < 0.1000 was ⩾ 2h of cold ischemic time (P = 0.055). A multivariate analysis including these variables identified ⩾ 2 h of cold ischemic time, no acute cellular rejection and ALT level of ⩾ 20 IU/L to be independent risk factors for a score of ⩾ A1 at two years after LDLT (P = 0.028, P = 0.033 and P = 0.012, respectively) (Table 3). The ROC curve analysis of the ALT level at two years after LDLT in the patients with a score of ⩾ A1, the recommended cutoff value for diagnosing a score of ⩾ A1 was 20 IU/L (sensitivity: 50.0%, specificity: 76.1%, area under the curve: 0.685 and 95%CI: 0.557-0.813) (Figure 1). Univariate analysis identified the risk factor for a score of ⩾ F1 at two years after LDLT to be the aspartate amino transferase level (P = 0.016). The variables with P < 0.100 included a recipient age of < 12 mo and an ALT level of ⩾ 20 IU/L (P = 0.062 and P = 0.075, respectively). A multivariate analysis of these variables found none to be independent risk factors for a score of ⩾ F1 at two years after LDLT (Table 3).

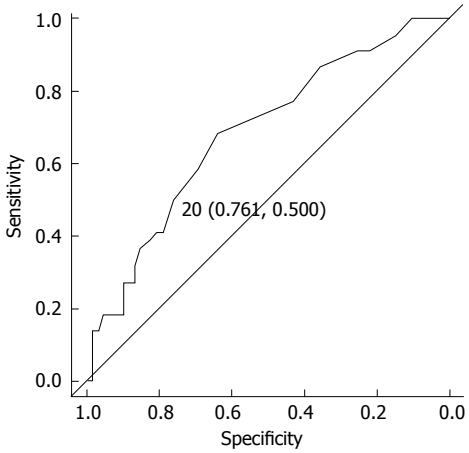

Figure 1 Receiver operating characteristic curve analysis of the alanine amino transaminase level at two years after living donor liver transplantation in the patients with a score of ⩾ A1. The recommended cutoff value for diagnosing a score of ⩾ A1 was set at 20 IU/L (sensitivity: 50.0%, specificity: 76.1%, area under the curve: 0.685 and 95%CI: 0.557-0.813).

The incidence of scores of ⩾ A2 or ⩾ F2 at two years after LDLT was 3.4% (three cases), and all patients had a score of ⩾ A2 (Table 4). In all cases, the dose of immunosuppressants was increased after the PLB, and two patients who underwent a follow-up liver biopsy improved to scores of ⩽ A1 and F1.

Results of PLB at five years after LDLT

The incidence of scores of ⩾ A1 and ⩾ F1 at five years after LDLT was 42.3% and 34.5%, respectively. The activity score was A0 in 29 patients, A1 in 23 patients and A2 in three patients, and the fibrosis score was F0 in 36 patients, F1 in 12 patients and F2 in seven patients.

The impact of various recipient and graft variables on the scores of ⩾ A1 and ⩾ F1 was assessed, and the results are summarized in Table 5. A univariate analysis identified no risk factors for scores of ⩾ A1 at five years after LDLT. The variables with P < 0.100 included ⩾ 2 h of cold ischemic time and acute cellular rejection (P = 0.061 and P = 0.087, respectively). Multivariate analysis of these variables found none to be independent risk factors for a score of ⩾ A1 at five years after LDLT (Table

Table 4 Clinical and hitopathological findings of cases with ⩾ A2 or ⩾ F2 of protocol liver biopsy at two or five years after living donor liver transplantation

