thology of liver transplantation. Necroinflammatory activity (A0-A4) and fibrosis stage (F0-F4) were assessed using the METAVIR score (*23, 24*). Fibrosis of stage 2 or higher was defined as significant fibrosis and was used as one of the endpoints in this study. A stage score of 2 was considered easily separable from stage 1 as a dividing point, as stage 1 involves fibrosis confined to the portal tract.

Prognostic Factors for Patient Survival and HCV Recurrence

A total of 18 variables potentially associated with patient survival and HCV recurrence were evaluated. Pretransplantation variables included: recipient age; recipient gender; Child-Pugh grade; MELD score; presence of HCC; HCV genotype (1b vs. non-1b); HCV viral load; and history of previous antiviral treatment with interferon. Donor-related variables comprised: age; gender; relation to the recipient (related vs. unrelated); ABO-blood type and HLA compatibilities; graft-to-recipient body weight ratio (GRWR: $<1.0\%$ vs. $\geq 1.0\%$). Posttransplant variables were: induction immunotherapy (tacrolimus vs. cyclosporine, with or without steroid); and administration of steroid boluses.

Statistical Analysis

Overall survival, time to reach fibrosis of stage 2 or more according to liver biopsy (with time to last biopsy for all patients who did not reach fibrosis of stage 2), and time to severe HCV recurrence were evaluated. Severe HCV recurrence was defined as the presence of liver cirrhosis (F4) in a liver biopsy and/or the development of clinical decompensation secondary to liver diseases with portal hypertension (*11*). Cumulative probability curves of survival or HCV recurrence were calculated using the Kaplan-Meier method, and differences between these curves were compared using the log-rank test. The cutoff chosen for quantitative variables was the median, unless stated otherwise. Any variable identified as significant ($P<0.05$) in univariate analysis by log-rank testing was considered a candidate for multivariate analysis using Cox's proportional hazards regression model. Values of $P<0.05$ were considered statistically significant.

RESULTS

Patient Survival

As of the end of May 2005, 65 patients were still alive. One patient had received re-LDLT for graft cirrhosis due to HCV recurrence 31 months after first LDLT and has survived 14 months since then. Causes of death for the 26 patients were: sepsis (n=11); peritonitis (n=4); pneumonia (n=3); recurrent HCC (n=4); chronic rejection (n=2); veno-occlusive disease (n=1); and recurrent HCV (fibrosing cholestatic hepatitis (FCH), n=1). Overall patient survival rate at 5 years was 69%, similar to that of 209 non-HCV patients (71%) who underwent right-lobe LDLT at our institute between February 1998 and June 2004 (PBC/PSC, n=56; HBV cirrhosis, n=53; fulminant hepatitis, n=38; cholestatic disease, n=19; and others, n=43; Fig. 1). Among HCV-positive patients, 5-year survival rate tended to be better in patients without HCC (n=34) than in patients with HCC (n=57), although no significant difference was identified (82% vs. 60%, $P=0.069$; Fig. 2). None of the variables listed above as prognostic factors for patient survival and HCV recurrence displayed any significantly associations with patient survival.

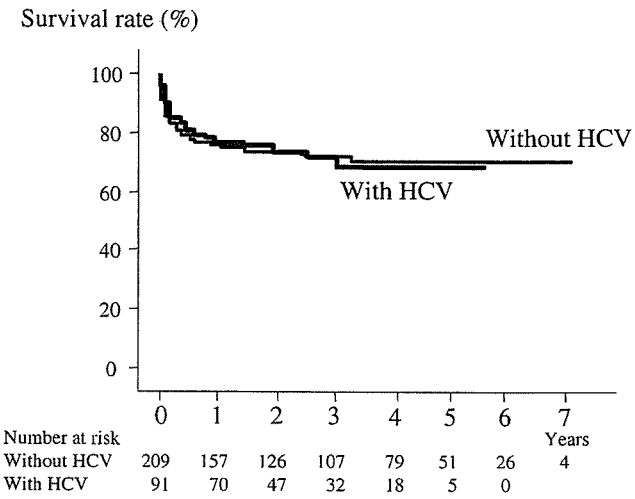

FIGURE 1. Patient survival after living donor liver transplantation (HCV vs. non-HCV). Overall patient survival rate for HCV-positive patients was 69% at 5 years, similar to that for non-HCV patients (71%, n=209) who underwent right-lobe LDLT at our institute between February 1998 and June 2004.

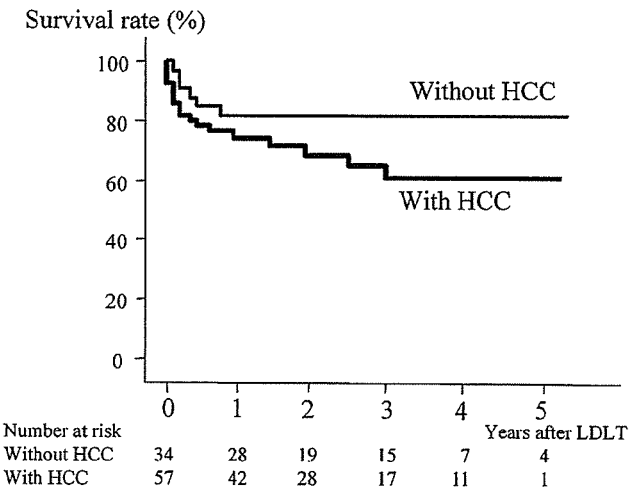

FIGURE 2. Patient survival for HCV patients (with vs. without HCC). Among HCV-positive patients, survival rate tended to be better for patients without HCC (n=34) than for patients with HCC (n=57), although no significant difference was found (82% vs. 60%, $P=0.069$).

Evaluation of Liver Histology

During the first year after LDLT, necroinflammatory changes suggesting recurrent hepatitis C were observed in 50 patients: A1 in 32 patients; A2 in 17 patients; and A3 in one patient. Afterwards, the percentage of patients who received biopsy among those alive at yearly intervals was as follows: 1 year, 86% (60/70); 2 years, 72% (34/47); 3 years, 44% (14/32); 4 years, 33% (6/18); and 5 years, 20% (1/5). Ten patients who were alive for >1 year (range, 22–72 months) without any

evidence of progressive liver disease never underwent any biopsy at 1 year or later. Significant fibrosis (stage 2 or more) was identified in 23 patients, including 3 patients who developed to fibrosis of stage 4 within 1 year. Excluding 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year, cumulative probability of significant fibrosis was 19% at 1 year after LDLT, 39% at 2 years, and 58% at 3 years. Follow-up was censored at the time of last biopsy for all patients who did not reach fibrosis of stage 2.

The results from univariate analysis of risk factors for significant fibrosis are summarized in Table 2. Female recipient and male donor were significantly associated with development of significant fibrosis. Analysis of quantitative variables, donor age and GRWR, demonstrated that rate of significant fibrosis was not significantly different even when cutoff levels were changed. Multivariate analysis with Cox's hazards model showed that neither female recipient nor male donor represented independent risk factors for significant fibrosis (data not shown).

Severe Recurrence of HCV

FCH was diagnosed in two patients, one of whom died of liver failure 7 months after LDLT. The other patient suffered from FCH 2 months after LDLT, but recovered from cholestasis and was still alive after 28 months. Final liver biopsies of both patients showed fibrosis of stage 4. Another three patients also developed fibrosis of stage 4 during follow-up. One patient whose liver biopsy led to a diagnosis of recurring chronic hepatitis with F3 fibrosis 10 month after LDLT also suffered from stenosis of duct-to-duct biliary anastomosis. This patient underwent hepaticojejunostomy, but died of fungal pneumonia 1 month later. Liver histology at autopsy revealed F4 fibrosis. Another patient received re-LDLT for recurrent decompensated cirrhosis, as described above. The other patient who developed to biopsy-proven stage 4 fibrosis at 50 months was alive without decompensation as of 63 months after LDLT. In total, severe recurrence (progression of biopsy-proven cirrhosis and/or occurrence of clinical decompensastion) was diagnosed in five patients, and cumulative probability of severe recurrence was 8% at 2 years. Of the five patients presenting with severe recurrence, three were female and all had received the liver graft from a male donor.

TABLE 2. Risk factors associated with fibrosis of stage 2 or higher

Factors	n	Recurrence rate (number of patients at risk) 1 year	2 years	3 years	*P* value
Total[a]	62	19% (50)	39% (14)	58% (5)	
Recipient sex					0.006
Male	40	10% (36)	27% (10)	48% (4)	
Female	22	36% (14)	60% (4)	70% (1)	
Donor sex					0.047
Male	36	25% (28)	49% (6)	59% (1)	
Female	26	12% (22)	26% (8)	47% (4)	

[a] The 19 patients who died within 1 year without identified fibrosis and the 10 patients alive without biopsy for >1 year were excluded.

DISCUSSION

In the present study, only one patient had died of recurrent HCV as of the time of writing, and the majority of posttransplant deaths were attributable to postoperative complications occurring within a few months after LDLT. Infectious complications such as sepsis, pneumonia and peritonitis represented the most common causes of early mortality, as was the case in HCV-negative recipients. One-year mortality rates were 23% and 25%, respectively. Currently, overall 5-year patient survival rate for HCV-positive patients appears similar to that for non-HCV patients in our adult LDLT series (69% vs. 71%; Fig. 1). Of the 91 patients, HCC was present in 57 (63%), including 25 patients who exceeded the Milan criteria. Four patients died of recurrent HCC after LDLT, and the survival rate tended to be lower for patients with HCC than for patients without HCC (82% vs. 74% at 1 year, and 82% vs. 60% at 5 years; Fig. 2). Only one patient in this cohort had to undergo re-transplantation, and 5-year graft survival rates were 68% for all patients and 82% for patients without HCC. These results are comparable to the reported DDLT outcomes in the UNOS database: patient and graft survival rates of HCV-positive patients (n=3955) at 2 years were 81% and 75%, respectively (*13*); and rates for HCV-positive but HCC-negative patients (n=5640) at 5 years were 74.6% and 69.9%, respectively (*7*).

Progression of fibrosis due to recurrent chronic hepatitis is key to determining graft prognosis after liver transplantation for HCV-positive recipients. In the present study, progression of fibrosis in the liver biopsy was assessed and fibrosis to stage 2 or more was defined as significant fibrosis. The probability of progression to significant fibrosis was 39% at 2 years after LDLT. Several risk factors associated with posttransplant recurrence of hepatitis C have been identified (*5, 25–27*). These include pretransplant viral load, genotype 1b, donor age and graft steatosis, recipient age, race, gender, coexistence of HCC, and rejection treatment using bolus steroid or antilymphocyte preparations. Among the 18 potential variables examined in our study, univariate analysis identified female recipient and male donor as closely related to significant fibrosis. However, multivariate analysis showed that neither variable represented a significant independent risk factors. Actually, some correlation among these two variables was noted. Of the 30 female recipients, 24 had received a liver graft from a male donor (son or husband). An association between female gender of the recipient and severity of recurrent HCV has been demonstrated in previous studies (*6, 7*). However, no previous reports have implicated gender of the donor as an involved factor. Although difficulty exists in determining which is the predominant factor, the combination of male donor and female recipient may exert a negative impact on HCV recurrence.

Rapid proliferation of hepatocytes during postoperative graft regeneration may contribute to a higher rates of both HCV replication and severe recurrence in LDLT (*11*). This seems to imply a higher risk of recurrence in cases involving smaller grafts, which are supposed to undergo regeneration at a higher rate. Our study, however, showed that progression of significant fibrosis was similar for patients who received grafts with GRWRs of <1.0% or ≥1.0%. This result is supported by a recent report (*28*) showing that liver

regeneration following partial liver transplant does not increase the risk of HCV recurrence. Likewise, neither the relationship between donor and recipient nor degree of HLA matching seemed to influence recurrence. The results of our study thus do not support the hypothesis that these factors may exert negative effects on HCV recurrence in LDLT patients.

Due to the small number of patients treated using DDLT in Japan, HCV recurrence rates could not be compared between DDLT and LDLT. In previous studies on HCV recurrence after DDLT (*1, 13, 25, 29, 30*), histologically diagnosed recurrence of chronic HCV occurred in 65–90% of HCV-positive DDLT recipients during the first 2 years. However, a lack of uniform definitions for recurrent HCV, even when histological liver biopsy findings are used as criteria, has been indicated as one reason for the difficulties in comparing studies on HCV recurrence (*31*). Recently, a report from Spain demonstrated that severe recurrence of hepatitis C, defined as the development of cirrhosis or clinically decompensated liver disease, is more frequent in LDLT recipients (*11*). According to this report, the 2-year probability of developing severe recurrence was 45% after LDLT, compared to 22% after DDLT ($P=0.019$). When the same definitions were applied, rate of severe recurrence was only 8% at 2 years in our study. Arguably as many as 19 patients (21%) died within 1 year before developing HCV recurrence in our series. However, considering that the probability of either death or severe recurrence was 29% at 2 years, the results for our LDLT series were not likely to be greatly inferior to other reported cases.

In conclusion, postoperative patient survival was similar for HCV-positive and -negative recipients in our adult LDLT series. Rate of recurrence for chronic HCV and prevalence of progression to severe disease for our LDLT recipients appeared comparable to those for DDLT reported in the literature. Although these results need to be confirmed with a longer follow-up period, the present findings suggest that LDLT can produce acceptable outcomes for patients suffering from end-stage liver disease due to chronic HCV.

