TABLE 3 Relationship between preoperative AFP mRNA level and clinical parameters

	Preoperative AFP mRNA		*P*
	Positive ratio	Level (mean, range)	
Tumor characteristics			
Microscopic vascular invasion			
Negative	9/103 (8.7%)	4.77E−7 (2.0E−8, 2.42E−06)	0.44
Positive	4/49 (8.2%)	6.17E−7 (2.5E−8, 1.27E−06)	
Edmondson			
1	0/2 (0%)	–	0.499
2	4/55 (7.3%)	6.12E−7 (2.0E−8, 2.42E−06)	
3	8/75 (10.7%)	16.7E−7 (2.47E−8, 1.09E−5)	
4	1/8 (12.5%)	12.7E−7	
Capsule formation			
−	2/22 (9.1%)	1.75E−7 (2.0E−8, 3.29E−7)	0.283
+	12/128 (9.4%)	17.2E−7 (2.4E−8, 1.09E−5)	
TNM stage[a]			
1	1/11 (9.1%)	24.2E−07	0.278
2	6/92 (6.5%)	3.85E−7 (2.0E−8, 1.24E−6)	
3	6/37 (16.2%)	21.4E−7 (4.42E−8, 1.09E−5)	
4a	1/11 (9.1%)	5.45E−8	
4b	0/2	–	
HCC recurrence			
No recurrence	3/68 (4.4%)	36.7E−7 (2.38E−8, 1.09E−5)	0.110
Single recurrence (SR)	3/28 (10.7%)	16.4E−7 (1.24E−6, 2.42E−6)	
Multiple recurrence (MR)	6/38 (15.8%)	2.72E−7 (2.5E−8, 9.05E−7)	
Extrahepatic recurrence (EX)	2/19 (10.5%)	3.7E−8 (2.4E−8, 5.5E−8)	

[a] According to the Liver Cancer Study Group of Japan (LCSGJ)

except it was associated with serum AFP level ($P = 0.039$; Table 2). Furthermore, AFP mRNA expression level did not correlate with tumor characteristics (microscopic vascular invasion, tumor differentiation by Edmondson classification, and capsule formation), TNM stage, or HCC recurrence (Table 3).

Figure 1a shows the recurrence-free survival rate of the SR, MR, and EX groups. The rate was significantly better in the SR group than the MR and EX groups ($P = 0.0015$), and it was almost identical in the latter two groups. Similarly, the survival rate was significantly better in the SR group than the MR and EX groups ($P < 0.0001$; Fig. 1b).

Univariate risk factor analysis showed that tumor size, tumor number, and microscopic vascular invasion were significantly related to HCC recurrence. Preoperative TACE was a candidate risk factor for HCC recurrence ($P = 0.067$); however, it correlated significantly with tumor size ($P < 0.0001$). Serum PIVKA-II level tended to

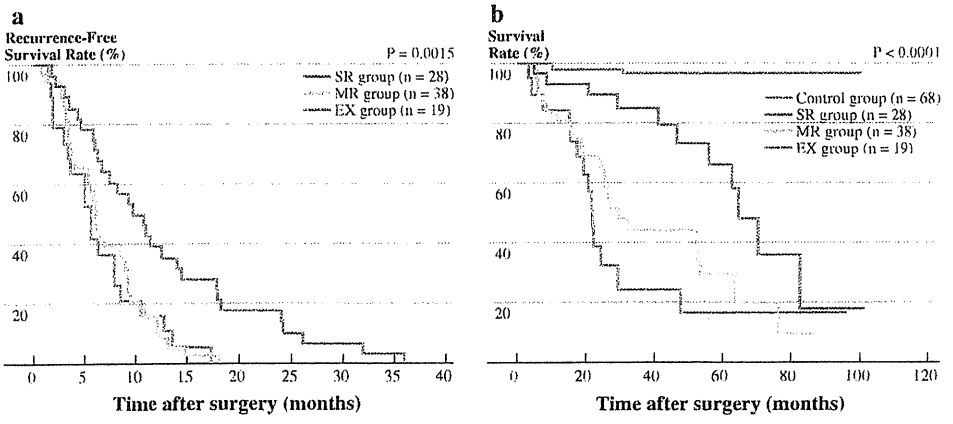

FIG. 1 Kaplan–Meier plot of recurrence-free and overall survival rates after liver resection. **a** There was a significant difference in the recurrence-free survival rate ($P = 0.0015$, log-rank test). **b** There was a significant difference in the overall survival rate ($P < 0.0001$, log-rank test). Control group: no recurrence; SR group: intrahepatic single recurrence after liver resection; MR group: multiple intrahepatic recurrence; EM group: extrahepatic HCC recurrence

be a risk factor for HCC recurrence ($P = 0.076$), whereas AFP was a significant risk factor for HCC recurrence ($P = 0.004$). AFP mRNA expression preoperatively was a risk factor for HCC recurrence ($P = 0.015$), whereas postoperative expression was not ($P = 0.082$). The small subset of both preoperative and postoperative AFP mRNA-positive patients ($n = 6$) did not show any characteristics in terms of incidence in each group (Table 1) and HCC recurrence (data not shown).

Multivariate Cox proportional hazard analysis identified tumor number, preoperative AFP mRNA, microscopic vascular invasion, and serum PIVKA-II level as independent risk factors for HCC recurrence (Table 4).

The HCC recurrence-free survival rate according to AFP mRNA status is shown in Fig. 2a and b. The difference in HCC recurrence-free survival based on preoperative AFP mRNA status was significant, whereas the rate was similar irrespective of the postoperative AFP mRNA status. The difference in the survival rate was more conspicuous when the data of all patients were analyzed (Fig. 2c, d). Patients with positive preoperative AFP mRNA status had significantly worse overall survival than those with negative status, whereas there was no difference in overall survival between patients with positive postoperative AFP mRNA status and those with negative postoperative AFP mRNA status ($P = 0.364$). Among the 85 patients with HCC recurrence, preoperative AFP mRNA was positive in 11 (12.9%) patients. Among 14 patients with positive preoperative AFP mRNA, 11 (78.6%) patients developed HCC recurrence.