Case	Original disease	Age at PLB/ sex	Previous ACR	Post-transplant complications	IS at PLB	Tac trough at PLB	ALT/HA at PLB	A/F at PLB	IS at follow-up biopsy	A/F at follow-up biopsy
PLB at two years after LDLT										
1	OTCD	71/female	-	-	Tac (3.0)	2.5	12/35	2/1	Tac (1.0)/MMF (400)	1/1
2	OTCD	164/female	-	BDS	Tac (2.0)/MMF (1000)	5.2	34/13	2/1	Tac (2.0)/MMF (1000)	1/0
3	OTCD	44/male	-	-	Tac (0.8)/MMF (250)	2	25/< 9	2/2	Tac (0.8)/MMF (500)	N.E.
PLB at five years after LDLT										
4	BA	70/female	+	Bowel perforation	Tac (0.6)	1.1	22/13	2/2	Tac (2.0)/MMF (1000)	0/0
5	BA	118/female	-	-	Tac (1.0)	2.3	20/24	2/2	Tac (2.0)/MMF (1000)	1/0
6	BA	70/female	+	HAT/IHBDS	Tac (0.8)/ MMF(500)	3.6	32/28	1/2	Tac (2.0)/MMF (500)	1/1
7	BA	71/female	-	CMV-I	Tac (0.4)	0	16/< 9	2/2	Tac (1.6)	N.E.
8	FHF	83/female	-	-	Tac (2.0)/MMF (500)	2.2	26/< 9	1/2	Tac (2.8)/MMF (500)	N.E.
9	BA	77/female	-	CMV-I	Tac (0.4)	2.6	14/29	2/3	Tac (0.8)	0/1
10	BA	84/female	+	Fungal infection	Tac (0.4)	2.1	26/11	2/2	Tac (0.4),MMF (500)	1/1
11	BA	89/male	+	PVS	Tac (1.6)/MP (4.0)/MMF (1500)	2.2	12/17	2/2	Tac (1.6)/MP (2.0)/ MMF (1500)	1/1
12	BA	174/male	-	BDS	Tac (3.0)	2.3	16/20	1/2	Tac (4.0)	0/1
13	BA	69/female	+	CMV-I	Tac (1.6)	2.8	18/< 9	1/2	Tac (2.0)/MMF (1000)	0/1
14	BA	84/male	-	HVS	Tac (2.0)/MP (1.0)/MMF (1000)	5.6	12/23	1/2	Tac (2.0)/MP (1.0)/ MMF (1000)	N.E.

PLB: Protocol liver biopsy; LDLT: Living donor liver transplantation; ACR: Acute cellular rejection; IS: Immunosuppressants; Tac: Tacrolimus; ALT: Alanine amino transferase; HA: Hyaluronic acid; A: Activity; F: Fibrosis; OTCD: Ornithine transcarbamylase deficiency; BA: Biliary atresia; FHF: Fulminant hepatic failure; BDS: Biliary duct anastomotic stenosis; HAT: Hepatic artery thrombosis; IHBDS: Intrahepatic biliary duct stenosis; CMV-I: Cytomegalovirus infection; PVS: Portal vein stenosis; HVS: Hepatic vein stenosis; MMF: Mycophenolate mofetil: MP: Methylprednisolone.

3). Univariate analysis identified no risk factors for a score of ⩾ F1 at five years after LDLT. The variable with $P < 0.100$ included acute cellular rejection ($P = 0.079$). Multivariate analysis of these variables found none to be independent risk factors for a score of ⩾ F1 at five years after LDLT (Table 3).

The incidence of scores of ⩾ A2 or ⩾ F2 at five years after LDLT was 20.0% (11 cases), and all patients had a score of ⩾ F2 (Table 4). In all cases, the dose of immunosuppressants was increased after the PLB, and all eight patients who underwent a follow-up liver biopsy improved to scores of ⩽ A1 and F1.

Clinical and histopathological findings in the patients who underwent PLB at both two and five years after LDLT

PLBs were performed at both two and five years after LDLT in 21 cases; the results are summarized in Table 6. The activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ⩾ A2 or ⩾ F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT maintained scores of A0 and F0 at five years; however, the remaining patients exhibited worse scores of ⩾ A1 or ⩾ F1. Three patients with a score of A1 or F1 at two years after LDLT maintained a score of A1 or F1 at five years; however, the remaining patients exhibited worse a score of ⩾ A2 or ⩾ F2.

Complications of PLB

Complications related to the PLB occurred in only one patient (0.7%) who developed acute cholangitis. This complication resolved following the administration of antibiotics for three days.

Case reports

We described two representative liver transplant recipients with abnormal histopathological findings and normal LFT results in whom the dose of immunosuppressants was increased, which led to improvements in the histopathological findings (Table 4).

Case 4: A seven-month-old female infant with biliary atresia underwent ABO-identical LDLT using a left lateral segment graft. Tac and MP were administered as the standard postoperative immunosuppressive regimen. The patient's postoperative course included an episode of small intestine perforation requiring surgical repair and acute cellular rejection requiring steroid pulse treatment; however, she was discharged from the hospital on POD 28 after LDLT. MP was withdrawn at 18 mo after LDLT, and thereafter, only Tac was administered for immuno-

Table 5 Risk factors for ⩾ A1 and ⩾ F1 of protocol liver biopsy at five years after living donor liver transplantation: univariable analysis