REFERENCES

1. Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. *Liver Transplant* 2003; 9: S28–S34.
2. Prieto M, Berenguer M, Rayon J, et al. High incidence of allograft cirrhosis in HCV genotype 1b following transplantation. *Hepatology* 1999; 29: 250–256.
3. Feray C, Caccamo L, Alexander G, et al. HCV and liver transplantation: Preliminary results of a European collaborative study on factors influencing outcome after liver transplantation for hepatitis C. *Gastroenterology* 1999; 117: 619–625.
4. Sanchez-Fueyo A, Restrepo J, Quinto L, et al. Impact of recurrence of HCV infection after liver transplantation on the long-term viability of the graft. *Transplantation* 2002; 73: 56–63.
5. Berenguer M, Prieto M, Juan FS, et al. Contribution of donor age to the recent decrease in patient survival among HCV-infected liver transplant recipients. *Hepatology* 2002; 36: 202–210.
6. Forman L, Lewis J. Association between hepatitis C infection and survival after 31orthotopic liver transplantation. *Gastroenterology* 2002; 122: 889–896.
7. Velidedeoglu E, Mange KC, Frank A, et al. Factors differentially correlated with the outcome of liver transplantation in HCV+ and HCV- recipients. *Transplantation* 2004; 77: 1834–1842.
8. Gaglio PJ, Malireddy S, Russo RS, et al. Hepatitis C recurrence in recipients of grafts from living vs cadaveric liver donors [abstract]. *Hepatology* 2002; 36: 265A.
9. Ghobrial RM, Amersi F, Farmer DG, et al. Rapid and severe early HCV recurrence following adult living donor liver transplantation [abstract]. *Am J Transplant* 2002; 2: 163A.
10. Gaglio PJ, Malireddy S, RusLevitt BS, et al. Increased risk of cholestatic hepatitis C in recipients of grafts from living versus cadaveric liver donors. *Liver Transpl* 2003; 9: 1028–1035.
11. Garcia-Retortillo M, Forns X, Llovet JM, et al. Hepatitis C recurrence is more severe after living donor compared to cadaveric liver transplantation. *Hepatology* 2004; 40: 699–707.
12. Everson GT, Trotter J. Role of adult living donor liver transplantation in patients with hepatitis C. *Liver Transpl* 2003; 9: S64–S68.
13. Russo MW, Galanko J, Beavers K, et al. Patient and graft survival in hepatitis C recipients after adult living donor liver transplantation in the United States. *Liver Transpl* 2004; 10: 340–346.
14. Bozorgzadeh A, Jain A, Ryan C, et al. Impact of hepatitis C viral infection in primary cadaveric liver allograft versus primary living-donor allograft in 100 consecutive liver transplant recipients receiving tacrolimus. *Transplantation* 2004; 77: 1066–1070.
15. Shiffman ML, Stravitz RT, Contos MJ, et al. Histologic recurrence of chronic hepatitis C virus in patients after living donor and deceased donor liver transplantation. *Liver Transpl* 2004; 10: 1248–1255.
16. Sugawara Y, Makuuchi M. Should living donor liver transplantation be offered to patients with hepatitis C virus cirrhosis? *J Hepatol* 2005; 42: 472–475.
17. Ohno T, Mizokami M, Wu RR, et al. New hepatitis C virus (HCV) genotyping system that allows for identification of HCV genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *J Clin Microbiol* 1997; 35; 201–207.
18. Inomata Y, Uemoto S, Asonuma K, et al. Right lobe graft in living donor liver transplantation. *Transplantation* 2000; 69: 258–264.
19. Kasahara M, Takada Y, Fujimoto Y, et al. Impact of with middle hepatic vein graft in right lobe living donor liver transplantation. *Am J Transpl* 2005; 5: 1339–1346.
20. Inomata Y, Tanaka K, Egawa H, et al. The evolution of immunosupression with FK506 in pediatric living related liver transplantation. *Transplantation* 1996; 61: 247–252.
21. Tanabe M, Shimazu M, Wakabayashi G, et al. Intraportal infusion therapy as a novel approach to adult ABO-incompatible liver transplantation. *Transplantation* 2002; 73: 1959–1961.
22. Banff schema for grading liver allograft rejection: an international consensus document. *Hepatology* 1997; 25: 658–663.
23. Bedossa P, Poynard T, for the METAVIR Cooperative Study Group. An algorithm for the grading of activity in chronic hepatitis C. *Hepatology* 1996; 24: 289–293.
24. Poynard T, Bedossa P, Opolon P, for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. *Lancet* 1997; 349: 825–832.
25. Berenguer M, Prieto M, Cordoba J, et al. Early development of chronic active hepatitis in recurrent hepatitis C virus infection after liver transplantation: association with treatment of rejection. *J Hepatol* 1998; 28: 756–763.
26. Wiesner RH, Rakela J, Ishitani MB, et al. Recent advances in liver transplantation. *Mayo Clin Proc* 2003; 78: 197–210.
27. Berenguer M. What determines the natural history of recurrent hepatitis C after liver transplantation. *J Hepatol* 2005; 42: 448–479.
28. Humar A, Horn K, Kalis A, et al. Living donor and split-liver transplants in hepatitis C recipients: Does liver regeneration increase the risk for recurrence? *Am J Transplant* 2005; 5: 399–405.
29. Gane EJ, Portmann BC, Naoumov NV, et al. Long-term outcome of hepatitis C infection after liver transplantation. *N Engl J Med* 1996; 334: 815–820.
30. Shuhart MC, Bronner MP, Gretch DR, et al. Histological and clinical outcome after liver transplantation for hepatitis C. *Hepatology* 1997; 26: 1646–1652.
31. Russo MW, Shrestha R. Is severe recurrent hepatitis C more common after adult living donor liver transplantation? *Hepatology* 2004; 40: 5245–5246.

Biliary Reconstruction in Right Lobe Living-Donor Liver Transplantation

Comparison of Different Techniques in 321 Recipients

Mureo Kasahara, MD, PhD, Hiroto Egawa, MD, PhD,* Yasutsugu Takada, MD, PhD,† Fumitaka Oike, MD, PhD,† Seisuke Sakamoto, MD, PhD,† Tetsuya Kiuchi, MD, PhD,‡ Syujiro Yazumi, MD, PhD,§ Toshiya Shibata, MD, PhD,‖ and Koichi Tanaka, MD, PhD*†*

Objective: To assess the incidence of biliary complications after right lobe living-donor liver transplantation (LDLT) in patients undergoing duct-to-duct choledochocholedochostomy or Roux-en-Y choledochojejunostomy reconstruction.

Summary Background Data: Biliary tract complications remain one of the most serious morbidities following liver transplantation. No large series has yet been carried out to compare the 2 techniques in LDLT. This study undertook a retrospective assessment of the relation between the method of biliary reconstruction used and the complications reported.

Methods: Between February 1998 and June 2004, 321 patients received right lobe LDLT. Biliary reconstruction was achieved with Roux-en-Y choledochojejunostomy in 121 patients, duct-to-duct choledochocholedochostomy in 192 patients, and combined Roux-en-Y and duct-to-duct choledochocholedochostomy in 8 patients. The number of graft bile duct and anastomosis, mode of anastomosis, use of stent tube, and management of biliary complications were analyzed.

Results: The overall incidence of biliary complications was 24.0%. Univariate analysis revealed that hepatic artery complications, cytomegalovirus infections, and blood type incompatibility were significant risk factors for biliary complications. The respective incidence of biliary leakage and stricture were 12.4% and 8.3% for Roux-en-Y, and 4.7% and 26.6% for duct-to-duct reconstruction. Duct-to-duct choledochocholedochostomy showed a significantly lower incidence of leakage and a higher incidence of stricture; however, 74.5% of the stricture was managed with endoscopic treatment.

Conclusions: The authors found an increase in the biliary stricture rate in the duct-to-duct choledochocholedochostomy group. Because of greater physiologic bilioenteric continuity, less incidence of leakage, and easy endoscopic access, duct-to-duct reconstruction represents a feasible technique in right lobe LDLT.

(*Ann Surg* 2006;243: 559–566)

Biliary tract complication remains one of the most serious morbidities following liver transplantation, with an incidence of 10% to 30% in deceased liver transplantation.[1–4] It has been reported that the pathogenesis of biliary leakage and stricture in deceased liver transplantation were related to preoperative patient condition, blood type incompatibility, ischemic time, hepatic artery complications, and cytomegalovirus (CMV) infection.[5–9] The published data have also suggested that the frequency of biliary complications is higher in post-living donor liver transplantation (LDLT) compared with deceased liver transplantation.[10,11] Major concerns are early leakage and late stricture at the anastomotic site, which are associated with technical, anatomic, or microcirculatory considerations. Particularly in the recipient with "small-for-size" graft or deteriorated preoperative status, early biliary complications readily result in a fatal outcome, and these conditions themselves may increase the risk of complications.

There remains considerable disparity in the reported cases with regard to the incidence of biliary complications after right lobe LDLT, with reported rates ranging from 24% to 60%.[11,12–15] In right liver graft, current controversy focuses on the selection between Roux-en-Y hepaticojejunostomy and duct-to-duct choledochocholedochostomy. Many technical issues, such as the method of dissection, selection of suture and mode, and the use of stenting tube, are still under discussion. Duct-to-duct is currently our standard technique of choice for biliary reconstruction in right lobe LDLT, with the following advantages over Roux-en-Y choledochojejunostomy: 1) no need for intestinal manipulation, serving as an

From the *Organ Transplant Unit, Department of Transplant Surgery, Kyoto University Hospital, Kyoto, Japan; †Department of Transplantation and Immunology, Kyoto University Faculty of Medicine, Kyoto, Japan; ‡Department of Transplant Surgery, Nagoya University Hospital, Nagoya, Japan; §Department of Gastroenterology and Hepatology, Kyoto University Faculty of Medicine, Kyoto, Japan; and ‖Department of Interventional Radiology, Kyoto University Faculty of Medicine, Kyoto, Japan.

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.

Reprints: Mureo Kasahara, MD, Department of Transplant Surgery, Kyoto University Hospital, 54 Kawara-cho, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. E-mail: mureo@kuhp.kyoto-u.ac.jp.

ISSN: 0003-4932/06/24304-0559
DOI: 10.1097/01.sla.0000206419.65678.2e

anatomic barrier to reflux of enteric contents into the biliary tract, and it may theoretically decrease the risk of ascending cholangitis and the morbidity is reduced even when early anastomotic leakage occurs; 2) technically faster and easier than Roux-en-Y; and 3) the physiologic bilioenteric continuity enables good endoscopic access postoperatively.[16,17]

This report describes surgical trials for biliary reconstruction in 321 consecutive right lobe LDLT, focusing on technical considerations regarding the biliary anatomy on graft, suture mode and stent tube in duct-to-duct/Roux-en-Y biliary reconstructions during long-term follow-up.

PATIENTS AND METHODS

Between June 1990 and June 2004, 953 patients underwent 1000 LDLTs at Kyoto University Hospital, Kyoto, Japan. Right lobe LDLT was first carried out at our institution in February 1998, and we have since performed 346 right lobe LDLTs. Of these, 25 patients died within 3 months of LDLT and are thus excluded from this study. A total of 321 patients were the subjects of the present study.

The patients were 164 males and 157 females, with a median age of 43.4 years (range, 15.5–70.3 years), and a median weight of 59.9 kg (range, 34.6–99.5 kg). Median model for end-stage liver disease[18] score was 18.0. The indication for liver transplantation was hepatocellular carcinoma in 86 patients, followed by viral hepatitis (n = 57), cholestatic liver disease (n = 57), fulminant hepatic failure (n = 39), biliary atresia (n = 34), metabolic liver disease (n = 9), metastatic liver tumor (n = 3), retransplantation (n = 16), and others (n = 20). Forty patients (12.5%) received blood type incompatible grafts. Thirty patients (9.3%) received right lobe with middle hepatic vein graft. A total of 121 patients (37.7%) received biliary reconstruction using Roux-en-Y and 192 (59.8%) had duct-to-duct anastomosis, while 8 (2.5%) patients underwent combined Roux-en-Y and duct-to-duct anastomosis. After the introduction of duct-to-duct anastomosis in July 1999, patients who had liver disease without extrahepatic biliary tract involvement were candidates for duct-to-duct anastomosis. The median follow-up period was 60 months (range, 7–80 months) in Roux-en-Y choledochojejunostomy and 34 months (range, 7–64 months) in duct-to-duct anastomosis ($P < 0.01$). There were no significant differences in patient characteristics between either group, except for the follow-up period and the patient age. Because of the patient population with biliary atresia in the Roux-en-Y group, the patient age was significantly younger in the Roux-en-Y group ($P < 0.01$) (Table 1).

Immunosuppression consisted of tacrolimus and low-dose steroids.[19] Patients who received blood type incompatible transplants had preoperative plasma exchange or double filtration plasmapheresis to reduce the anti-ABH antibody titer. Prostaglandin E1, cyclophosphamide, and additional steroids were administered from the portal vein or hepatic artery postoperatively.[20,21]

Statistical analysis was performed using the generalized Wilcoxon test. Actuarial survival rate was calculated with the nonparametric Kaplan-Meier method and was compared with the Wilcoxon test throughout the study. − values of less than 0.01 were considered significant.