We also divided HCC recurrence into two different patterns—solitary intrahepatic recurrence in the SR group, and multicentric or extrahepatic recurrence in the MR/EX group—because the HCC recurrence-free survival rates of the MR and EX groups were almost identical. In the analysis of "time to solitary intrahepatic recurrence," both preoperative and postoperative AFP mRNA statuses did not correlate with HCC recurrence, whereas only preoperative AFP mRNA status correlated significantly with HCC recurrence in the analysis of "multicentric or extrahepatic recurrence" (Fig. 3a–d).

DISCUSSION

The diagnosis of tumor recurrence by detecting circulating tumor cells is already applied in various cancers, such as breast cancer, prostate cancer, and HCC.[2] AFP mRNA has been reported to be a suitable marker for prediction of tumor recurrence, and the efficacy of predicting HCC recurrence after curative resection using AFP mRNA detection has been confirmed in many studies, although others did not.[5,9–14] The reasons for the differences in the utility of AFP mRNA in predicting HCC recurrence are (1) differences in sampling time points among the studies, and (2) differences in RT-PCR technique, using conventional RT-PCR, nested RT-PCR, or quantitative RT-PCR. We consistently used the quantitative RT-PCR method, which is described in detail in several previous studies.[5,6,8]

We reported previously that AFP mRNA is a useful predictor of HCC recurrence in both liver resection and liver transplant patients.[5,6,8] In liver transplant patients, we showed that preoperative detection of AFP mRNA-positive cells in peripheral blood was associated with high incidence of postoperative HCC recurrence, whereas it was not in the anhepatic phase or immediate postoperative period, although the detection rate of AFP mRNA-positive cells in peripheral blood was increased from 9.1 to 30.1%. Based on these results, we hypothesized that the operative maneuver during liver transplantation results in squeezing normal hepatocytes or impotent tumor cells into the blood stream, resulting in detection of AFP mRNA in peripheral blood regardless of the presence of potent CTC.

In the present study, we evaluated the AFP mRNA in a larger population sample of liver resection, limiting the sample to those who underwent complete resection and showed no macroscopic vascular invasion. More importantly, we analyzed these patients with reference to the pattern of HCC recurrence, i.e., SR group, MR group, and EX group, compared with the no recurrence group (control group). The overall recurrence rate in this cohort was 43.2% at 1 year and 63.7% at 3 years, which is similar to the previous report.[1,15]

We reported two major findings in the present study. First, preoperative AFP mRNA was an independent risk factor for HCC recurrence, whereas postoperative AFP mRNA was not. As we pointed out in our previous study of transplant patients, this result supported the notion that detection of AFP mRNA in the immediate postoperative period was not significant. Overall survival was significantly worse in the preoperative AFP mRNA-positive group than mRNA-negative group, whereas it was similar between postoperative AFP mRNA-positive and mRNA-negative groups (Fig. 2b, d), supporting the above-mentioned results. The false positivity of AFP mRNA could simply represent normal hepatocytes or HCC cells being squeezed from the liver into the systemic circulation, and detected by RT-PCR method, rather than representing the complex process of tumor recurrence and systemic spread. This is important because circulating tumor cells are not always viable or proliferative, and most CTCs disappear without causing micrometastasis.[16–19]

The second major finding of the present study was that the recurrence-free survival curve was almost similar for

TABLE 4 Univariate and multivariate risk factor analyses for HCC recurrence

	Relative risk	95% CI	*P*	Relative risk	95% CI	*P*
Age (years)						
≤60	1					
>60	1.13	0.692–1.86	0.619			
Gender						
Male	1					
Female	1.54	0.879–2.68	0.132			
HCV						
−	1					
+	0.92	0.599–1.42	0.712			
HBV (HBsAg+)						
−	1					
+	0.92	0.538–1.56	0.746			
Preoperative TACE						
−	1					
+	1.5	0.97–2.33	0.067			
Tumor size (cm)						
≤5	1			1		
>5	1.64	1.03–2.6	0.037	1.413	0.88–2.28	0.156
Number						
Single	1			1		
Multiple	1.95	1.26–3.01	0.0026	2.07	1.33–3.23	0.001
Microscopic vascular invasion						
−	1			1		
+	1.72	1.1–2.68	0.016	1.83	1.16–2.88	0.009
Histological differentiation (Edmondson classification)						
1, 2	1					
3, 4	1.11	0.72–1.71	0.652			
Capsule formation						
−	1					
+	1.04	0.575–1.88	0.896			
Hepatectomy (HR)[a]						
s, 0	1					
1, 2, 3	0.996	0.64–1.55	0.987			
Blood loss (median; range) (ml)						
≤1000	1					
>1000	1.23	0.51–1.29	0.377			
AFP						
≤20	1					
>20	1.96	1.24–3.01	0.004			
PIVKA-II						
≤200	1			1		
>200	3.58	0.87–14.71	0.076	5.62	1.33–23.8	0.019
Preoperative AFP mRNA						
−	1			1		
+	1.98	1.05–3.73	0.036	2.54	1.31–4.93	0.006
Postoperative AFP mRNA						
−	1			1		
+	1.48	0.95–2.3	0.082	1.37	0.88–2.15	0.166

CI Confidence interval, *AFP* alpha-fetoprotein, *PIVKA* protein induced by vitamin K antagonist

[a] According to the Liver Cancer Study Group of Japan (LCSGJ)