Variables	Incidence of ⩾ A1 (%)	*P*-value	Incidence of ⩾ F1 (%)	*P*-value
Recipient age at LDLT				
< 12 mo (n = 18) *vs* ⩾ 12 mo (n = 37)	38.9 *vs* 51.4	0.385	38.9 *vs* 32.4	0.637
Recipient body weight at LDLT				
< 10 kg (n = 29) *vs* ⩾ 10 kg (n = 26)	41.4 *vs* 53.8	0.355	55.0 *vs* 30.8	0.577
Original disease				
Cholestatic diseases (n = 45) *vs* others (n = 10)	46.7 *vs* 50.0	0.850	35.6 *vs* 33.3	0.738
PELD or MELD				
⩾ 20 (n = 12) *vs* < 20 (n = 43)	41.7 *vs* 48.8	0.660	41.7 *vs* 52.6	0.558
Donor age				
⩾ 35 yr (n = 22) *vs* < 35 yr (n = 33)	40.9 *vs* 51.5	0.440	36.4 *vs* 33.3	0.816
Gender combinations between donor and recipient				
Mismatch (n = 30) *vs* match (n = 25)	53.3 *vs* 40.0	0.324	40.0 *vs* 28.0	0.352
ABO compatibility				
incompatible (n = 7) *vs* others (n = 48)	42.9 *vs* 47.9	0.802	28.6 *vs* 35.4	0.722
HLA-A				
Mismatch (n = 41) *vs* match (n = 14)	51.2 *vs* 35.7	0.316	39.0 *vs* 21.4	0.232
HLA-B				
Mismatch (n = 52) *vs* match (n = 3)	48.1 *vs* 33.3	0.619	32.7 *vs* 66.7	0.229
HLA-DRB[1]				
Mismatch (n = 47) *vs* match (n = 8)	51.1 *vs* 25.0	0.172	36.2 *vs* 25.0	0.539
Lymphocyte cross-matching				
⩾ 4 × (n = 16) *vs* negative (n = 39)	31.3 *vs* 53.8	0.127	18.8 *vs* 41.0	0.115
GV/SLV				
< 40 % (n = 2) *vs* ⩾ 40 % (n = 53)	0.0 *vs* 49.1	0.173	0.0 *vs* 35.8	0.295
Graft type				
Lateral segment graft (n = 43) *vs* others (n = 12)	51.2 *vs* 33.3	0.274	39.5 *vs* 16.7	0.141
Operation time				
⩾ 20 h (n = 16) *vs* < 20 h (n = 39)	37.5 *vs* 51.3	0.352	31.3 *vs* 35.9	0.742
Cold ischemic time				
⩾ 2 h (n = 40) *vs* < 2 h (n = 15)	55.0 *vs* 26.7	0.061	40.0 *vs* 20.0	0.165
Warm ischemic time				
⩾ 1 h (n = 42) *vs* < 1 h (n = 13)	42.9 *vs* 61.5	0.238	31.0 *vs* 46.2	0.314
Blood loss volume				
⩾ 150 mL/kg (n = 11) *vs* < 150 mL/kg (n = 44)	27.3 *vs* 52.3	0.137	27.3 *vs* 36.4	0.57
Transfusion volume				
⩾ 100 mL/kg (n = 15) *vs* < 100 mL/kg (n = 40)	40.0 *vs* 50.0	0.508	40.0 *vs* 32.5	0.603
Splenectomy				
Yes (n = 2) *vs* No (n = 53)	100.0 *vs* 45.3	0.128	0.0 *vs* 35.8	0.295
Portal vein complications				
Yes (n = 9) *vs* No (n = 46)	44.4 *vs* 47.8	0.852	33.3 *vs* 34.8	0.933
Hepatic arterial complications				
Yes (n = 4) *vs* No (n = 51)	25.0 *vs* 49.0	0.354	25.0 *vs* 35.3	0.677
Hepaticojejunostomic anastomotic stricture				
Yes (n = 16) *vs* No (n = 39)	31.3 *vs* 53.8	0.127	25.0 *vs* 38.5	0.340
Cytomegalovirus infection				
Yes (n = 17) *vs* No (n = 38)	47.1 *vs* 47.4	0.999	47.1 *vs* 28.9	0.192
Acute cellular rejection				
Yes (n = 23) *vs* No (n = 32)	60.9 *vs* 37.5	0.087	47.8 *vs* 25.0	0.079
Total bilirubin at PLB				
⩾ 0.7 mg/dL (n = 25) *vs* < 0.7 mg/dL (n = 30)	48.0 *vs* 46.7	0.920	36.0 *vs* 33.3	0.836
AST at PLB				
⩾ 30 IU/L (n = 22) *vs* < 30 IU/L (n = 33)	54.5 *vs* 42.4	0.378	36.4 *vs* 33.3	0.816
ALT at PLB				
⩾ 20 IU/L (n = 21) *vs* < 20 IU/L (n = 34)	57.1 *vs* 41.2	0.249	28.6 *vs* 38.2	0.464
γ-GTP at PLB				
⩾ 20 IU/L (n = 20) *vs* < 20 IU/L (n = 35)	45.0 *vs* 48.6	0.799	30.0 *vs* 37.1	0.592
Hyaluronic acid at PLB				
⩾ 20 ng/mL (n = 22) *vs* < 20 ng/mL (n = 33)	50.0 *vs* 45.5	0.741	36.4 *vs* 33.3	0.816
IgG at PLB				
⩾ 1200 mg/dL (n = 24) *vs* < 1200 mg/dL (n = 31)	54.2 *vs* 41.9	0.368	41.7 *vs* 29.0	0.328
ANA at PLB				
⩾ 20 × (n = 14) *vs* < 20 × (n = 41)	35.7 *vs* 51.2	0.316	28.6 *vs* 36.6	0.586
ASMA at PLB				
⩾ 20 × (n = 10) *vs* < 20 × (n = 45)	70.0 *vs* 42.2	0.111	40.0 *vs* 33.3	0.688