TABLE 1. Patient Characteristics

Characteristic	Roux-en-Y (n = 121)	DD (n = 192)	P
Age (yr)	35.2 ± 13.5	48.8 ± 11.3	<0.01
MELD score	16.9 ± 11.4	18.4 ± 10.5	NS
Donor age (yr)	43.1 ± 10.9	41.4 ± 11.9	NS
ABO incompatibility (%)	11.6	13.5	NS
GRWR (%)	1.19 ± 0.29	1.13 ± 0.26	NS
Cold ischemic time (min)	123 ± 103	97 ± 77	NS
Warm ischemic time (min)	42 ± 13	49 ± 17	NS
Operation time (min)	789 ± 192	693 ± 173	NS
Blood loss (g)	7855 ± 10582	6060 ± 8082	NS
Median follow-up period (mo)	60.0	34.0	<0.01

DD indicates duct-to-duct choledochocholedochostomy; GRWR, graft-to-recipient weight ratio; NS, not significant.

The study was approved by the international review board and informed consent was obtained in all the cases.

Donor Operation

Standard right lobe technique was previously described.[12,22,23] Before parenchymal transection, the right lobe was mobilized and the sizeable (>5 mm) right inferior hepatic vein was preserved with a caval cuff for reconstruction. After careful definition of biliary anatomy in the hepatic hilum using intraoperative cholangiography, the right hepatic duct was transected 2 to 3 mm away from the bifurcation. Minimal dissection of pericholedochal tissue was required at this point to maintain blood supply around the hepatic duct, and the hepatic duct was separated with fine scissors. Right-sided liver has a higher incidence of vascular and biliary variant. Overall, 39.6% of grafts had multiple bile ducts in our right lobe LDLT series, which poses a further difficulty in reconstruction.[16,24] The right portal vein and the right hepatic artery were temporally clamped to clarify the parenchymal transection line. An 8-mm Penrose drain was passed between the right hepatic vein superiorly and the portal bifurcation inferiorly to maintain the cutting plane during parenchymal dissection. The end of remnant hepatic ducts were closed with a continuous suture using 6–0 polydioxanone absorbable monofilament, and cholangiogram was performed to ensure that there was no leakage or stricture.

Recipient Operation

Hilar dissection was carefully performed to preserve adequate blood supply of the epicholedodochal arterial plexus and the 2 distinct intramural arteries (3 and 9 o'clock arteries),[25,26] and the bile duct was divided above the hilar bifurcation. Biliary anastomosis was performed with 6–0 polydioxanone absorbable monofilament suture after completion of vascular anastomosis. The graft hepatic duct was anastomosed to Roux-en-Y limb and/or bile duct. When bile ducts in a graft were far apart, they were anastomosed separately. In 8 grafts, the bile ducts were so far apart that both duct-to-duct and Roux-en-Y reconstructions were indi-

cated. If the blood supply of the recipient cystic duct was sufficient and the recipient cystic duct was a better size match, the recipient cystic duct was used for the posterior duct reconstruction. Variations in technical preference remain, and modifications may be necessary to meet anatomic variants. The anastomosis was started at the posterior wall with interrupted or continuous suture, after which the anterior anastomosis was completed. A 4-French polyethylene tube was inserted for anastomotic decompression in some cases.

For internal stent in Roux-en-Y, 2 cm of 18G silicon vascular catheter was placed in the anastomosis. For external stent in Roux-en-Y, the 4-French tube was inserted through the jejunum and the tip was placed through the anastomosis. The stent tube was removed 8 weeks after transplantation.[27]

Biliary complications were diagnosed clinically and radiologically. Biliary leakage was defined by bilirubin level in the bilious ascites higher than the serum level, and stricture was diagnosed by dilated intrahepatic bile ducts with ultrasonography, hepatobiliary scan with Tc-99m Sn-*N*-pyridoxyl-5-methyltryptophan (^{99m}Tc-PMT), and radiologic intervention in all cases.[28]

RESULTS

Overall Incidence of Biliary Complication and Risk Factor

Of 321 right lobe LDLTs, 77 patients (24.0%) experienced 87 biliary complications (leakage: n = 27, 8.4%; stenosis: n = 60, 18.7%). There were no significant differences between the patient with or without biliary complication (n = 77 versus n = 244, respectively) in model for end-stage liver disease score (18.3 ± 9.3 versus 18.0 ± 11.3); donor age (40.9 ± 11.6 years versus 42.5 ± 11.5 years); percentage of blood type incompatibility (16.9% versus 12.3%); graft-to-recipient weight ratio (1.11 ± 0.24% versus 1.16 ± 0.29%); cold ischemic time (112 ± 92 minutes versus 87 ± 67 minutes); and warm ischemic time (48 ± 26 minutes versus 48 ± 16 minutes). However, the respective incidence of hepatic artery complications (28.6% versus 0.4%) and CMV infection (39.0% versus 22.5%) was significantly higher in the patients with biliary complications ($P < 0.01$) (Table 2). Blood type incompatibility was not a significant risk factor in overall right lobe LDLT series.

Overall incidence of biliary leakage and stricture were 12.4% and 8.3% in Roux-en-Y (n = 121), 4.7% and 26.6% in duct-to-duct (n = 192), and 0% in combined Roux-en-Y and duct-to-duct (n = 8), respectively. Duct-to-duct anastomosis showed significantly lower incidence of leakage and a higher incidence of stricture ($P < 0.01$). The onset of biliary leakage and stricture were 19.0 ± 7.7 days (range, 8–35 days; median, 17.5 days) and 12.3 ± 12.2 months (range, 2–36 months; median, 7.5 months) in Roux-en-Y, and 26.5 ± 26.1 day (range, 2–90 days; median, 20 days) and 8.7 ± 5.4 months (range, 2–35 months; median, 8 months) in duct-to-duct (P = not significant), respectively.

TABLE 2. Potential Risk Factor for Biliary Complication in 321 Consequent Right Lobe Living Donor Liver Transplantations

	Biliary Complications		
	Yes (n = 77)	No (n = 244)	*P*
MELD score	18.3 ' 9.3	18.0 ' 11.3	NS
Donor age (yr)	40.9 ± 11.6	42.5 ± 11.5	NS
Blood type incompatibility (%)	13 (16.9%)	30 (12.3%)	NS
GRWR (%)	1.11 ± 0.24	1.16 ± 0.29	NS
Cold ischemic time (min)	112 ± 92	87 ± 67	NS
Warm ischemic time (min)	48 ± 26	48 ± 16	NS
Hepatic artery stenosis/ thrombosis (%)	22 (28.6%)	1 (0.4%)	<0.01
CMV infection (%)	30 (39.0%)	55 (22.5%)	<0.01

GRWR indicates graft-to-recipient weight ratio; CMV, cytomegalovirus; NS, not significant.

Analysis of Biliary Complication According to the Type of Anastomosis

A total of 121 patients received Roux-en-Y biliary reconstruction (Table 3). There was no significant difference in biliary complications among the number of bile ducts in the graft and mode of anastomotic suture (P = not significant). There was a high incidence of biliary complications in the graft with 3 ducts. There was a trend toward a lower incidence of leakage and a higher incidence of stricture in continuous suture, but no significant difference was found among the groups. The patients with external stent (n = 103) showed lower incidence of biliary leakage compared with those with internal stent (n = 5), but this observation did not achieve statistical significance. The incidence of biliary stricture in the patients with external stent was significantly lower than in the patients without stent (n = 13) ($P < 0.01$).

TABLE 3. Biliary Complication in Roux-en-Y Choledochojejunostomy (n = 121)

	n	Leakage (%)	Stricture (%)
No. of graft bile ducts and anastomosis			
1 duct/1 anastomosis	66	7 (10.6)	4 (6.1)
2 ducts/2 anastomoses	64	7 (10.9)	5 (7.8)
3 ducts/1 anastomosis	1	1 (100)	1 (100)
Mode of anastomosis suture			
Interrupted	68	10 (14.7)	5 (7.4)
Continuous	48	4 (8.3)	5 (10.4)
Posterior: continuous/anterior: interrupted	5	1 (20.0)	0 (0.0)
Stent use for biliary reconstruction			
No stent	13	3 (23.1)	3 (23.1)
Internal stent	5	3 (60.0)	2 (40.0)
External stent	103	9 (8.7)	5 (4.9)*

*$P < 0.01$.

TABLE 4. Biliary Complication in Duct-to-Duct Choledochocholedochostomy (n = 192)

	n	Leakage (%)	Stricture (%)
No. of graft bile ducts and anastomosis			
1 duct/1 anastomosis	117	8 (6.8)	38 (32.4)
2 ducts/1 anastomosis	32	0 (0.0)	5 (15.6)
2 ducts/2 anastomoses	41	0 (0.0)	7 (17.0)
3 ducts/1 anastomosis	1	1 (100)	1 (100)
3 ducts/2 anastomoses	1	1 (100)	0 (0.0)
Mode of anastomosis suture			
Interrupted	25	2 (8.0)	9 (36.0)
Continuous	148	7 (4.7)	37 (25.0)
Posterior: continuous/anterior: interrupted	19	1 (5.3)	5 (26.3)
Stent type for biliary reconstruction (12)			
No stent	6	1 (16.7)	2 (33.3)
Cystic drainage	16	2 (12.5)	6 (37.5)
Cystic stent	9	0 (0.0)	2 (22.2)
External stent	163	7 (4.3)	41 (25.1)

Duct-to-duct biliary reconstruction was achieved in 192 cases (Table 4). If we focus on blood type incompatibility in biliary complication with duct-to-duct reconstruction, leakage and stricture was observed in 11.5% and 38.5% of the patients with blood type incompatibility; the incidence of biliary complications was significantly higher in duct-to-duct patients with blood+ type incompatibility ($P < 0.01$).

In 117 recipients (60.9%) of duct-to-duct anastomosis, the common bile duct was used to perform the reconstruction with a single right bile duct. In 11 of 117 patients with single duct-to-duct anastomosis (9.4%) and 8 of 41 patients with 2 anastomoses for 2 ducts (19.5%), a small incision (1–2 mm) in the anterior wall of the graft bile duct was made to accommodate the size mismatch. In 5 patients with one anastomosis for 2 ducts (15.6%), a ductoplasty was performed to enable a single anastomosis to the recipient common bile duct. In 6 of 41 patients with 2 anastomoses for 2 ducts (14.6%), the recipient cystic duct was used to perform the posterior duct reconstruction for better size matching. Two of them (33.3%) had biliary stricture at 2 and 6 months after transplantation. In another case with 2 ducts, the ducts were anastomosed to the recipient left and right hepatic ducts. Totally, there was no significant difference in biliary complications among the number of bile ducts in the graft and mode of anastomosis suture in duct-to-duct reconstruction. However, if the graft had 3 ducts, there was a high incidence of biliary complication.

In 188 cases, the biliary stent tube was inserted for anastomotic decompression in duct-to-duct anastomosis. For cystic drainage (n = 16), the stent was inserted through the remaining cystic duct and pushed downward into the recipient common bile duct. For cystic stent (n = 9), the tube was inserted through the remaining cystic duct and was placed through the anastomosis as a splint. For external stent (n = 163), the tube was placed through the anastomosis and was pulled out through the common bile duct.[16] There was no significant difference in biliary complications according to the type of biliary stent in duct-to-duct reconstruction. If we compare the incidence of anastomotic complication in single duct-to-duct reconstruction (n = 117), the incidence of biliary leakage and stricture was 10.0% and 40% in interrupted suture, 7.2% and 31.3% in continuous suture, 0% and 28.6% in combined interrupted and continuous suture (P = not significant), respectively. Also, the use of the stent tube did not reduce biliary complications in single duct-to-duct anastomosis.

Clinical Outcome of Patients After Biliary Complication in Roux-en-Y Hepaticojejunostomy

The clinical outcome of the patients with biliary complications in Roux-en-Y reconstruction is summarized in Figure 1. Two patients with bile leaks and one with biliary stricture died of sepsis. Biliary leakage was first treated with percutaneous drainage. When the amylase level of aspirated fluid was high or the patient's condition was critical, relaparotomy was indicated. Because the anastomosis appeared to be too fragile for revision, we put drains and carried out a Roux-en-Y enterostomy to isolate/rest the biliary anastomosis (Roux-en-Y diversion). Five patients received Roux-en-Y diversion. The enterostomy was removed after the leak had

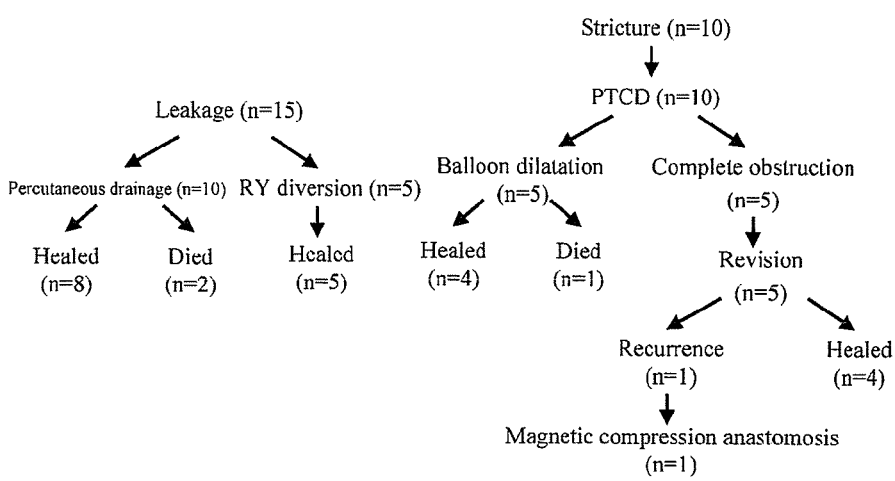

FIGURE 1. Summary of clinical outcome after biliary complications in Roux-en-Y choledochojejunostomy. PTCD, percutaneous transhepatic cholangiography and drainage.

been successfully treated. Two patients died of septic complication after biliary leakage at 14 and 3 months after transplantation.