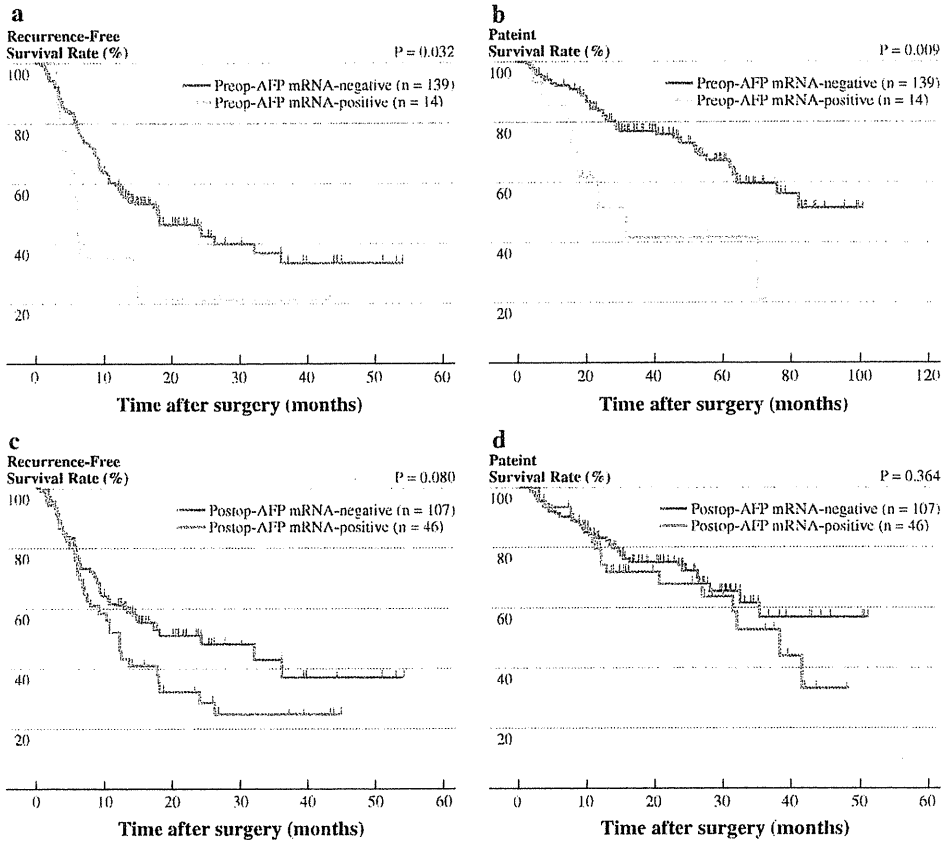

FIG. 2 Kaplan–Meier plot of recurrence-free and overall survival rates after liver resection according to pre- and postoperative AFP mRNA status. **a** The recurrence-free survival rate was worst for patients positive for preoperative AFP mRNA expression in peripheral blood ($P = 0.032$). **b** The overall survival rate was worst for patients positive for preoperative AFP mRNA expression in peripheral blood ($P = 0.009$). **c** Postoperative AFP mRNA-positive patients tended to have the worst recurrence-free survival rates ($P = 0.080$). **d** There was no difference in the overall survival rates between postoperative AFP mRNA-positive and mRNA-negative patients

the MR group and EX group, suggesting that the mech anism of multiple intrahepatic HCC recurrence could be associated with circulating tumor cells as in the EX group, which was compatible with the statement in our previous report.[20] Furthermore, preoperative AFP mRNA was not associated with HCC recurrence in the SR group, whereas it was strongly associated with HCC recurrence in the MR and EX groups. These result are quite reasonable because HCC recurrence in the EX group develops with circulating tumor cells that can be detected by measuring AFP mRNA in peripheral blood. Furthermore, as the result of MR group showed, it is possible that HCC recurrence results from circulating tumor cells detectable in peripheral blood homing residual liver tissue.

Multivariate analysis identified well-known risk factors, including tumor number, and microscopic vascular invasion as independent risk factors for HCC recurrence, which was compatible with other reports published previously, although tumor size was not an independent risk factor.[21] We applied TACE preoperatively to patients with tumors measuring >5 cm, and there was a strong correlation between TACE and tumor size ($P < 0.0001$). One reason why tumor size was not an independent risk factor for HCC recurrence in our series is that TACE was effective in reducing HCC recurrence postoperatively. AFP correlated with AFP mRNA expression in the peripheral blood. AFP is a well-known predictor of HCC recurrence. However, the presence of circulating tumor cells has a quite different meaning from the release of AFP from tumor cells. The association of these predictors can be interpreted to reflect the release of large amounts of AFP from advanced HCC and transmigration of HCC cells from the liver into the systemic circulation.

AFP mRNA positivity was defined using a cutoff level (relative ratio to GAPDH more than 1.0×10^{-8}) of AFP mRNA quantification in our studies. Although there may be considered potential increase of the risk of HCC recurrence associated with higher level of preoperative AFP mRNA, there was no relation between preoperative AFP mRNA level and these risk factors of tumor characteristics and HCC recurrence (Table 3).

The ability to predict HCC recurrence preoperatively is certainly clinically useful. Although there is solid evidence that preoperative intervention, such as TACE or radiofrequency ablation, results in suppression of tumor recurrence and improves prognosis, it is possible that the status of AFP mRNA would predict the efficacy of preoperative