Trough of tacrolimus at PLB ≥ 3.0 ng/mL (n = 19) *vs* < 3.0 ng/mL (n = 33)[1]	52.6 *vs* 42.4	0.477	36.8 *vs* 33.3	0.797

[1]Three cases which were used a cyclosporine were removed. LDLT: Living donor liver transplantation; PELD: Pediatric end-stage liver disease; MELD: Model for end-stage liver disease; GV/SLV: Ratio of graft volume to standard liver volume; PLB: Protocol liver biopsy; AST: Aspartate amino transferase; ALT: Alanine amino transferase; IgG: Immunoglobulin G; ANA: Antinuclear antibody; ASMA: Antismooth nuclear antibody.

Table 6 Clinical and histopathological findings of cases who performed protocol liver biopsy at both two and five years after living donor liver transplantation

Case	Original disease	Age at LT/sex	Previous ACR	Post-transplant complications	IS at two years PLB	Tac trough at PLB	ALT/HA at PLB	A/F at PLB	IS at five years PLB	Tac trough at PLB	ALT/HA at PLB	A/F at PLB
1	OTCD	46/female	-	-	Tac (3.0)	2.5	12/35	2/1	Tac (1.0)/MMF (400)	0.5	11/52	1/1
11	BA	26/male	+	PVS	Tac (0.8)/MP (4.0)/MMF (500)	3.2	20/11	0/1	Tac (1.6)/MP (4.0)/MMF (1500)	2.2	12/17	2/2
12	BA	114/male	-	BDS	Tac (2.0)	2.6	14/21	1/0	Tac (3.0)	2.3	16/20	1/2
13	BA	10/female	+	CMV-I	Tac (0.4)	3.8	19/11	0/0	Tac (1.6)	2.8	18/< 9	1/2
14	BA	30/male	-	HVS	Tac (1.2)	5.3	18/29	1/1	Tac (2.0)/MP (1.0)/MMF (1000)	5.6	12/23	1/2
15	BA	120/female	-	BDS	Tac (1.5)/PSL (2.5)	4.4	15/14	0/0	Tac (4.0)	7.0	17/< 9	0/0
16	BA	163/male M	+	BDS/CMV-I	CsA (150)	CsA 50	9/27	0/0	CsA (150)/MMF (1000)	CsA 83	91020	0/1
17	BA	8/female	+/OKT3	PVS/CMV-I	Tac (0.8)/MP (0.5)	2.4	30/36	0/0	Tac (2.0)	5.3	15/18	1/1
18	BA	12/male	+	-	Tac (0.8)	3.8	14/58	0/0	Tac (0.8)	0.2	8/20	1/1
19	BA	13/female	+	CMV-I	Tac (1.6)/MP (2.0)	9.3	22/11	0/0	Tac (1.4)/MP (3.0)/MMF (500)	2.1	15/15	0/1
20	AD	19/female	+	CMV-I	Tac (0.8)/MMF (500)	2.3	19/13	0/1	Tac (2.4)/MMF (500)	3.8	14/15	1/1
21	WD	112/male	-	-	Tac (4.0)	1.3	16/16	0/0	Tac (5.0)	1.4	19/13	0/0
22	BA	170/female	+	BDS	Tac (2.0)	6.3	17/16	0/1	Tac (6.0)/MP (12)/MMF (2000)	15.5	39/22	1/0
23	BA	33/F	+	HVS	Tac (1.0)/MP (2.0)/MMF (400)	3.7	10/< 9	0/0	Tac (1.5)/MMF (1000)	5.4	41/19	1/0
24	BA	9/female	-	HAT	Tac (0.6)	0.3	14/24	0/0	Tac (0.8)	0	10/10	0/0
25	BA	28/female	-	-	Tac (0.4)	2.8	18/< 9	0/0	Tac (1.0)	0.9	15/10	1/0
26	BA	9/female	-	IHBDS	Tac (0.4)	2.1	23/< 9	0/1	Tac (2.0)/MP (0.5)/MMF (500)	5.3	41/19	1/1
27	AD	19/male	-	-	Tac (0.6)	3.3	12/17	0/0	Tac (2.0)/MMF (500)	1.5	11/59	0/0
28	BA	45/female	-	BDS	Tac (1.2)	3.6	13/15	0/0	Tac (1.5)	4.6	11/10	0/0
29	BA	9/female	-	-	Tac (0.4)	0.9	11/17	0/0	Tac (0.8)	1.1	13/25	0/0
30	CEPS	37/male	-	-	Tac (2.0)/MP (2.5)/MMF (500)	2.5	13/< 9	0/0	Tac (2.0)/MP (1.5)/MMF (500)	3.9	11/< 9	0/0