Four of the 10 biliary strictures were secondary to biliary leakage. Anastomotic stricture was initially managed with percutaneous transhepatic cholangiodrainage (PTCD). Five patients were successfully treated with balloon dilatation. Five patients (50%) with complete anastomotic obstruction required surgical revision. One patient developed biliary stricture after surgical revision and was treated with magnetic compression anastomosis between the hepatic duct and Roux-en-Y loop, as proposed by Yamanouchi et al.[29] One patient with biliary stricture died of sepsis after several courses of PTCD and balloon dilatation 11 months after transplantation. One patient who underwent revision surgery for biliary stricture died of recurrence of hepatocellular carcinoma 30 months after transplantation.

Clinical Outcome of Patients After Biliary Complication in Duct-to-Duct Choledochocholedochostomy

Figure 2 shows clinical outcome of the patients with biliary complications in duct-to-duct reconstruction. In patients with biliary leakage, endoscopic retrograde nasobiliary drainage (ENBD) was indicated as an initial treatment. Four of the 10 patients with biliary leakage required conversion to Roux-en-Y (n = 3) or reoperation with duct-to-duct reconstruction (n = 1). One patient with a blood type incompatible graft died of sepsis 11 months after transplantation. Five of 10 patients were successfully treated with ENBD.

Six of the 51 biliary strictures (15.7%) were secondary to biliary leakage. Initially, anastomotic stricture was referred for endoscopic retrograde cholangiography (ERC). Thirteen of 51 patients (25.5%) could not receive endoscopic treatment because of the difficulty in accessing the papilla of Vater and the difficulty of passing a guidewire through the tight anastomotic stricture. All of them required PTCD. Consequently, 5 patients underwent revision surgery with Roux-en-Y reconstruction to repair the stricture. Two patients with tight anastomotic stricture were closely observed for a week with PTCD for anastomotic decompression, and were successfully treated with endoscopic retrograde biliary drainage (ERBD). Eight patients were treated with ERC balloon dilatation without placing inside stents. One patient died of sepsis secondary to chronic cholangitis 5 months after transplantation. The remaining 27 of 51 patients (52.9%) were treated by placing inside stents endoscopically above the sphincter of Oddi. One patient with a blood type incompatible graft underwent conversion to Roux-en-Y after ERBD because of acute cholangitis and hemobilia. As shown in Figure 2, 9 of 51 patients (17.6%) with duct-to-duct anastomotic stricture required surgical revision. The need for surgical revision due to biliary stricture tended to be lower in the duct-to-duct group compared with the Roux-en-Y group (50.0%), but this did not reach statistical significance ($P = 0.03$).

DISCUSSION

Right-lobe LDLT can provide an adequate graft size to compensate for the metabolic demands in most adult recipients, and the clinical outcome has improved in our series.[22] Among the controversies in right lobe LDLT, techniques of biliary reconstruction remain an open question. Right-sided liver has a higher incidence of vascular and biliary variants, this was explained by the relative consistency between the left umbilical vein and the liver. Multiple biliary orifices are encountered in 26.0 to 39.6% of the cases, which presents a further difficulty in reconstruction in right lobe LDLT.[16,24,30] For safe biliary reconstruction, precise evaluation of the biliary anatomy is essential.

The method for preoperative or intraoperative biliary duct evaluation remains a controversial topic for discussion. We have performed preoperative biliary duct evaluation with three-dimensional drip infusion cholangiographic computed tomography (CT) or magnetic resonance (MR) cholangiography in the evaluation of the potential donor. Although it provides adequate anatomic information of the biliary system, adaptation of these valuable methods for potential donor candidates is not always possible because of the risk of allergic reaction to contrast medium and the cost. In our experience, intraoperative cholangiography is an adequate and convenient way to evaluate the donor biliary tree.

The blood supply for biliary anastomosis is a major concern in LDLT. The arterial blood supply of the biliary system has been described by several investigators. A previous study using fine casts showed that 60% of the arterial

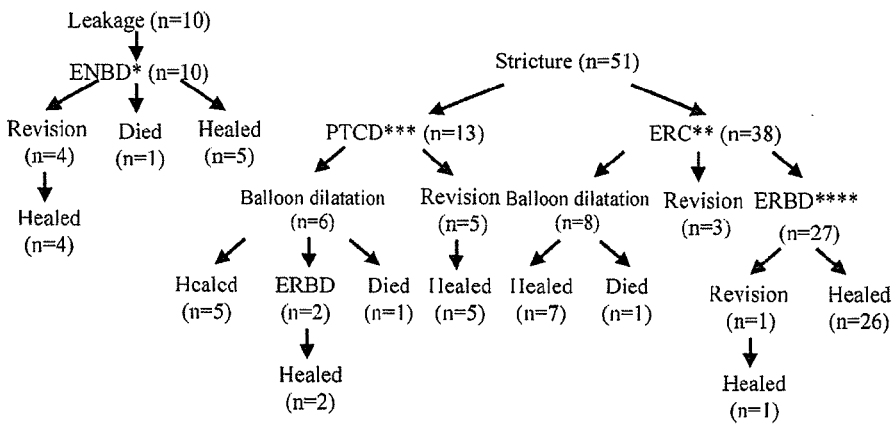

FIGURE 2. Summary of clinical outcome after biliary complications in duct-to-duct choledochocholedochostomy. ENBD, endoscopic nasobiliary drainage; ERC, endoscopic retrograde cholangiography; PTCD, percutaneous transhepatic cholangiography and drainage; ERBD, endoscopic retrograde biliary drainage.

supply for the bile duct comes from the caudal side through periduodenal arteries, 38% from the cranial side and only 2% from the hepatic artery itself. The 3 o'clock, 9 o'clock, and retroportal arteries give rise to multiple arteriolar branches, which form a free anastomosis within the wall of the bile duct.[26] In the absence of any attachments in transplanted liver recipients, the blood supply to the graft bile duct is derived solely from the hepatic artery. Histologic examination of disrupted duct-to-duct reconstruction often shows the loss of 3 o'clock and 9 o'clock intramural arteries on the recipient side (Fig. 3). Preservation of periductal microcirculation in the recipient duct and excellent hepatic artery reconstruction might be a key factor for successful duct-to-duct anastomosis.

Our current study confirmed that arterial complications, CMV infection, and blood type incompatibility were significant and important etiologic variables in biliary complications.[16,27] We do not use prophylactic administration of ganciclovir. However, the results of this study underline the importance of prophylaxis. In our LDLT program, a blood type incompatible graft was unavoidable in 12% of the recipients.[21] Despite the application of preoperative plasma exchange, splenectomy, and enhanced immunosuppression, the 5-year survival rate in adult patients was less than 50% and nearly 70% of the adult patients had biliary complications. We started the intrahepatic arterial immunosuppression protocol from December 2001 and the preconditioning regimen with anti-CD20 monoclonal antibody infusion from April 2004. Although it is still a tentative trial, these protocols have dramatically improved the outcome, with a 1-year graft survival rate of 85.7% and a biliary complication rate of 38.8%.[31]

There is still no consensus among transplant surgeons with regard to the type of biliary reconstruction in right lobe LDLT. Recently, the use of duct-to-duct reconstruction has been increasingly reported in LDLT.[12,14,16,32] We have reported our initial experience of 51 cases of duct-to-duct biliary reconstruction and concluded that it represents a useful technique for right lobe LDLT.[16] In July 1999, duct-to-duct reconstruction became the first choice for biliary reconstruction in our institution. In the series reported here, duct-to-duct technique had a lower incidence of biliary leakage. In cases of biliary leakage with duct-to-duct, peritoneal contamination from intestinal contents was minimized. In addition to the physiologic bilioenteric continuity and later good access by endoscopy, duct-to-duct reconstruction has an advantage over Roux-en-Y that the morbidity is reduced even when early anastomotic leakage occurs.

Biliary stricture was encountered in 26.6% of the patients with duct-to-duct reconstruction in this series, which was significantly higher than the Roux-en-Y group (4.7%). Although strictures seemed to develop more frequently in the duct-to-duct group, the requirement for surgical revision tended to be lower in that group. Because of easy access and imaging through endoscopy, 38 of 51 patients (74.5%) could be treated with ERC. Once ERBD was initiated, 26 of 27 patients (96.3%) were successfully treated. Recently, Gondolesi et al reported the largest Western experience with biliary complications in right lobe LDLT, and demonstrated that duct-to-duct reconstruction had higher incidence of stricture (31.7%) and lower incidence of leakage (16.3%), while the opposite was true following Roux-en-Y reconstruction (7.3% and 18.2%). Also, they recommended early and aggressive use of interventional treatment of biliary complications.[32] We agree with this suggestion that early interventional treatment could avoid further operative intervention. Endoscopic biliary intervention is useful for most anastomotic strictures. Unless the anastomotic site is completely necrotic, insertion of a long-term short stent is very effective in securing bile drainage without increased risk of ascending cholangitis.[17]

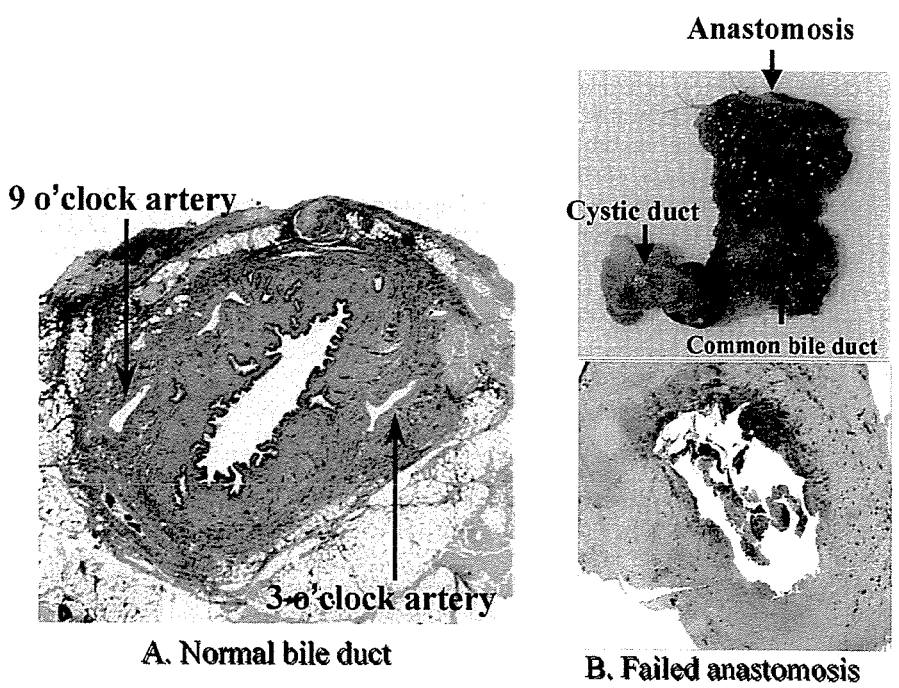

FIGURE 3. Histologic examination of the failed duct-to-duct choledocho-choledochostomy often shows the loss of the 3 and 9 o'clock arteries on the recipient side. Preservation of periductal microcirculation on the recipient side is a key factor for successful anastomosis. A, Normal common bile duct with patent 3 and 9 o'clock intramural arteries. B, Failed duct-to-duct choledochocholedochostomy with loss of 3 and 9 o'clock intramural arteries.

Variations in technical preference remain and modifications may be necessary to take account of anatomic variants in biliary reconstruction. Biliary complications seemed to develop more frequently in graft with multiple bile duct; however, this did not reach statistical significance in the present series. Duct-to-duct reconstruction is safely applied even in multiple bile duct reconstruction with plasty of the graft bile duct or with combined duct-to-duct and Roux-en-Y anastomosis. Contrary to an old concept, duct-to-duct reconstruction has been successfully performed even with the recipient cystic duct,[33] although the incidence of stricture in the cystic duct anastomosis was revealed to be high in our series (33.3%), and not just in a few left lobe grafts.[34]

With regard to biliary morbidity according to the reconstruction method used, we did not find any conclusive tendency to favor any mode or suture. The use of synthetic monofilament suture material was reported to be feasible for biliary reconstruction because of reduced tissue reaction by synthetic materials, as well as bacterial adherence.[35] The Paul Brousse group recommended nonabsorbable suture rather than absorbable material because the resorption of the latter might induce local inflammation and subsequent stenosis.[36] Trends remain in the Roux-en-Y group toward lower incidence of stricture in interrupted suture and lower incidence of leakage in continuous suture in the present study. Our current preference is the use of 6–0 or 7–0 nonabsorbable running suture at the posterior wall and interrupted suture at the anterior wall.