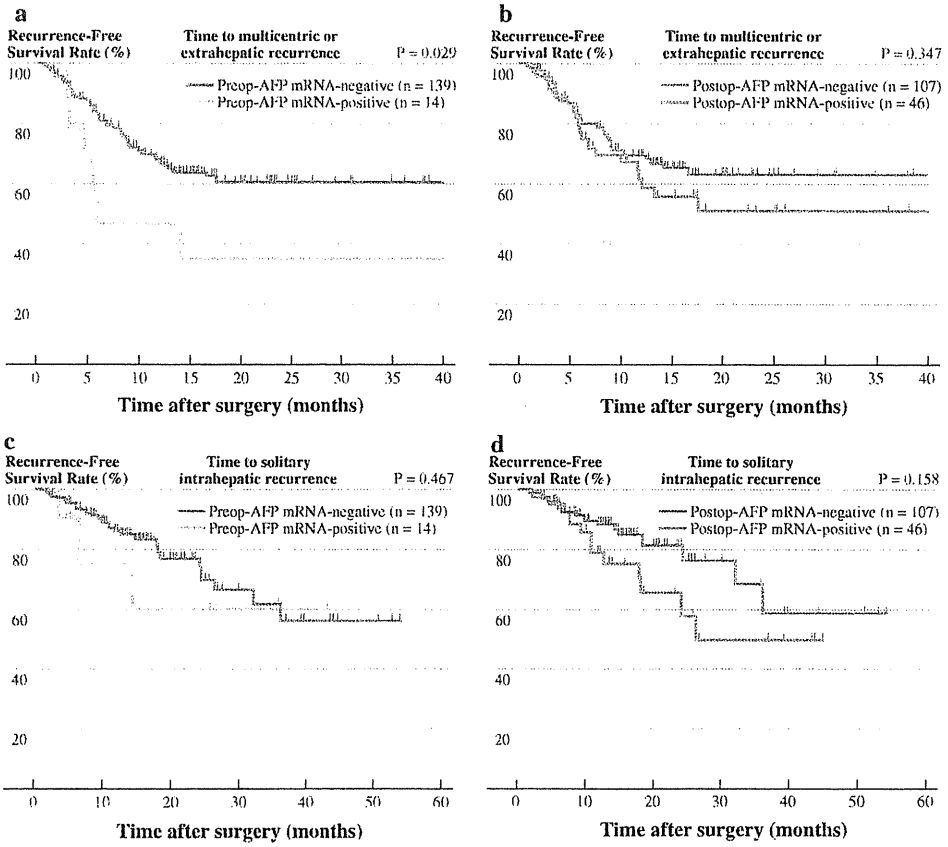

FIG. 3 Kaplan–Meier plot of recurrence-free survival rates after liver resection, censored by HCC recurrence in the SR group (="time to solitary intrahepatic recurrence") and MR/EX combination group (="time to multicentric or extrahepatic recurrence"). **a** MR/EX recurrence-free survival rate according to "time to multicentric or extrahepatic recurrence." HCC recurrence in the MR/EX group was censored. Patients were divided according to the status of preoperative AFP mRNA expression. Preoperative AFP mRNA-positive patients had the worst recurrence-free survival rate ($P = 0.029$). **b** Recurrence-free survival rates for the MR/EX group, divided according to the status of postoperative AFP mRNA according to "time to solitary intrahepatic recurrence." HCC recurrence in the SR group was censored. The status of postoperative AFP mRNA did not influence the recurrence-free survival rate. **c** SR recurrence-free survival curves. Patients were divided according to the status of preoperative AFP mRNA. HCC recurrence of the MR/EX group was censored. **d** SR recurrence-free curves, divided according to the status of preoperative AFP mRNA. HCC recurrence of the MR/EX group was censored. The status of AFP mRNA did not influence the recurrence-free survival rate

locoregional treatment in patients found preoperatively positive for AFP mRNA. Furthermore, the indication for hepatectomy in patients found preoperatively positive for AFP mRNA is an important issue, which requires comparative cohort study or a randomized, clinical trial.

One of the limitations of detecting AFP mRNA preoperatively to predict HCC recurrence is the low sensitivity of the RT-PCR used for AFP mRNA (as low as 12.9%) and relatively high specificity (78.6%). The low sensitivity of this method to detect circulating tumor cells could be due to the small sample of blood obtained (16 ml), which may not be adequate for detecting viable circulating tumor cells. Another possibility is that patients with very advanced HCC and macroscopic vascular invasion were excluded from this study to simplify the study design. An alternative approach would be to find a better biomarker to distinguish potent CTCs from dying CTCs, which may explain the high detectability of AFP mRNA postoperatively without association with HCC recurrence. Nonetheless, it is notable and quite important finding that the specificity of preoperative AFP mRNA to predict HCC recurrence was high, and that recurrence-free or overall survival was significantly worse according to the preoperative AFP mRNA status.

We evaluated bone marrow samples for detection of AFP mRNA in a previous study.[2] However, the expression of AFP mRNA in the bone marrow did not correlate with HCC recurrence; therefore, we did not evaluate bone marrow samples in the present study.

In conclusion, the results of the present study show that detection of AFP mRNA preoperatively in peripheral blood

is a useful predictor of multiple intrahepatic and extrahepatic HCC recurrence and that the expression of AFP mRNA does not predict de novo HCC.

CONFLICT OF INTEREST None.