PLB: Protocol liver biopsy; LDLT: Living donor liver transplantation; ACR: Acute cellular rejection; IS: Immunosuppressants; Tac: Tacrolimus; ALT: Alanine amino transferase; HA: Hyaluronic acid; A: Activity; F: Fibrosis; OTCD: Ornithine transcarbamylase deficiency; BA: Biliary atresia; AD: Alagille syndrome; WD: Wilson disease; CEPS: Congenital extrahepatic portsystemic shunt; OKT3: Muromonab-CD3; PVS: Portal vein stenosis; BDS: Biliary duct anastomotic stenosis; CMV-I: Cytomegalovirus infection; HVS: Hepatic vein stenosis; HAT: Hepatic artery thrombosis; IHBDS: Intrahepatic biliary duct stenosis; MP: Methylprednisolone; MMF: Mycophenolate mofetil; PSL: Prednisolone; CsA: Cyclosporin A.

suppression. In postoperative year (POY) 5, a PLB was performed; the LFT data were normal, but the Metavir scores were A2 and F2 (Figure 2A). The immunosuppression was subsequently strengthened by increasing the dose of Tac and adding MMF because the PLB histopathology was considered to be abnormal. A follow-up liver biopsy was performed 18 mo after the PLB, at which time the scores were A0 and F0 (Figure 2B).

Case 5: A 58-month-old female girl with biliary atresia underwent ABO-identical LDLT using a left lateral segment graft. Tac and MP were administered as the standard postoperative immunosuppressive regimen. The patient's postoperative course was uneventful, except for an episode of acute respiratory distress, and she was discharged from the hospital on POD 56 after LDLT. MP was withdrawn at 18 mo after LDLT, and thereafter, only Tac was administered for immunosuppression. In POY 5, PLB was performed; the LFT data were normal, but the Metavir scores were A2 and F2 (Figure 2C). The