Stenting of the anastomosis is another topic for discussion in LDLT. The rationale of stent is the maintenance of biliary flow despite swelling of anastomosis and easy access for control cholangiography in case of suspected leakage or stricture.[37] The external stent tends to reduce biliary complication in the Roux-en-Y reconstruction, which was consistent with our previous series of 400 pediatric LDLTs.[27] Although our preliminary right lobe with duct-to-duct series demonstrated that the external stent significantly reduced the incidence of biliary stricture,[12,16] overall incidence of biliary stricture was considerably high (26.6%) in long-term follow-up. Scatton et al reported that employment of a T tube increased incidence of biliary complications and recommended the performance of duct-to-duct without a T tube in deceased liver transplantation.[38] The most frequent complication was leakage after T tube removal. We do not experience leakage after removal of 4-Fr biliary tube in the present series. To confirm this finding, while we formerly used a small stent tube, we ceased to use it from July 2004 and are monitoring the results.

CONCLUSION

Duct-to-duct biliary reconstruction in right lobe LDLT appears to be a feasible option with better endoscopic access for treating biliary stricture. Long-term observation may be necessary to collect sufficient data for the establishment of this treatment modality. It is hoped that increased experience and continuing refinements of the technique will lead to improved outcomes in right lobe LDLT.

REFERENCES

1. Sutcliffe R, Maguire D, Mroz A, et al. Bile duct stricture after adult liver transplantation: a role for biliary reconstructive surgery? *Liver Transplantation.* 2004;10:928–934.
2. Lerut J, Gordon RD, Iwatsuki S, et al. Biliary tract complications in human orthotopic liver transplantation. *Transplantation.* 1987;43:47–51.
3. Stratta RJ, Wood RP, Langnas AN, et al. Diagnosis and treatment of biliary tract complications after orthotopic liver transplantation. *Surgery.* 1989;106:675–683.
4. Chardot C, Candinas D, Miza D, et al. Biliary complications after liver transplantation: Birmingham's experience. *Transplant Int.* 1995;8:133–140.
5. Tung BY, Kimmery MB. Biliary complications of orthotopic liver transplantation. *Dig Dis.* 1999;17:133–144.
6. Colonna JO, Shaked A, Gomes AS, et al. Biliary strictures complicating liver transplantation: incidence, pathogenesis, management and outcome. *Ann Surg.* 1992;216:344–350.
7. Sanchez-Urdazpal L, Balls KP, Gores GJ, et al. Increased bile duct complications in liver transplantation across the ABO barrier. *Ann Surg.* 1993;218:152–158.
8. Abbasogla O, Levy MF, Vodapally MS, et al. Hepatic artery stenosis after liver transplantation: incidence, presentation, treatment and long-term outcome. *Transplantation.* 1997;63:250–255.
9. Halme L, Hockerstedt K, Lautenschlager I. Cytomegalovirus infection and development of biliary complications after liver transplantation. *Transplantation.* 2003;75:1853–1958.
10. Trotter JF, Wachs M, Everson GT, et al. Adult-to-adult transplantation of the right hepatic lobe from a living donor. *N Engl J Med.* 2002;346:1074–1082.
11. Qian YB, Liu CL, Lo CM, et al. Risk factors for biliary reconstructions after liver transplantation. *Arch Surg.* 2004;139:1101–1105.
12. Nakamura T, Tanaka K, Kiuchi T, et al. Anatomical variations and surgical strategies in right lobe living donor liver transplantation: lessons from 120 cases. *Transplantation.* 2002;73:1896–1903.
13. Marcos A, Fisher RA, Ham JM, et al. Right lobe living donor liver transplantation. *Transplantation.* 1999;68:798–803.
14. Dulundu E, Sugawara Y, Sano K, et al. Duct-to-duct biliary reconstruction in adult living-donor liver transplantation. *Transplantation.* 2004; 78:574–579.
15. Grewal HP, Shokouh-Amiri MH, Vera S, et al. Surgical technique for right lobe adult living donor liver transplantation without venovenous bypass or portocaval shunting and with duct-to-duct biliary reconstruction. *Ann Surg.* 2001;233:502–508.
16. Ishiko T, Egawa H, Kasahara M, et al. Duct-to-duct biliary reconstruction in living donor liver transplantation utilizing right lobe graft. *Ann Surg.* 2002;236:235–240.
17. Hisatsune H, Yazumi S, Egawa H, et al. Endoscopic management of biliary strictures after duct-to-duct biliary reconstruction in right-lobe living donor liver transplantation. *Transplantation.* 2003;76:810–815.
18. Malinchoc M, Kamath PS, Gordon FD, et al. A model to predict poor survival in patients undergoing transjugular intrahepatic portosystemic shunts. *Hepatology.* 2000;31:864–871.
19. Inomata Y, Tanaka K, Egawa H, et al. The evolution of immunosuppression with FK506 in pediatric living related liver transplantation. *Transplantation.* 1996;61:247–252.
20. Tanabe M, Shimazu M, Wakabayashi G, et al. Intraportal infusion therapy as a novel approach to adult ABO-incompatible liver transplantation. *Transplantation.* 2002;73:1959–1961.
21. Egawa H, Oike F, Buhler L, et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. *Transplantation.* 2004;77:403–411.
22. Inomata Y, Uemoto S, Asonuma K, et al. Right lobe graft in living donor liver transplantation. *Transplantation.* 2000;69:258–264.
23. Inomata Y, Egawa H. Surgical procedure for right lobectomy. In: Tanaka K, Inomata Y, eds.. *Living-Donor Liver Transplantation.* Barcelona: Prous Science, 2003:43–58.
24. Kasahara M, Egawa H, Ogawa K, et al. Variations in biliary anatomy associated with trifurcated portal vein in right lobe living donor liver transplantation. *Transplantation.* 2005;79:626–627.
25. Stapleton GN, Hickman R, Terblanche J. Blood supply of the right and left hepatic ducts. *Br J Surg.* 1998;85:202–207.
26. Northover JM, Terblanche J. A new look at the arterial supply of the bile duct in man and its surgical implications. *Br J Surg.* 1979;66:379–384.

27. Egawa H, Inomata Y, Uemoto S, et al. Biliary anastomotic complications in 400 living related liver transplantation. *World J Surg.* 2001;25: 1300–1307.
28. Kanazawa A, Kubo S, Tanaka H, et al. Bile leakage after living donor liver transplantation demonstrated with hepatobiliary scan using 99mTc-PMT. *Ann Nucl Med.* 2003;17:507–509.
29. Takao S, Matsuo Y, Shinchi H, et al. Magnetic compression anastomosis for benign obstruction of common bile duct. *Endoscopy.* 2001;33:988–990.
30. Ohkubo M, Nagino M, Kajima J, et al. Surgical anatomy of the bile ducts at the hepatic hilum as applied to living donor liver transplantation. *Ann Surg.* 2004;239:82–86.
31. Oike F, Egawa H, Kozaki K, et al. Dramatic improvement in the outcome of ABO-incompatible liver transplantation from living donor by hepatic arterial infusion therapy. *Am J Transplant.* 2004;8(suppl):293.
32. Gondolesi GE, Varotti G, Florman SS, et al. Biliary complications in 96 consecutive right lobe living donor transplant recipients. *Transplantation.* 2004;77:1842–1848.
33. Suh KS, Choi SH, Yi NJ, et al. Biliary reconstruction using the cystic duct in right lobe living donor liver transplantation. *J Am Coll Surg.* 2004;199:661–664.
34. Soejima Y, Shimada M, Suehiro T, et al. Feasibility of duct-to-duct biliary reconstruction in left-lobe adult-living-donor liver transplantation. *Transplantation.* 2003;75:557–559.
35. Wilson BJ, Marsh JW, Makowka L, et al. Biliary tract complications in orthotopic adult liver transplantation. *Am J Surg.* 1989;158:68–70.
36. Azoulay D, Hargreaves GM, Castaing D, et al. Duct-to-duct biliary anastomosis in living related liver transplantation: the Paul Brousse Technique. *Arch Surg.* 2001;136:1197–1200.
37. Settmacher U, Steinmuller TH, Schmidt SC, et al. Technique of bile duct reconstruction and management of biliary complications in right lobe living donor liver transplantation. *Clin Transplant.* 2003;17:37–42.
38. Scatton O, Meunier B, Cherqui D, et al. Randomized trial of choledochocholedochostomy with or without a T tube in orthotopic liver transplantation. *Ann Surg.* 2001;233:432–437.

ORIGINAL ARTICLE

Acute Humoral Rejection and C4d Immunostaining in ABO Blood Type-Incompatible Liver Transplantation

Hironori Haga,[1,2] Hiroto Egawa,[2] Yasuhiro Fujimoto,[2] Mikiko Ueda,[2] Aya Miyagawa-Hayashino,[1] Takaki Sakurai,[1] Tomoko Okuno,[1] Itsuko Koyanagi,[1] Yasutsugu Takada,[2] and Toshiaki Manabe[1]
[1]Laboratory of Anatomic Pathology and [2]Department of Transplantation and Immunology, Kyoto University, Kyoto, Japan

Complement C4d deposition in graft capillaries has been reported to be associated with antibody-mediated rejection in kidney and other solid organ transplantation. The correlation of C4d deposits and humoral rejection in liver transplants, however, is not well understood. We investigated the C4d immunostaining pattern in 34 patients whose liver biopsy was taken within the first 3 postoperative weeks for suspected acute rejection after ABO blood type-incompatible liver transplantation. The staining pattern was classified as positive (portal stromal staining), indeterminate (endothelial staining only), and negative (no staining). Positive C4d immunostaining was seen in 17 (50%) patients and was significantly associated with high (×64 or more) postoperative antidonor A/B antibody (immunoglobulin M (IgM)) titers (88 vs. 35%, $P = 0.002$) and poorer overall survival rate (41 vs. 88%, $P = 0.007$). Ten of 11 (91%) cases with histological acute humoral rejection (periportal edema and necrosis (PEN) or portal hemorrhagic edema) were positive for C4d, all of which showed high postoperative antibody titers. The other histologies associated with C4d positivity was purulent cholangitis (n = 4), coagulative hepatocyte necrosis (n = 1), acute cellular rejection (n = 1), and hepatocanalicular cholestasis (n = 1). Full clinical recovery was observed in only 6 of 17 (35%) C4d-positive patients, and tended to be associated with a lower rejection activity index (RAI). In conclusion, our study indicates that C4d deposits in the portal stroma can be a hallmark of acute humoral rejection in ABO-incompatible liver transplantation, and allograft damage can be reversible in a minority of cases. *Liver Transpl 12:457-464, 2006.* © 2006 AASLD.

Received June 29, 2005; accepted September 15, 2005.

See Editorial on Page 356

In ABO blood type-incompatible liver transplantation, acute humoral rejection triggered by antibodies against donor-type isoagglutinins is the most serious form of rejection and is often associated with graft loss. With the introduction of effective antihumoral rejection therapy such as the arterial/portal infusion of prostaglandin E1 and use of anti-CD20 monoclonal antibody, as well as preoperative plasma exchange, ABO-incompatible liver transplantation is becoming a choice to overcome the paucity of liver allografts from deceased donors in Japan.[1-4] Acute humoral rejection, however, is still a major problem in patients with ABO blood type-incompatible grafts and the evaluation of humoral rejection is necessary.

We previously reported that periportal edema and necrosis (PEN) could be histological indications of the early phase of severe humoral rejection.[5] In that report, all grafts with PEN resulted in massive parenchymal or biliary necrosis. Recent papers from other institutions, however, reported that histologically proven humoral rejection could be reversible.[3,6] Biopsies also demonstrated portal edema or hemorrhage, but no significant necrosis or endothelialitis has been documented. Portal hemorrhagic edema without significant necroinflammation may represent a milder degree of humoral rejection.[6]

To obtain the full histological spectrum of humoral

Abbreviations: Ig, immunoglobulin; PEN, periportal edema and necrosis; RAI, rejection activity index.

Supported in part by grants from the Japanese Ministry of Education, Culture, Sports, Science and Technology.

Address reprint requests to Hironori Haga, MD, Laboratory of Anatomic Pathology, Kyoto University Hospital, 54 Kawahara-cho, Shogoin Sakyo-ku, Kyoto 606-8507, Japan. Telephone: 81-75-751-3488; FAX: 81-75-751-3499; E-mail: haga@kuhp.kyoto-u.ac.jp

DOI 10.1002/lt.20652
Published online in Wiley InterScience (www.interscience.wiley.com).

rejection of ABO blood type-incompatible liver transplantation, we performed clinicopathological analysis with complement C4d immunostaining. C4d is a molecule that covalently binds to tissues after activation of the complement system, and immunostaining has been widely used to demonstrate humoral immunoreactivity or antibody-mediated rejection in other solid organ transplantations.[7-10] The aim of this study was to clarify the clinicopathological features of acute humoral rejection caused by antidonor ABO blood group antigens using C4d immunostaining.

MATERIALS AND METHODS

Patient Selection

Between January 1999 and December 2004, 82 patients (35 children and 47 adults) received primary ABO blood type-incompatible living donor liver transplantation. Informed consent for ABO blood type-incompatible liver transplantation was obtained from the recipient and donor, and the Ethical Committee of the Medical School of Kyoto University approved the surgery and treatment procedures. Among them, 35 patients (13 children and 21 adults) underwent liver biopsy within the first 3 postoperative weeks. One adult patient was excluded from this study because the paraffin-embedded tissue was not available. The other 34 patients were enrolled in this study. As of April 2005, the clinical follow-up period for these patients ranged from 0.4 to 74 months (median, 16 months).