REFERENCES

1. Kumada T, Nakano S, Takeda I, Sugiyama K, Osada T, Kiriyama S, et al. Patterns of recurrence after initial treatment in patients with small hepatocellular carcinoma. *Hepatology*. 1997;25(1): 87–92.
2. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer*. 2008;8(5):329–40.
3. Roayaie S, Schwartz JD, Sung MW, Emre SH, Miller CM, Gondolesi GE, et al. Recurrence of hepatocellular carcinoma after liver transplant: patterns and prognosis. *Liver Transpl*. 2004; 10(4):534–40.
4. Todo S, Furukawa H. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. *Ann Surg*. 2004;240(3):451–9; discussion 459–61.
5. Morimoto O, Nagano H, Miyamoto A, Fujiwara Y, Kondo M, Yamamoto T, et al. Association between recurrence of hepatocellular carcinoma and alpha-fetoprotein messenger RNA levels in peripheral blood. *Surg Today*. 2005;35(12):1033–41.
6. Marubashi S, Dono K, Nagano H, Sugita Y, Asaoka T, Hama N, et al. Detection of AFP mRNA-expressing cells in the peripheral blood for prediction of HCC recurrence after living donor liver transplantation. *Transpl Int*. 2007;20(7):576–82.
7. Miyamoto A, Fujiwara Y, Sakon M, Nagano H, Sugita Y, Kondo M, et al. Development of a multiple-marker RT-PCR assay for detection of micrometastases of hepatocellular carcinoma. *Dig Dis Sci*. 2000;45(7):1376–82.
8. Miyamoto A, Nagano H, Sakon M, Fujiwara Y, Sugita Y, Eguchi H, et al. Clinical application of quantitative analysis for detection of hematogenous spread of hepatocellular carcinoma by real-time PCR. *Int J Oncol*. 2001;18(3):527–32.
9. Ijichi M, Takayama T, Matsumura M, Shiratori Y, Omata M, Makuuchi M. alpha-Fetoprotein mRNA in the circulation as a predictor of postsurgical recurrence of hepatocellular carcinoma: a prospective study. *Hepatology*. 2002;35(4):853–60.
10. Kamiyama T, Takahashi M, Nakagawa T, Nakanishi K, Kamachi H, Suzuki T, et al. AFP mRNA detected in bone marrow by real-time quantitative RT-PCR analysis predicts survival and recurrence after curative hepatectomy for hepatocellular carcinoma. *Ann Surg*. 2006;244(3):451–63.
11. Okuda N, Nakao A, Takeda S, Oshima K, Kanazumi N, Nonami T, et al. Clinical significance of alpha-fetoprotein mRNA during perioperative period in HCC. *Hepatogastroenterology*. 1999;46(25): 381–6.
12. Jeng KS, Sheen IS, Tsai YC. Circulating messenger RNA of alpha-fetoprotein: a possible risk factor of recurrence after resection of hepatocellular carcinoma. *Arch Surg*. 2004;139(10): 1055–60.
13. Lemoine A, Le Bricon T, Salvucci M, Azoulay D, Pham P, Raccuia J, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein mRNA in humans during liver surgery. *Ann Surg*. 1997;226(1):43–50.
14. Witzigmann H, Geissler F, Benedix F, Thiery J, Uhlmann D, Tannapfel A, et al. Prospective evaluation of circulating hepatocytes by alpha-fetoprotein messenger RNA in patients with hepatocellular carcinoma. *Surgery*. 2002;131(1):34–43.
15. Sakon M, Umeshita K, Nagano H, Eguchi H, Kishimoto S, Miyamoto A, et al. Clinical significance of hepatic resection in hepatocellular carcinoma: analysis by disease-free survival curves. *Arch Surg*. 2000;135(12):1456–9.
16. Mehes G, Witt A, Kubista E, Ambros PF. Circulating breast cancer cells are frequently apoptotic. *Am J Pathol*. 2001;159(1): 17–20.
17. Schmidt H, De Angelis G, Bettendorf O, Eltze E, Semjonow A, Knichwitz G, et al. Frequent detection and immunophenotyping of prostate-derived cell clusters in the peripheral blood of prostate cancer patients. *Int J Biol Markers*. 2004;19(2):93–9.
18. Solakoglu O, Maierhofer C, Lahr G, Breit E, Scheunemann P, Heumos I, et al. Heterogeneous proliferative potential of occult metastatic cells in bone marrow of patients with solid epithelial tumors. *Proc Natl Acad Sci USA*. 2002;99(4):2246–51.
19. Muller V, Stahmann N, Riethdorf S, Rau T, Zabel T, Goetz A, et al. Circulating tumor cells in breast cancer: correlation to bone marrow micrometastases, heterogeneous response to systemic therapy and low proliferative activity. *Clin Cancer Res*. 2005;11(10): 3678–85.
20. Sakon M, Nagano H, Nakamori S, Dono K, Umeshita K, et al. Intrahepatic recurrences of hepatocellular carcinoma after hepatectomy: analysis based on tumor hemodynamics. *Arch Surg*. 2002;137(1):94–9.
21. Minagawa M, Ikai I, Matsuyama Y, Yamaoka Y, Makuuchi M. Staging of hepatocellular carcinoma: assessment of the Japanese TNM and AJCC/UICC TNM systems in a cohort of 13,772 patients in Japan. *Ann Surg*. 2007;245(6):909–22.

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Successful Adult ABO Incompatible Living Donor Liver Transplantation: Experience with Double Infusion through the Hepatic Artery and Portal Vein

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KEY WORDS: ABO-incompatible donor, liver transplantation, double infusion, antibody-mediated rejection.

ABBREVIATIONS: Antibody-mediated rejection (AMR); Disseminated intravascular coagulation (DIC); Hepatic artery (HA); Hepatocellular carcinoma (HCC); Living donor (LD); Liver transplantation (LT); Plasma exchange (PE); Portal vein (PV).

ABSTRACT

Background/Aims: Multiple immunosuppressants, plasma exchanges (PEs), splenectomy, and/or local infusion therapy (either hepatic artery (HA) or portal vein (PV) infusion) are needed in patients undergoing ABO-incompatible living donor liver transplantation (LDLT). Local infusion therapy is commonly undertaken using the single route, and double-infusion therapy has scarcely been investigated. Herein, we describe our experience with five ABO-incompatible LDLT patients who received double-infusion therapy via both the HA and PV. **Methodology:** Five patients (age 43-67 years, with HBV, HBV+HCC, HCV+HCC, PBC, cryptogenic) underwent ABO-incompatible LDLT. Following multiple PEs, LDLT with splenectomy was performed. Triple-immunosuppressant and double-infusion therapy, namely, methylprednisolone and PGE1 via the HA and gabexate mesilate via the PV were employed. **Results:** All five patients achieved accommodation (22-66 months). One patient experienced transient AMR, and recovered after PE and intraarterial steroid infusion. Four of the five patients experienced PV mural thrombi near the PV catheter and recovered with pharmacological treatment. **Conclusions:** Thus, double-infusion therapy could also be useful for ABO-incompatible LDLT under control against PV mural thrombi.