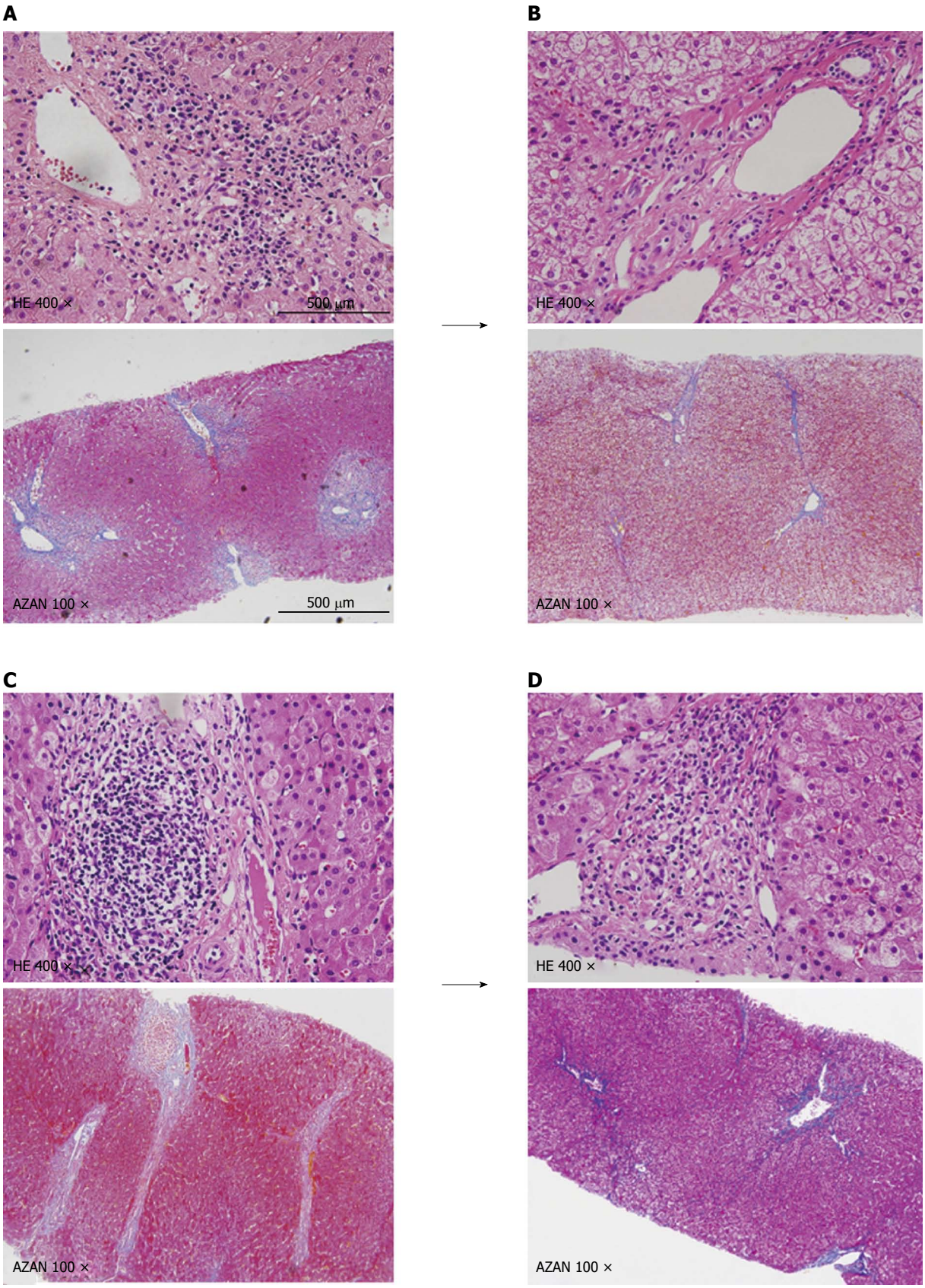

Figure 2 In postoperative year 5, a protocol liver biopsy was performed. A: At which time the Metavir scores were abnormal: A2 (portal inflammation) and F2 (portal and pericellular fibrosis); B: Follow-up liver biopsy was performed at 18 mo after the protocol liver biopsy (PLB), at which time the scores were A0 and F0; C: At which time the Metavir scores were abnormal: A2 (portal inflammation) and F2 (portal fibrosis); D: A follow-up liver biopsy was performed at 20 mo after the PLB, at which time the scores were A1 (portal inflammation) and F0. HE: Hematoxylin and eosin stain; AZAN: Azan stain.

immunosuppression was then strengthened by increasing the dose of Tac and adding MMF because the PLB histopathology was considered to be abnormal. A follow-up liver biopsy was performed 20 mo after the PLB, at which time the scores were A1 and F0 (Figure 2D).

DISCUSSION

LT is an established curative treatment for pediatric patients with end-stage liver disease or acute liver failure[1-3]. However, histopathological assessments performed during the mid- and long-term period after LT remain insufficient, and it is necessary to further improve the prognosis by maintaining the function of the liver graft using a minimum degree of immunosuppression to obtain an optimal balance between the effectiveness and side effects of individual immunosuppressants.