Antirejection Regimen and Clinical Monitoring

Baseline antihumoral rejection therapies included intravenous steroids and oral tacrolimus. Most adult patients underwent continuous prostaglandin E1 infusion via an intra-arterial or intravenous catheter for 2 to 3 weeks posttransplantation.[1,11]

Splenectomy was performed for adult patients whose transplantation was performed between May 1999 and January 2003. To avoid splenectomy-related portal vein thrombosis, the administration of preoperative rituximab (anti-CD 20 monoclonal antibody) without splenectomy was introduced after April 2004.[11] In addition, 2 adult patients were given postoperative rituximab as a therapy for humoral rejection.

Patients underwent preoperative plasmapheresis or blood exchange in order to reduce antidonor blood group A/B antibody (immunoglobulin M (IgM) and IgG) titers to ×8 or lower. A microhemagglutination assay was used to monitor the serum levels of antidonor ABO blood group antibodies at least 3 times per week during the first postoperative month. Patients were assigned to high-titer groups if the peak of the postoperative antidonor blood group IgM titers was ×64 or more, and to the low-titer group if it was less.[5] In this study, there were 21 patients with a high titer and 13 with a low titer. Postoperative plasmapheresis was performed when severe humoral rejection was suggested clinically or histologically. Elevation of postoperative antidonor antibody titer not associated with liver dysfunction was treated by steroid bolus therapy or was managed with watchful observation alone.

Hepatic necrosis was diagnosed by enhanced computed tomography. The findings were diffusely spreading low-density lesions in the graft. Intrahepatic biliary complications were diagnosed by cholangiography, showing irregularity and beading throughout the intrahepatic biliary tree.

Pretransplant T-cell cross-match tests were performed as previously described.[12] The test was interpreted as positive when 41% or more of donor lymphocytes were killed, weakly positive when 21 to 40% of donor lymphocytes were killed, and negative when no more than 20% of donor lymphocytes were killed. Cross-match tests were negative in 33 of 34 patients. One patient was interpreted as weakly positive.

Histological Diagnosis

Pathological diagnosis was made on a routine basis by 3 pathologists (H.H., T.S., and A.M.), with no information about immunostaining of immunoglobulins or complements, including C4d. The minimal quantitative requirement for the diagnosis of rejection was a biopsy containing at least 5 portal areas. Acute cellular rejection was diagnosed using Banff criteria.[13] Histological acute humoral rejection was suspected when PEN or portal hemorrhagic edema was associated with the elevation of antidonor A/B antibody titers.[5,6] In each case of acute cellular rejection and acute humoral rejection, portal inflammation, bile duct damage, and venular endothelialitis were evaluated separately with the rejection activity index (RAI) of Banff criteria.[13] To apply the RAI to humoral rejection, we regarded any leukocyte infiltration as portal inflammation and gave a score of 3 on periportal coagulative necrosis as part of portal inflammation.

C4d Immunostaining and Control Materials

Eighteen-gauge liver core tissue biopsies were placed in 10% buffered formalin from several hours to overnight, processed routinely, and sliced into 3-μm paraffin sections. Staining methods for routine histological evaluation included hematoxylin and eosin, Masson trichrome, and immunostaining of cytokeratin 7 (OV-TL 12/30, Dako, Glostrup, Denmark; dilution, 1:200). The polyclonal antibody against C4d complement (BI-RC4D, Biomedica, Vienna, Austria; 1:50) was used for immunostaining with an automated immunostainer (BENCHMARK XT, Ventana, Tucson, AZ). For antigen retrieval, deparaffinized and rehydrated sections were treated with protease I (Ventana; 0.5 units/mL) at 37°C for 20 minutes.

We used lymphoid tissue with follicular hyperplasia as a positive control for C4d staining.[14] The reticular staining pattern in the germinal centers was confirmed in every C4d immunostaining. Ten wedge biopsies of the liver allografts taken during graft resection (time 0

biopsy) were used as negative controls. For comparison of ABO blood type-incompatible and non-ABO blood type-incompatible cases, 10 needle biopsies with typical acute cellular rejection from ABO-identical transplants were assessed for C4d immunostaining.

Evaluation of C4d Immunostaining

Three pathologists (H.H., T.S., and A.M.) independently evaluated the C4d immunostaining slides of the initial biopsies. The identification numbers were removed from the slides and the pathologists were not given any clinical information. C4d staining was semiquantitatively evaluated in terms of the percentage of portal tracts containing distinctly stained stroma and/or endothelium. Biopsies containing 50% or more stromal-positive portal tracts were evaluated as positive. Specimens with less than 50% stromal-positive portal tracts or positive staining only in the vascular endothelium or sinusoids were classified as indeterminate. Completely negative staining was evaluated as negative. Extraportal endothelial staining was recorded but not graded. Any staining in the liver capsule or extraliver tissue was omitted to evaluate.

A couple of discordant cases were classified as indeterminate, and we did not perform additional C4d immunostaining because of the small size of the specimens. Immunostaining of the follow-up biopsies of C4d-positive cases (n = 21) and the second biopsies of C4d-negative cases (n = 17) was evaluated with clinical data and previous biopsies.

Statistical Analysis

Statistical significance of differences among the groups was assessed by Fisher's exact test. Laboratory data were analyzed using Mann-Whitney's U test. Patient survival was determined by Kaplan-Meier analysis, and differences in survival were analyzed with a log-rank test. For all analyses, P values of less than 0.05 were taken as significant.

RESULTS

C4d Immunostaining and Clinical Outcomes

Five of 10 time 0 allograft biopsies obtained at liver transplantation showed focal endothelial or stromal C4d staining. These positive stainings did not exceed 10% of the number of the portal tracts and were classified as indeterminate. The other 5 time 0 biopsies were negative for C4d. In ABO blood type-identical specimens with a diagnosis of acute rejection, 1 specimen showed strong and diffuse portal stromal staining (positive), 6 showed focal or diffuse endothelial staining (indeterminate), and 3 negative. Distinct C4d staining was seen in the perivenular areas in the C4d-positive case.

Among 34 initial biopsies from ABO blood type-incompatible transplants, 17 cases (50%) were positive for C4d in the portal stroma, 8 (24%) were indeterminate, and 9 (26%) were negative. Representative staining patterns are shown in Figure 1. Preoperative clinical features were not significantly different between the groups (Table 1). However, high-postoperative antidonor antibody titer (×64 or more) was more frequently seen in C4d-positive patients than in C4d-indeterminate and -negative ones (Table 1). When the C4d-indeterminate group was regarded as negative for C4d, C4d positivity was significantly associated with high titers (88 vs. 35%; $P = 0.002$). Hepatic necrosis demonstrated by computed tomography was seen only in the C4d-positive patients (24 vs. 0%; $P = 0.052$). Patients who underwent postoperative plasma exchange were statistically more frequently seen in C4d-positive patients than in C4d-indeterminate and -negative patients (53 vs. 6%; $P = 0.003$). The timing of postoperative plasma exchange was clinical humoral rejection, which occurred within 14 postoperative days. One to 6 (median, 3.5) courses of plasma exchange were performed, and 4 of 10 (40%) grafts recovered.

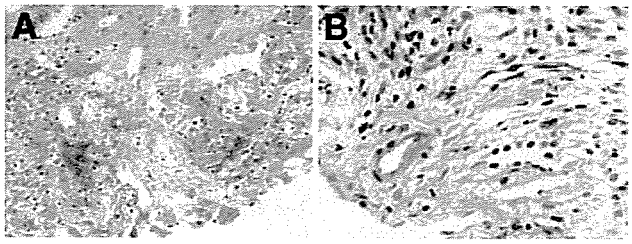

Figure 1. (A) C4d-positive staining. C4d deposited in the periportal area. Surrounding parenchyma showed coagulative necrosis (original magnification, ×200). (B) Indeterminate endothelial staining in the small artery and capillaries (×400).

Overall, patient survival was significantly worse for C4d-positive than for C4d-indeterminate and -negative patients (41 vs. 88%; $P = 0.007$; Fig. 2A). The high-titer group (n = 21) also showed poorer overall survival than the low-titer group (n = 13), which was not significant in this study (56 vs. 83%; $P = 0.072$; Fig. 2B).

C4d Immunostaining and Histology

Findings of initial biopsies are summarized in Table 2. In C4d-positive patients, the most common histology at initial biopsy was PEN/portal hemorrhagic edema. Ten of 11 cases (91%) with PEN/portal hemorrhagic edema showed C4d stromal deposition. The median postoperative day of C4d-positive PEN cases was day 7 (Table 3).

The other histology in C4d-positive cases included purulent cholangitis (n = 4; median posttransplant day 18), coagulative hepatocyte necrosis (n = 1; posttransplant day 17; clinically hepatic artery thrombosis), moderate acute cellular rejection (n = 1; posttransplant day 15), and hepatocanalicular cholestasis (n = 1; posttransplant day 13).

In C4d-indeterminate and -negative patients, the most common histology was hepatocanalicular cholestasis (n = 5; median posttransplant day 13), followed by purulent cholangitis (n = 4; median posttransplant day 18), mild lobular inflammation (n = 4; median posttransplant day 16), mild to moderate acute cellular rejection (n = 3; median posttransplant day 12), and PEN (n = 1; posttransplant day 10; RAI = 6).

TABLE 1. C4d Immunostaining Pattern and Clinical Features

C4d immunostaining	Positive (n = 17)	Indeterminate / negative (n = 17)	
		Indeterminate (n = 8)	Negative (n = 9)
Female: Male	13:4 (76%: 24%)	6:2 (75%: 25%)	6:3 (67%: 33%)
Age group			
<3 yr	3 (18%)	3 (38%)	4 (44%)
3–50 yr	10 (60%)	1 (13%)	2 (22%)
>50 yr	4 (24%)	4 (50%)	3 (33%)
Original diseases			
Chronic liver diseases	17 (100%)	6 (75%)	7 (78%)
Fulminant liver failure	0 (0%)	2 (25%)	2 (22%)
Mean HLA-A, B, DR mismatches	2.9	2.8	2.8
Preoperative treatment			
Splenectomy	4 (24%)	2 (25%)	3 (33%)
Rituximab	6 (35%)*	2 (25%)	1 (11%)
Peak of postoperative antibody titer (IgM)			
High-titer cases (x64 or more)	15 (88%)	3 (38%)	3 (30%)
Median (range)	x256 (x8–x8,192)	x12 (x1–x512)	x8 (x2–x1,024)
Mean postoperative day (range)	7 (4–13)	6 (1–10)	5 (1–18)
Radiological findings			
Hepatic necrosis	4 (24%)	0 (0%)	0 (0%)
Intrahepatic biliary complications	1 (6%)	0 (0%)	2 (22%)
Postoperative plasma exchange	9 (53%)	0 (0%)	1 (11%)
Overall patient survival	41%	100%	78%

*Used postoperatively in 2 cases.

PEN/portal hemorrhagic edema was statistically more frequently observed in C4d-positive patients than in non-C4d-positive patients (59 vs. 6%; $P = 0.0012$). Levels of serum transaminases tended to be higher in C4d-positive patients but did not reach statistical significance. Total bilirubin level was significantly higher in C4d-positive patients ($P = 0.009$).

PEN/Portal Hemorrhagic Edema Cases

Among 11 PEN/portal hemorrhagic edema cases, the histology of 5 patients (cases 3, 5, 12, 15, and 1 C4d-negative case) was PEN (Table 3). PEN included portal hemorrhage, mild to moderate portal neutrophilic infiltration, perivenular endothelialitis without significant perivenular necrosis, and focal periportal necrosis (Fig. 3A). The portal stroma adjacent to the parenchyma and peribiliary stroma were positive for C4d (Fig. 3B). The terminal hepatic venules showed occasional faint positivity, but the sinusoids in zones 2 and 3 were negative for C4d. Cholangitis with mild to moderate neutrophilic infiltration was seen in all cases, but ductopenia or duct atrophy was not observed with CK7 immunostaining. The RAI score of PEN cases was 6 or 7, and all patients with PEN resulted in graft failure or severe graft damage.

The other 6 patients showed portal hemorrhagic edema (cases 8, 11, 13, 14, 16, and 17). Cellular infiltration was mild (Fig. 3C), but all revealed a C4d staining pattern identical to that of PEN (Fig. 3D). Some cases had perivenular C4d deposition, but perivenular inflammation was none or minimal. The RAI of portal hemorrhagic edema was 4 or less (Table 3). Only 1 patient resulted in graft failure (case 14; RAI = 2).

Follow-up Biopsies

Among 17 C4d-positive patients, 5 patients (3 with PEN, 1 with periportal hemorrhagic edema, and 1 with cholangitis) resulted in rapid graft failure and follow-up biopsies were not available (cases 1, 3, 12, 14, and 15; Fig. 4).

In the other 12 C4d-positive patients, at least 1 follow-up biopsy was available at various times. Five patients (cases 2, 4, 10, 13, and 16) were negative for C4d at the last follow-up biopsy (range, 34-792 posttransplant days). The other 7 showed positive C4d immunostaining in the last follow-up biopsy (range, 52-508 posttransplant days). Six patients (case 4 with acute cellular rejection and cases 8, 11, 13, 16, and 17 with portal hemorrhagic edema) were clinically free of symptoms and achieved normalization of the levels of serum transaminases and bilirubin as of April 2005. Among them, 3 (cases 8, 11, and 17) were positive for C4d at the last biopsy (posttransplant days 77, 336, and 209, respectively).

Any second biopsy from patients whose initial biopsies were indeterminate or negative for C4d did not reveal C4d positivity in the portal stroma (median posttransplant day 31; range, 19 - 603).