INTRODUCTION

ABO incompatible living donor (LD) is a hopeful source in liver transplantation (LT), however, antibody-mediated rejection (AMR) commonly occurs in adult recipients, which may induce severe bile duct and/or vascular complications (1-3). The morbidity and mobility has gradually improved in recent years based on the use of various protocols such as multiple plasma exchange (PE) during the perioperative period, splenectomy to reduce plasmacytes intraoperatively, and use of triple or quadruple immunosuppressants (4-6). In addition to these protocols, Tanabe *et al.* (7) used portal vein (PV) infusion of steroids and anticoagulants, as well as multiple PEs and splenectomy, and their protocol resulted in marked improvement of survival of ABO incompatible LD (7). Researchers at Kyoto University, Japan, started to use PV infusions from 2000, tried the combination of hepatic artery (HA) and PV infusions in 2001, and decided on HA infusion alone from 2003 (8). The use of PV infusion improved patients' survival with a 3-year survival rate of 56-70%, and almost all patients were since managed with either HA or PV infusion (9). However, to our knowledge, there is little or no information on the merits and demerits of HA/PV combination infusion therapy.

At Osaka University, we started the ABO incompatible LDLT program in 2004, which comprised multiple PEs, triple immunosuppressants, intraoperative splenectomy, and HA/PV combination infusion. We experienced 5 ABO incompatible LDLTs from 2005 through 2007 under this management program. We report herein the results of these cases with rejection and perioperative complications.

METHODOLOGY

Recipients and Donors

Between 2005 and 2007, 5 patients underwent LDLT from ABO incompatible donors (**Table 1**). Their ages were 43-67 (median 57) and the Model for End-Stage Liver Disease (MELD) scores ranged from 14 to 37 (median 18). **Table 1** also lists the background liver diseases, blood types, and anti-A or B antibody titers. Two recipients had hepatocellular carcinoma (HCC); with a maximum tumor size of 3.7cm and uncountable number of tumors (exceeding Milan criteria) in Patient #2, and maximum size of 2cm with two tumors (within Milan) in Patient #3. Patient #2 was reported by Shimoda M, *et al.* (10).

Protocol

The treatment protocol was approved by the institutional ethics review board. All recipients were managed with the protocol described below. Before surgery, anti-A or B antibody titers

(IgM and IgG) were reduced to ≤8 by repeat PEs. Patients with titers >1024 were treated with 375mg/m^2 of rituximab (anti-CD20 antibody) before preoperative PE. Splenectomy was performed during liver transplantation.

Perioperative immunosuppression was achieved by triple immunosuppressants (methyl-prednisolone (mPSL), tacrolimus hydrate (FK-506), and cyclophosphamide (CPA) or micophenolate mofetil (MMF)). Intravenous mPSL was used [1000mg after reperfusion, then 100, 80, 60, 40 and 20mg/day on postoperative day (POD) 1-5, respectively, and 20mg/day after POD 6] and then replaced with 20mg/day of oral PSL. The target trough level of FK-506 was 15-20ng/mL on POD 1-7, 10-15ng/mL on day 8-21 and 8-10ng/mL thereafter. CPA was administered at 2mg/kg body weight/day on POD 0-14 and then switched to oral 2000mg of MMF.

In addition to the systemic immunosuppressants, the liver was directly infused *via* HA with 125mg/day mPSL on POD 0-7 and 50mg/day on POD 8-14, and PGE1 0.02μg/kg/min on POD 0-21, and *via* the PV with gabexate mesilate, a protease inhibitor, at 1000mg/day on POD 0-21 for local anticoagulation (7, 8).

Perioperatively, anti-A and -B antibody titers were monitored, and plasma exchange was performed when the titers were >128 or 4 times the previous antibody titer. Three months after transplantation, liver needle biopsy specimens were obtained for histopathological diagnosis of cellular/antibody-mediated rejection or accommodation. Following LT, we applied the immunosuppressant protocol used regularly at our institution.

RESULTS

Antibody-mediated Rejection and management

Table 2 describes the clinical course and perioperative complications. None of the recipients experienced acute cellular rejection, but one (Patient #1) developed AMR, which was diagnosed with liver biopsy specimen (**Figure 1**) on POD 7 (11). In this patient, high titers of anti-B IgM and IgG antibody were associated with notable hyperbilirubinemia. PE was performed after AMR over a 3-day period, and 925mg/day of mPSL was administrated *via* the HA. Hyperbilirubinemia improved gradually with decrements in IgM and IgG titers after repeat PE. AMR improved histologically on POD 14. Postoperatively, two patients (Patients #2 and #5) experienced elevation of anti-A antibody titer, serum alanine transaminase and bilirubin, and underwent 12 and 11 PEs, respectively. PE resulted in improvement of both alanine transaminase and bilirubin in both patients.

Vascular and other complications

Systemic heparin was administered postoperatively to control activated coagulation time between 150 and 180 seconds. However, three of the patients experienced postoperative hemorrhage (Patients #2, #3, and #5) that required surgical intervention.

Three patients developed HA complications and four developed PV thrombosis. One patient developed HA aneurysm (Patient #1) at the site of insertion of the infusion catheter, while the other two patients developed HA stenosis (Patient #3 and #4). The HA aneurysm was treated with surgical resection while HA stenosis was treated with interventional radiology. PV mural thrombus was noted near the tip of the catheter used for PV infusion (**Figure 2**, Patient #4). In Patient #5, we administrated the same dosage of gabexate mesilate systemically instead of PV infusion and no PV thrombosis was noted. PV mural thrombi were treated successfully in all patients by removal of the catheter and anti-coagulation.

With regard to bacterial and virus infections, only Patient #5 experienced pancreatic fluid leakage. All patients were treated with prophylactic ganciclovir, and no CMV infection was recorded.

Recurrence of primary disease and outcome

Four of the five patients remain alive at the time of preparation of this report after a follow-up period ranging from 22 and 66 months. All recipients are free from the primary disease except for HCC (**Table 2**). Both patients with HCC experienced recurrence of HCC (Patient #2, multiple lung metastases; Patient #3, multiple metastatic tumors in the liver, and distant metastases in the left adrenal gland and brain) and Patient #3 died 22 months postoperatively due to multiple liver metastases.