Histopathological assessments using PLB have recently been reported to be important in adult recipient[12-15], because the occurrence of graft fibrosis or the recurrence of the original disease cannot be predicted using standard LFTs alone. However, in pediatric recipients, the need for PLB is controversial due to the low incidence of recurrent original diseases. Liver graft fibrosis has recently been reported to be present in 43%-65% and 25%-69% of patients at two and five years after LT, respectively, even if the LFT data are normal[4-6]. Moreover, there is a relationship between liver graft fibrosis and chronic rejection[4,5], and the progression to severe fibrosis has been reported to occur in 14%-25% of patients at ten years after LT[4,6]. Furthermore, the development of liver graft fibrosis after pediatric LT occurs in 69%-97% of cases, including cases of mild fibrosis[4-8]. The risk factors for fibrosis include an increasingly long interval after LT[4,7], positivity for antinuclear antibodies[4], long cold ischemic time[6], young age at LT[6], a high donor to recipient graft ratio[6] and partial LT[6]. In the present study, independent risk factors in a multivariate analysis of a score of $\geqslant$ A1 at two years after LDLT included $\geqslant$ 2 h of cold ischemic time, no acute cellular rejection and an ALT level of $\geqslant$ 20 IU/L ($P = 0.028$, $P = 0.033$ and $P = 0.012$, respectively); however, no risk factors were identified for a score of $\geqslant$ F1. Furthermore, no independent risk factors were identified in a multivariate analysis of scores of $\geqslant$ A1 and $\geqslant$ F1 at five years. We believe that $\geqslant$ 2 h of cold ischemic time was found to be an independent risk factor for a score of $\geqslant$ A1 at two years after LDLT because a prolonged cold ischemic time may induce an immune response by affecting graft liver dysfunction. In addition, we believe that no acute cellular rejection was found to be an independent risk factor for a score of $\geqslant$ A1 at two years after LDLT because acute cellular rejection may cause an immune response due to the use of less immunosuppression. However, as a result of the ROC curve analysis of ALT at two years after LDLT in the patients with a score of $\geqslant$ A1, the recommended cutoff value for diagnosing a score of $\geqslant$ A1 was set at 20 IU/L (sensitivity: 50.0% and specificity: 76.1%). Therefore, the serum ALT level reflects the degree of portal inflammation in PLB patients at two years after LDLT with an ALT level of $\geqslant$ 20 IU/L.

With respect to concrete assessment methods for evaluating graft liver fibrosis, portal fibrosis-based liver fibrosis staging systems, such as those reported by Ishak *et al*[16] and the Metavir Study Group[11], are widely used, even in studies of pediatric LT recipients[7,8,17]. Therefore, we applied histopathological assessments using the Metavir score in the present study. Recent reports have indicated that centrilobular perisinusoidal fibrosis occurs in pediatric LT recipients in association with tacrolimus withdrawal or in the presence of donor-specific anti-human leukocyte antigen antibodies[18,19]. Venturi *et al*[17] recently developed a novel histopathological scoring system based on the detection of fibrosis in three areas: portal tracts, sinusoids and centrilobular veins. However, the significance of these histopathological findings with respect to morbidity has yet to be clarified and is the most important issue that should be addressed in the future. In the present study, using the Metavir scoring system, the incidence of the scores of $\geqslant$ F1 at two and five years after LDLT was 24.7% and 34.5%, respectively. However, no risk factors for graft fibrosis were identified, and no serum markers reflected the degree of graft fibrosis. Therefore, detecting graft fibrosis by performing a histopathological assessment using a liver biopsy is important. Furthermore, the PLB represents an important periodic examination in long-term recipients after LDLT because it enables the assessment of the effectiveness of the current immunosuppressive regimen, even when the PLB histopathology is normal. Therefore, at present, PLB is an indispensable examination for the management of patients who have undergone LDLT.

Potential problems associated with PLB include the following: (1) timing; (2) invasiveness; and (3) the obscure definition of abnormal PLB histopathology. The timing of PLB after LT is not definitive. In our department, we performed PLB at two, five, ten and 15 years after LT, considering the examination's effectiveness, invasiveness and potential complications. In the present study, the PLB performed two years after LDLT was found to be an unnecessary examination because the serum ALT level reflected the degree of portal inflammation. At the time, the immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. Gelson *et al*[20] reported that the histological inflammatory index is correlated with the ALT level. A PLB performed at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect the degree of graft fibrosis.

PLB suffers, however, from a disadvantage. PLB is an invasive procedure that is potentially associated with severe complications, with an incidence of 0.57%[21]. In the present study, although the rate of PLB-associated complications was only 0.7%, this rate may nevertheless be considered high. Non-invasive examinations, such as imaging, may be used instead of PLB if such examinations

become more effective than PLB in the future. Acoustic radiation force impulse and transient elastography imaging have been reported to exhibit good accuracy in the noninvasive diagnosis of liver fibrosis in the setting of pediatric LT[22,23].