Cases with Stromal C4d Immunostaining without Elevation of the Antidonor Antibody (IgM) Titers

Cases 6 and 9 showed C4d stromal positivity, but their postoperative titer of antidonor IgM antibody remained low. In case 6, the peak antidonor IgM titer was ×16, but the antidonor IgG titer was elevated up to ×256 and

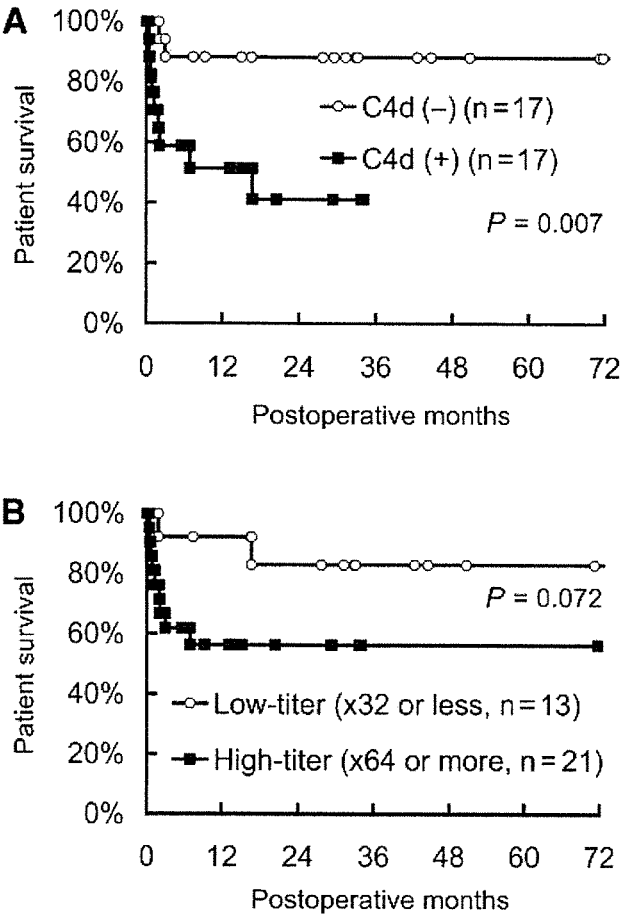

Figure 2. Survival curve in relation to (A) C4d immunostaining and (B) postoperative peak titers of antidonor A/B antibodies. Indeterminate C4d staining was regarded as negative.

lasted for 4 days. Every biopsy of case 6 showed purulent cholangitis, and subsequent computed tomography revealed hepatic necrosis. The patient died of chronic cholangitis.

Case 9 was a patient with multivisceral transplantation from 2 different donors; the liver transplantation was ABO blood type incompatible (AB to B), while the small intestine graft was ABO blood type compatible (blood type O). The postoperative peak antidonor IgM titer was low (×8) and the antidonor IgG was not detected. However, preoperative cross-match tests were interpreted as weakly positive against both donors. Liver biopsies revealed purulent cholangitis with ductular cholestasis, which was attributed to sepsis (Fig. 5A). C4d staining was positive (Fig. 5B). The ABO blood type-compatible small intestine graft showed severe acute rejection with microthrombi, and C4d deposits were observed in the endothelium (Fig. 5C and D). The patient died on posttransplant day 58. Necropsy revealed severe mucosal hemorrhage in the small intestine and monoclonal posttransplant lymphoproliferative disorder. Preoperative blood exchange and induction therapy with basiliximab were used for this patient, but no postoperative cross-match test was performed.

DISCUSSION

In ABO blood type-incompatible liver transplantation, preoperative plasma exchange was performed to lower the titers of antidonor blood group A/B antigen antibodies. The onset of acute humoral rejection is thus suspected when liver dysfunction occurs along with the elevation of titers of those antibodies. Liver biopsies soon after titer elevation usually demonstrated characteristic features such as portal edema, hemorrhage, and periportal necrosis. In some cases, however, elevation of the antidonor antibody titers was not associated with graft dysfunction, or late graft dysfunction occurred even when patients did not show significant titer elevation. In such cases, histological demonstration of deposition of immunoglobulin or complements in the allografts is important to demonstrate humoral reactivity and to decide patients' treatment.[12,15,16] Immunostaining of IgM or other complements such as C3c, however, was technically difficult in paraffin-embedded formalin-fixed liver tissue, and we were unable to make reproducible results.[4,5] Moreover, severe tissue damage other than humoral rejection can cause immunoglobulin and complement deposition.[12,17] Interpretation of the staining results can be difficult if you do not routinely perform immunofluorescent study of the allograft liver. On the other hand, C4d immunoperoxidase staining was relatively easy to perform, and the results were reliable since positive and negative controls were readily available.

Still, there have been few data about the significance of antibody-mediated rejection in clinical liver transplantation, and the criteria of histological diagnosis and therapeutic options remain unclear. Recently, a couple of studies demonstrated C4d deposition in liver allografts with acute rejection using paraffin-embedded sections, although the presence of alloreactive antibodies was not clearly stated.[18,19] Instead, they suggested that the local B-cell response might be specifically involved in C4d deposition in human liver allograft rejection. In their reports, C4d deposited in the pericapillary or periportal areas of the portal tracts, and the staining patterns were nearly identical to those of our cases. These staining patterns denoted that periportal areas may be the main targets of both humoral and acute cellular rejection, and that lobular deposition of immunoglobulin and complement may be a secondary change. In ABO blood type-incompatible transplantation, the findings may be compatible with the observation that capillaries in the portal tracts are the primary sites of ABH blood type antigen expression in the liver allografts.[20] The staining pattern may also support that periportal necrosis seen in experimental and clinical rejection can be a diagnostic histological feature of humoral rejection.[5,21]

On the other hand, the significance of C4d staining only in the endothelium was not clear in this study. Since the endothelial staining pattern was reproduc-

TABLE 2. C4d Immunostaining and Initial Biopsy Within the First 3 Postoperative Weeks

C4d immunostaining	Positive (n = 17)	Negative / indeterminate (n = 17)	*P* value
Mean postoperative day (range)	8 (5–19)	13 (5–20)	0.052
Histology			
PEN / portal hemorrhagic edema	10 (59%)	1 (6%)	0.0012
Cholangitis	4 (24%)	4 (24%)	n.s.
Cholestasis, hepatocanalicular	1 (6%)	5 (29%)	n.s.
Acute cellular rejection	1 (6%)	3 (18%)	n.s.
Coagulative hepatocyte necrosis	1 (6%)	0 (0%)	n.s.
Mild lobular inflammation	0 (0%)	4 (24%)	n.s.
Laboratory data at biopsy (median ± S.D.)			
AST (IU/L)	222 ± 305	101 ± 51	0.32
ALT (IU/L)	357 ± 388	179 ± 151	0.19
Total bilirubin (mg/dL)	11.8 ± 9.7	5.2 ± 5.6	0.009

Abbreviation: n.s., not significant.

TABLE 3. C4d-Positive Patients

Case	Age/gender	ABO (R/D)	Titer peak	Initial biopsy (POD)	Follow-up biopsy* and C4d status (POD)
1	46/F	A/AB	x8192	Cholangitis (17)	Not available
2	48/M	O/A	x128	Cholestasis (13)	Cholangitis, C4d (–) (34)
3	32/F	O/AB	x512	PEN, RAI = 6 (7)	Not available
4	1/F	B/A	x256	ACR, RAI = 6 (15)	No remarkable change, C4d (–) (792)
5	3/F	O/B	x2,048	PEN, RAI = 7 (8)	Cholangitis, C4d (+) (508)
6	55/F	O/B	x16	Cholangitis (16)	Cholangitis, C4d (+) (230)
7	11mo/M	B/AB	x128	Coagulative necrosis (17)	No remarkable change, C4d (+) (387)
8	13/M	B/A	x128	PHE, RAI = 2 (5)	No remarkable change, C4d (+) (209)
9†	1/F	B/A	x8	Cholangitis (19)	Cholangitis, C4d (+) (52)
10	56/F	B/A	x512	Cholangitis (6)	Cholangitis, C4d (–) (129)
11	41/F	A/B	x2,048	PHE, RAI – 1 (6)	Recurrent PBC or ACR, RAI – 6, C4d (+) (336)
12	50/M	O/A	x2,048	PEN, RAI = 7 (8)	Not available
13	33/F	A/AB	x256	PHE, RAI = 4 (7)	No remarkable change, C4d (–) (421)
14	29/F	O/B	x2,048	PHE, RAI = 2 (6)	Not available
15	16/F	O/A	x256	PEN, RAI = 6 (13)	Not available
16	56/F	A/AB	x1,024	PHE, RAI = 2 (6)	ACR, RAI = 5, C4d (–) (80)
17	39/F	O/AB	x256	PHE, RAI – 4 (6)	Cholangitis, C4d (+) (77)

Abbreviations: ACR, acute cellular rejection; PBC, primary biliary cirrhosis; PEN, periportal edema and necrosis; PHE, portal hemorrhagic edema; POD, postoperative day; RAI, rejection activity index (the Banff schema).
*In cases where multiple follow-up biopsies were available, the last follow-up histology is shown.
†Multivisceral transplantation case (liver and small intestine).

ible, and was common in acute cellular rejection of ABO blood type-identical cases, we speculated that it represented mild humoral reaction of the grafts. Neither patient survival nor severe liver damage, however, was associated with the endothelial staining pattern.

We were able to demonstrate in this study that C4d deposition in the portal stroma was correlated with poor patient survival and characteristic histology, as well as with postoperative titers of ABO blood type alloantibodies. Conversely, PEN/portal hemorrhagic edema showed a high percentage of C4d positivity and was probed as a useful histological feature in establishing the diagnosis of acute humoral rejection. The histology of PEN/portal hemorrhagic edema was transient, and there was a spontaneous decrease of ABO blood type alloantibody titers in the first postoperative month, but some follow-up biopsies more than a year after transplantation remained positive for C4d. This suggests that nonspecific histology such as cholangitis or coagulative necrosis can be regarded as a result of humoral rejection when C4d was detected in the allografts. For example, case 7, an 11-month-old boy, was the youngest patient with humoral rejection in this study, while hepatic artery thrombosis was a clinical cause of hepatocyte necrosis.

C4d staining was also correlated with radiological findings. It is known that there are 2 major complications associated with ABO blood type-incompatible liver transplantation.[4,15] One is massive hepatocyte necrosis occurring within the first 2 weeks, which is re-

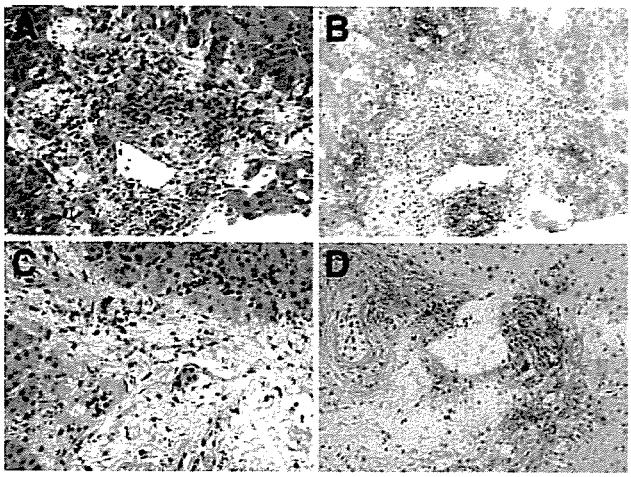

Figure 3. (A) Typical PEN (RAI = 7) and (B) C4d staining mainly in the areas of periportal edema. (C) Portal hemorrhagic edema (RAI = 2) and (D) C4d staining.