DISCUSSION

Before the local infusion era, Demetiris *et al.* (1) described liver damage in ABO-incompatible LT as "single organ disseminated intravascular coagulation (DIC)". To avoid such "single organ DIC", three different local infusion therapy protocols (PV infusion alone, HA infusion alone, and combined HA + PV combination infusion) have been described (7, 8). However, their advantages with regard to survival and AMR remain controversial. According to experience at the Kyoto University, the one-year survival rates of HA, PV and combined HA/PV infusion therapy are approximately 80%, 60% and 80%, respectively (8). Data from the Japan Registry for ABO-Incompatible Transplantation indicate that although PV infusion therapy offered advantages against AMR as compared to HA infusion, there were no statistically significant differences between the results of HA and PV infusion (9). In our program, the results were similar to previous reports in regard to not only combined

infusion therapy, but also in relation to HA and PV infusion therapy. These results indicate that local infusion is necessary to prevent local DIC, however, the route of infusion does not seem to influence the frequency of occurrence of AMR.

Meanwhile, our five cases showed a high frequency of vascular complications, although the frequencies of biliary complications and infection were lower (in the Japan Registry, the incidences of biliary complications, bacterial infection, and viral infection are approximately 10%, 30% and 50%, respectively (9)). These vascular complications were mainly related to the coagulation system, and consisted of postoperative hemorrhage and PV mural thrombi. To control local DIC and prevent the formation of PV thrombi after splenectomy (12), we administered anticoagulation therapy, both by parenteral and local infusion. The postoperative coagulopathy associated with hemorrhage and PV mural thrombi might have occurred because of the interaction between the anticoagulant therapy and insufficient liver function. It is important to tightly control coagulation during the preoperative period to prevent postoperative hemorrhage and formation of PV mural thrombi.

Another cause of PV mural thrombi may also be speculated. PV infusion in double-infusion therapy may cause PV mural thrombi, although no such thrombus formation is seen in association with PV infusion alone (7, 8). In Patient #4, computed tomography demonstrated mural thrombi around the tip of the catheter. In contrast, although a PV catheter is inserted in every patient during regular LT to monitor the PV pressure, the rate of PV thrombosis in that population is quite low (data not shown). The formation of the PV mural thrombi seemed to be related to the positioning of the catheter tip and/or drug- induced vascular cell injury (13). Thus, in double infusion through the HA and PV, care should be taken regarding the positioning of the catheter tip to prevent the formation of PV mural thrombi. Further studies are needed to select the ideal local infusion therapy, including the drugs used for infusion.

After the accommodation, appropriate follow-up and maintenance for the primary disease are important. In our cases, three of the five patients did not suffer from recurrent primary disease, however, both of the remaining two patients with HCC suffered from recurrent HCC. One of these patients was within the Milan criteria (patient #3, two tumors with a maximum tumor size of 2cm). Not only recurrent HCC, but also *de novo* malignancy has been reported rarely in ABO- incompatible LT. In an ABO-incompatible renal transplantation series, the incidence of *de novo* malignancy was similar to that associated with regular renal transplantation, but 8% of the patients suffered from *de novo* malignancy (14). In ABO-incompatible LT, one early recurrence of HCC was reported among 8 cases (6 months after LT) (15). Since the protocol for ABO-incompatible LT includes multiple

immunosuppressants, further discussion of HCC recurrence and *de novo* malignancy in LT is necessary.

Recently, a local-infusion-free protocol was introduced for ABO-incompatible LT (16), based on the use of rituximab, an intravenous immune globulin, and PE. ABO-incompatible LT has also recently been extended to the treatment of urgent patients, such as those with fulminant liver failure. In a previous series of LT using a rituximab-based protocol, three of four patients were not urgent cases, but one of them was a case of fulminant liver failure; successfully accommodation was also achieved in this case after an episode of AMR (16). Usually, rituximab is administered a few weeks before the transplantation. The local-infusion-free protocol is also expected for urgent series, however, in cases without a chance to administer rituximab, the local infusion protocols are still considered to be useful at present.

In conclusion, double-infusion therapy through HA and PV seems to be also useful for ABO-incompatible LT, however, vascular complications, namely, postoperative hemorrhage and PV mural thrombi, seem to occur at a high incidence. Further research is required for the prevention of these complications and improvement of the long-term outcome in patients with HCC.