The most problematic aspect of PLB is the obscure definition of abnormal histopathology. The histopathological findings of PLB after LT include idiopathic post-transplantation hepatitis (4.4%-64.0%)[4,24-26], central venulitis (16.0%-27.0%)[13,27], interface hepatitis (14.0%-24.4%)[28-30] and fibrosis (69.0%-97.0%)[4-8]. However, the indication for treatment with respect to each histopathological finding is unclear and controversial. In general, liver fibrosis is thought to be irreversible and resistant to treatment. However, in the present cases, the liver fibrosis was reversible, and portal inflammation was ameliorated after strengthening the immunosuppressive regimen. Immunosuppression can be strengthened effectively by increasing the dose of Tac and introducing MMF, given concerns about the side effects of MP[31-34] and the proven effectiveness of MMF[35,36]. Our present findings suggest that the early detection of graft liver fibrosis can be achieved using a liver biopsy and that liver fibrosis may be reversible if early treatment is initiated. In our department, we initially defined a histopathological abnormality as a Metavir score of ≥ A2 or ≥ F2. However, among 21 patients who underwent PLB at both two and five years after LDLT, the activity and fibrosis scores at two years after LDLT were A0 and F0 in 14 patients, A1 or F1 in six patients and ≥ A2 or ≥ F2 in one patient. Seven patients with scores of A0 and F0 at two years after LDLT exhibited worse a score of ≥ A1 or ≥ F1. Three patients with a score of A1 or F1 at two years after LDLT exhibited worse a score of ≥ A2 or ≥ F2. Therefore, we currently define a histopathological abnormality as a Metavir score of ≥ A1 or ≥ F1 and consider such scores to indicate the need for treatment because liver fibrosis is reversible if early treatment is initiated. Both further investigations and the accumulation of more LT cases are required to confirm our present findings.

In a conclusion, A PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. A PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect the degree of graft fibrosis.

COMMENTS

Background

Histopathological assessments using protocol liver biopsy (PLB) after liver transplantation (LT) have recently been reported to be important. However, few studies have investigated the impact of the timing and effectiveness of PLBs in the field of pediatric LT. This retrospective study assessed the clinical significance of PLBs in pediatric LT.

Research frontiers

The development of liver graft fibrosis after pediatric LT has been reported to occur in 69%-97% of cases, including cases of mild fibrosis. Because graft dysfunction does not occur unless the fibrosis becomes advanced and because the occurrence of graft liver fibrosis or portal inflammation cannot be predicted using standard liver function test (LFT) alone, histopathological assessments using PLB have recently been reported to be important. However, the significance of mild to severe fibrosis is unknown, and the indications for the treatment of abnormal PLB findings are controversial. In addition, few studies have investigated the impact of the timing and effectiveness of PLB.

Innovations and breakthroughs

The development of liver graft fibrosis after pediatric LT has been reported to occur in 69%-97% of cases, including cases of mild fibrosis. Because graft liver dysfunction does not occur unless the fibrosis becomes advanced and because the occurrence of graft liver fibrosis or portal inflammation cannot be predicted using standard LFT alone, histopathological assessments using PLB have recently been reported to be important. However, the significance of mild to severe fibrosis is unknown, and the indications for the treatment of abnormal PLB findings are controversial. In addition, few studies have investigated the impact of the timing and effectiveness of PLB. This retrospective study assessed the clinical significance of the timing and effectiveness of PLB after pediatric living donor liver transplantation (LDLT). In conclusion, a PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. A PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect the degree of graft fibrosis.

Applications

The study results suggest the following contents. A PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. A PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect the degree of graft fibrosis.

Terminology

Protocol liver biopsy: Protocol liver biopsy is a liver biopsy that is periodically performed at two and five years after LT.

Peer review

This is a good descriptive study in which the authors analyzed the histopathological findings using the Metavir scoring system and identified factors associated with scores of ≥ A1 and ≥ F1. They, thereafter, clarified the timing and effectiveness of PLB. The results are interesting and suggest the following. A PLB performed at two years after LDLT is an unnecessary examination because the serum ALT level reflects the degree of portal inflammation. In addition, immunosuppressive therapy should be modulated to maintain the ALT concentration at a level less than 20 IU/L. A PLB at five years is an excellent examination for the detection of early reversible graft fibrosis because no serum markers reflect the degree of graft fibrosis.

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P- Reviewers: Alsolaiman MM, Cichoz-Lach H, Hubscher SG, Li JD, Marino IR, Morioka D, Schuurman HJ **S- Editor**: Wen LL **L- Editor**: A **E- Editor**: Zhang DN

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ISSN 1007-9327