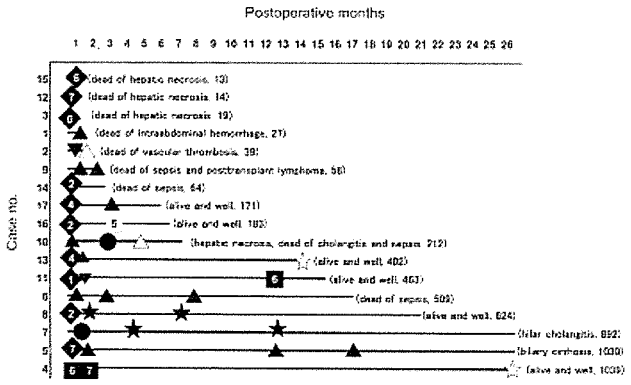

Figure 4. Histology changes in patients with C4d positivity. The histology of each case is marked on a horizontal line, the length of which denotes the follow-up period. Final patient status and postoperative day of the last follow-up are shown in parentheses. Number represents the RAI. Closed diamond (♦), PEN/portal hemorrhagic edema; closed triangle (▲/▼), cholangitis/cholestasis with C4d staining; open triangle (△), cholangitis without C4d staining; closed circle (●), coagulative parenchymal necrosis with C4d staining; closed square (■), acute cellular rejection with C4d; open square (□), acute cellular rejection without C4d; closed star (★), no remarkable finding with C4d; open star (☆), no remarkable finding without C4d.

fractory to postoperative therapy and fatal. The other is intrahepatic biliary complication that is not often associated with early liver graft dysfunction, but becomes evident several months after liver transplantation. In this study, hepatocyte necrosis found by computed tomography was associated with only C4d positivity and 3 of 4 patients revealed PEN, while no hepatocyte necrosis was found in C4d-indeterminate and -negative patients. This suggests that hepatocyte necrosis is a result of severe humoral rejection. Intrahepatic biliary complications, on the other hand, occurred in both C4d-positive and -negative patients. Since the focus of this study was concentrated on the patients showing

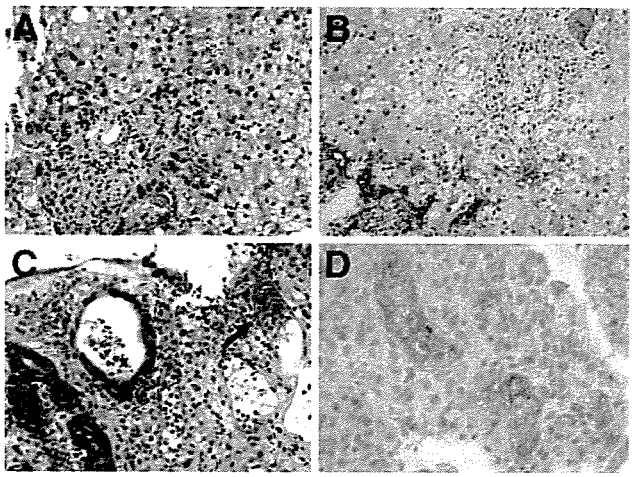

Figure 5. Multivisceral transplantation case without significant elevation of ABO alloantibodies (case 9). (A) Cholangitis with ductular cholestasis and mild portal fibrosis (×200; postoperative day 19). Clinically no biliary complications were noted and the original diagnosis was septic cholangitis. (B) C4d immunostaining revealed periportal staining (×200). (C) Small intestine allograft showing crypt apoptosis and a thrombus (arrow) (day 33; ×200). (D) The capillaries in the intestinal graft mucosa were positive for C4d (×400).

early graft dysfunction and receiving biopsy within the first 3 postoperative weeks, we think that a study based on protocol biopsy is necessary to elucidate late-onset biliary complications associated with ABO blood type-incompatible transplantation.

Once humoral rejection was defined by both morphology and C4d deposition, the RAI of the Banff schema with minor modification reliably predicted the outcome of humoral rejection. PEN corresponded to acute humoral rejection with a RAI of 6 or 7 and was associated with graft failure. On the other hand, portal hemorrhagic edema had minimal inflammatory cell infiltrate and an RAI score of 4 or less, and was related to better prognosis and complete recovery in some cases. The results suggested that the Banff schema could be useful for the evaluation of both acute cellular and acute humoral rejection. It must be kept in mind, however, that the prognosis of acute humoral rejection was much poorer than acute cellular rejection, and that even acute humoral rejection with low RAI required treatment, including repeated plasma exchange, to rescue the patients.

C4d stromal deposition in the liver allograft was demonstrated without elevation of the ABO blood type alloantibody titer in case 9. Since this patient showed a weakly positive T-cell cytotoxic cross-match and severe rejection in the ABO blood type-compatible small intestine graft, this humoral response might be mediated by lymphocytotoxic antibodies.[22,23]

We conclude from this study that the immunohistochemical detection of C4d has both diagnostic and prognostic value and could be a hallmark of antibody-mediated rejection in liver biopsy. In combination with conventional histological criteria, the demonstration of

C4d in the portal stroma may be useful to determine the indication of postoperative plasma exchange and to assess the current and future protocols for ABO blood type-incompatible liver transplantation. Further studies are needed to clarify the significance of C4d and other markers of humoral immunity in ABO blood type-matched liver transplantation.

ACKNOWLEDGMENTS

We thank Dr. Daisuke Morioka (Department of Gastroenterological Surgery, Yokohama City University Graduate School of Medicine) for his useful advice about the histology and treatment of the patients.

REFERENCES

1. Tanabe M, Shimazu M, Wakabayashi G, Hoshino K, Kawachi S, Kadomura T, et al. Intraportal infusion therapy as a novel approach to adult ABO-incompatible liver transplantation. Transplantation 2002;73:1959-1961.
2. Sekido H, Matsuo K, Takeda K, Morioka D, Kubota T, Tanaka K, et al. Successful adult ABO-incompatible liver transplantation: therapeutic strategy for thrombotic microangiopathy is the key to success. Transplantation 2003;75:1605-1607.
3. Usuda M, Fujimori K, Koyamada N, Fukumori T, Sekiguchi S, Kawagishi N, et al. Successful use of anti-CD20 monoclonal antibody (rituximab) for ABO-incompatible living-related liver transplantation. Transplantation 2005; 79:12-16.
4. Egawa H, Oike F, Buhler L, Shapiro AM, Minamiguchi S, Haga H, et al. Impact of recipient age on outcome of ABO-incompatible living-donor liver transplantation. Transplantation 2004;77:403-411.
5. Haga H, Egawa H, Shirase T, Miyagawa A, Sakurai T, Minamiguchi S, et al. Periportal edema and necrosis as diagnostic histological features of early humoral rejection in ABO-incompatible liver transplantation. Liver Transpl 2004;10:16-27.
6. Morioka D, Sekido H, Kubota K, Sugita M, Tanaka K, Togo S, et al. Antibody-mediated rejection after adult ABO-incompatible liver transplantation remedied by gammaglobulin bolus infusion combined with plasmapheresis. Transplantation 2004;78:1225-1228.
7. Collins AB, Schneeberger EE, Pascual MA, Saidman SL, Williams WW, Tolkoff-Rubin N, et al. Complement activation in acute humoral renal allograft rejection: diagnostic significance of C4d deposits in peritubular capillaries. J Am Soc Nephrol 1999;10:2208-2214.
8. Behr TM, Feucht HE, Richter K, Reiter C, Spes CH, Pongratz D, et al. Detection of humoral rejection in human cardiac allografts by assessing the capillary deposition of complement fragment C4d in endomyocardial biopsies. J Heart Lung Transplant 1999;18:904-912.
9. Magro CM, Pope Harman A, Klinger D, Orosz C, Adams P, Waldman J, et al. Use of C4d as a diagnostic adjunct in lung allograft biopsies. Am J Transplant 2003;3:1143-1154.
10. Ruiz P, Garcia M, Pappas P, Berney T, Esquenazi V, Kato T, et al. Mucosal vascular alterations in isolated small-bowel allografts: relationship to humoral sensitization. Am J Transplant 2003;3:43-49.
11. Yoshizawa A, Sakamoto S, Ogawa K, Kasahara M, Uryuhara K, Oike F, et al. New protocol of immunosuppression for liver transplantation across ABO barrier: the use of rituximab, hepatic arterial infusion, and preservation of spleen. Transplant Proc 2005;37:1718-1719.
12. Demetris AJ, Nakamura K, Yagihashi A, Iwaki Y, Takaya S, Hartman GG, et al. A clinicopathological study of human liver allograft recipients harboring preformed IgG lymphocytotoxic antibodies. Hepatology 1992;16:671-681.
13. International Panel. Banff schema for grading liver allograft rejection: an international consensus document. Hepatology 1997;25:658-663.
14. Zwirner J, Felber E, Schmidt P, Riethmuller G, Feucht HE. Complement activation in human lymphoid germinal centres. Immunology 1989;66:270-277.
15. Gugenheim J, Samuel D, Reynes M, Bismuth H. Liver transplantation across ABO blood group barriers. Lancet 1990;336:519-523.
16. Farges O, Kalil AN, Samuel D, Saliba F, Arulnaden JL, Debat P, et al. The use of ABO-incompatible grafts in liver transplantation: a life-saving procedure in highly selected patients. Transplantation 1995;59:1124-1133.
17. Straatsburg IH, Boermeester MA, Wolbink GJ, van Gulik TM, Gouma DJ, Frederiks WM, Hack CE. Complement activation induced by ischemia-reperfusion in humans: a study in patients undergoing partial hepatectomy. J Hepatol 2000;32:783-791.
18. Krukemeyer MG, Moeller J, Morawietz L, Rudolph B, Neumann U, Theruvath T, et al. Description of B lymphocytes and plasma cells, complement, and chemokines/receptors in acute liver allograft rejection. Transplantation 2004;78:65-70.
19. Moeller J, Krukemeyer MG, Morawietz L, Schmeding M, Dankof A, Neumann U, et al. Molecular case report: IgVH analysis in acute humoral and cellular liver allograft rejection suggests a selected accumulation of effector B cells and plasma cells. Virchows Arch 2005;446:325-332.
20. Tanaka Y, Haga H, Egawa H, Okuno T, Miyagawa-Hayashino A, Tsuruyama T, et al. Intragraft expression of recipient-type ABO blood group antigens: long-term follow-up and histological features after liver transplantation. Liver Transpl 2005;11:547-554.
21. Nakamura K, Murase N, Becich MJ, Furuya T, Todo S, Fung JJ, et al. Liver allograft rejection in sensitized recipients. Observations in a clinically relevant small animal model. Am J Pathol 1993;142:1383-1391.
22. Takaya S, Bronsther O, Iwaki Y, Nakamura K, Abu-Elmagd K, Yagihashi A, et al. The adverse impact on liver transplantation of using positive cytotoxic crossmatch donors. Transplantation 1992;53:400-406.
23. Wu T, Abu-Elmagd K, Bond G, Demetris AJ. A clinicopathologic study of isolated intestinal allografts with preformed IgG lymphocytotoxic antibodies. Hum Pathol 2004;35: 1332-1339.

LIVER TRANSPLANTATION 12:912-919, 2006

ORIGINAL ARTICLE

Living Donor Liver Transplantation as a Second-Line Therapeutic Strategy for Patients With Hepatocellular Carcinoma

Yasutsugu Takada,[1] Mikiko Ueda,[1] Takashi Ito,[1] Seisuke Sakamoto,[1] Hironori Haga,[2] Yoji Maetani,[3] Kohei Ogawa,[1] Mureo Kasahara,[1] Fumitaka Oike,[1] Hiroto Egawa,[1] and Koichi Tanaka[1]

[1]Department of Transplantation and Immunology, [2]Department of Pathology, and [3]Department of Radiology, Kyoto University, Kyoto, Japan

Living donor liver transplantation (LDLT) has evolved to represent an important surgical strategy for patients with hepatocellular carcinoma (HCC). However, due to disadvantages, including donor risks and higher rates of perioperative complications, LDLT has been considered as a second-line treatment in Japan. The present study retrospectively evaluated clinical outcomes for 93 patients with HCC who underwent LDLT at our institute, including 44 patients who exceeded Milan criteria (MC). A total of 73 patients (78%) displayed a history of previous treatment for HCC using various nontransplant methods. Median follow-up was 24 months (range, 1-76 months). At 4 years after LDLT, overall patient survival rate was 64%, with similar rates for within-MC and over-MC groups (68% vs. 59%, respectively; $P = 0.6548$). However, cumulative recurrence rate was significantly higher in the over-MC group than in the within-MC group (35% vs. 15%, $P = 0.0190$). Regarding history of conventional treatment for HCC before LDLT, patients who had received only 1-2 previous treatments showed significantly lower recurrence rates than patients with ≥3 treatments (9% vs. 37%, $P = 0.0411$). In conclusion, LDLT may constitute an optional treatment with a chance of cure for patients with otherwise uncontrolled disease. However, repeated nontransplant treatments for recurrent HCC prior to LDLT may increase the risk of recurrence and impair the survival advantages conferred by LDLT. *Liver Transpl 12:912-919, 2006.* © 2006 AASLD.

See Editorial on Page 896

The role of orthotopic liver transplantation in the management of hepatocellular carcinoma (HCC) has evolved significantly over the past decades. Initial experiences with orthotopic liver transplantation were limited to patients with extensive unresectable tumors, and were marked by uniformly dismal outcomes due to high rates of tumor recurrence.[1,2] Those results evoked considerable interest in reexamining the staging guidelines to determine eligibility for orthotopic liver transplantation. This lead to a study by Mazzaferro et al.[3] who reported that 48 patients with a single tumor ≤5 cm in diameter or with ≤3 tumors all ≤3 cm in diameter, as identified by preoperative imaging, displayed survival rates comparable to those of non-HCC liver transplant recipients. These Milan criteria (MC) are currently widely accepted as an effective method of selecting patients with early-stage HCC for curative orthotopic liver transplantation and have been incorporated into organ allocation systems.[4]

The prevalence of HCC is high in Japan, with around 30,000 deaths annually.[5] While deceased donor liver transplantation has barely been available for patients with HCC, various treatment modalities, such as hepatic resection, percutaneous ethanol injection, radiofrequency ablation, and transarterial chemoembolization, have been developed, leading to improved outcomes.[5-8] Against this background, living donor liver transplantation (LDLT) has recently emerged as a

Abbreviations: LDLT, living donor liver transplantation; HCC, hepatocellular carcinoma; MC, Milan criteria; MELD, model for end-stage liver disease; TNM, tumor, node, metastasis; JIS, Japan Integrated Staging.

Supported in part by grants from the Scientific Research Fund of the Ministry of Education and by Health and Labor Sciences Research Grants from the Ministry of Health, Labor and Welfare, Japan.

Address reprint requests to: Yasutsugu Takada, MD, Department of Transplantation and Immunology, Kyoto University, Kawara-cho 54, Shogoin, Sakyo-ku, Kyoto 606-8507, Japan. Telephone: 81-75-751-3660; FAX: 81-75-751-4877; E-mail: takaday@kuhp.kyoto-u.ac.jp

DOI 10.1002/lt.20642
Published online in Wiley InterScience (www.interscience.wiley.com).