REFERENCES

1. **Demetris AJ, Jaffe R, Tzakis A, Ramsey G, Todo S, Belle S, Esquivel C, Shapiro R, Markus B, Mroczek E, van Thiel DH, Sysyn G, Gordon R, Makowka L, Starzl TE:** Antibody-mediated rejection of human orthotopic liver allografts. A study of liver transplantation across ABO blood group barriers. Am J Pathol 1988; 132:489-502.
2. **Gugenheim J, Samuel D, Reynes M, Bismuth H:** Liver transplantation across ABO blood group barriers. Lancet 1990; 336:519-523.
3. **Farges O, Kalil AN, Samuel D, Saliba F, Arulnaden JL, Debat P, Bismuth A, Castaing D, Bismuth H:** The use of ABO-incompatible grafts in liver transplantation: a life-saving procedure in highly selected patients. Transplantation 1995; 59:1124-1133.
4. **Lo CM, Shaked A, Busuttil R:** Risk factors for liver transplantation across the ABO barrier. Transplantation 1994; 58:543-547.
5. **Hanto DW, Fecteau AH, Alonso MH, Valente JF, Whiting JF:** ABO incompatible liver transplantation with no immunological graft losses using total plasma exchange, splenectomy, and quadruple immunosuppression: Evidence for accommodation. Liver Transplantation 2003; 9:22-30.
6. **Troisi R, Noens L, Montalti R, Ricciardi S, Philippé J, Praet M, Conoscitore P, Centra M, de Hemptinne B:** ABO-mismatch adult living donor liver transplantation using antigen specific immunoadsorption and quadruple immunosuppression without splenectomy. Liver Transpl 2006; 12:1412-1417.
7. **Tanabe M, Shimazu M, Wakabayashi G, Hoshino K, Kawachi S, Kadomura T, Seki H, Morikawa Y, Kitajima M:** Intraportal infusion therapy as a novel approach to adult ABO-incompatible liver transplantation. Transplantation 2002; 73:1959-1961.
8. **Kozaki K, Egawa H, Kasahara M, Oike F, Yoshizawa A, Fukatsu A, Tanaka K:** Therapeutic strategy and the role of apheresis therapy for ABO incompatible living donor liver transplantation. Ther Apher Dial 2005; 9:285-291.
9. **Egawa H, Teramukai S, Haga H, Tanabe M, Fukushima M, Shimazu M:** Present status of ABO-incompatible living donor liver transplantation in Japan. Hepatology 2008; 47:143-152.
10. **Shimoda M, Marubashi S, Dono K, Miyamoto A, Takeda Y, Umeshita K, Nagano H, Hoshida Y, Monden M:** ABO-incompatible adult liver transplantation when the anti-ABO antibody titer is high. Hepato-gastroenterology 2009; 56:1174-1177.
11. **Haga H, Egawa H, Shirase T, Miyagawa A, Sakurai T, Minamiguchi S, Yamabe H, Manabe T, Tanaka K:** Periportal edema and necrosis as diagnostic histological features of early humoral rejection in ABO-incompatible liver transplantation. Liver Transplantation 2004; 10:16-27.
12. **Ikeda M, Sekimoto M, Takiguchi S, Kubota M, Ikenaga M, Yamamoto H, Fujiwara Y, Ohue M, Yasuda T, Imamura H, Tatsuta M, Yano M, Furukawa H, Monden M:** High incidence of thrombosis of the portal venous system after laparoscopic splenectomy: a prospective study with contrast-enhanced CT scan. Ann Surg 2005; 241:208-216.
13. **Aki T, Egashira N, Hama M, Yamauchi Y, Yano T, Itoh Y, Oishi R:** Characteristics of gabexate mesilate-induced cell injury in porcine aorta endothelial cells. J Pharmacol Sci 2008; 106:415-422.
14. **Popov Z, Ivanovski O, Kolevski P, Stankov O, Petrovski D, Cakalaroski K, Ivanovski N:** *De novo* malignancies after renal transplantation--a single-center experience in the Balkans. Transplant Proc 2007; 39:2589-2591.

15. **Matsuno N, Iwamoto H, Nakamura Y, Hama K, Kihara Y, Konno O, Jojima Y, Akashi I, Mijiti A, Ashizawa T, Nagao T:** ABO-incompatible adult living donor liver transplantation for hepatocellular carcinoma. Transplant Proc 2008; 40:2497-2500.
16. **Ikegami T, Taketomi A, Soejima Y, Yoshizumi T, Uchiyama H, Harada N, Iguchi T, Hashimoto N, Maehara Y:** Rituximab, IVIG, and plasma exchange without graft local infusion treatment: a new protocol in ABO incompatible living donor liver transplantation. Transplantation 2009; 88:303-307.

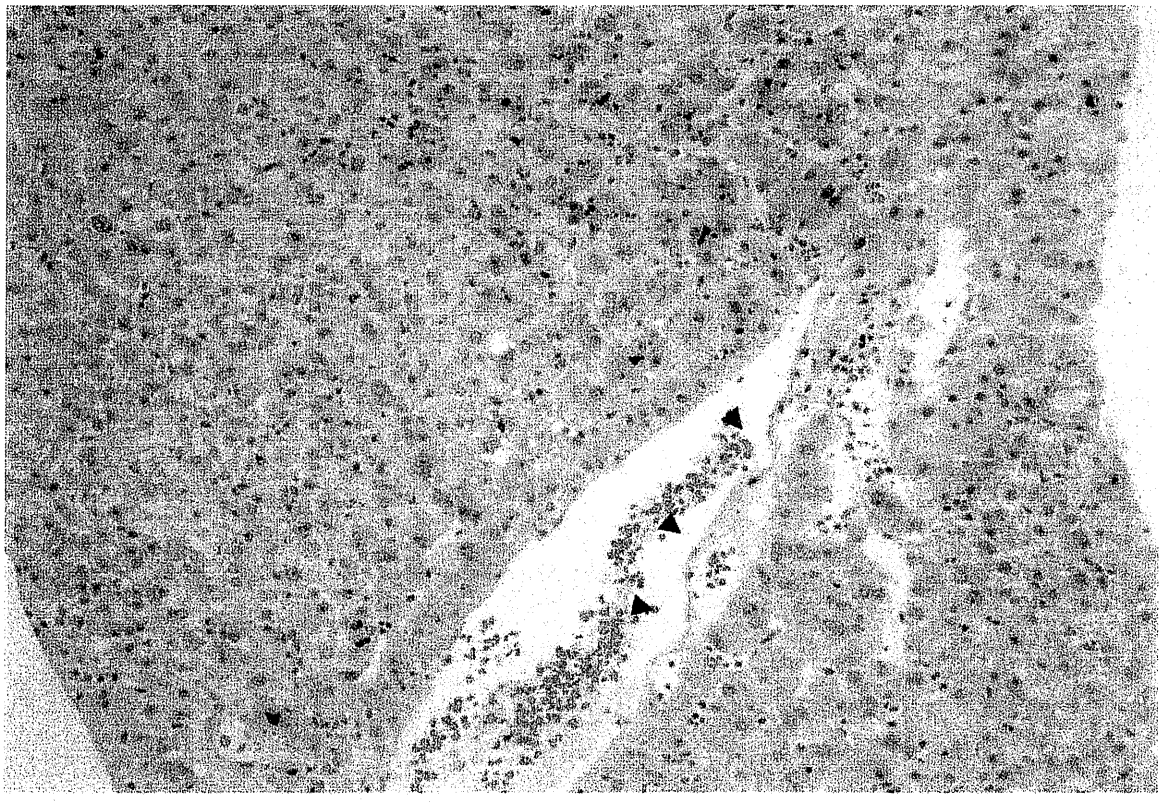

FIGURE 1. Patient 1. Liver biopsy of suspected antibody-mediated rejection on postoperative day 7. Note detachment of endothelial cells (arrowhead). Hematoxylin-eosin staining (x200).

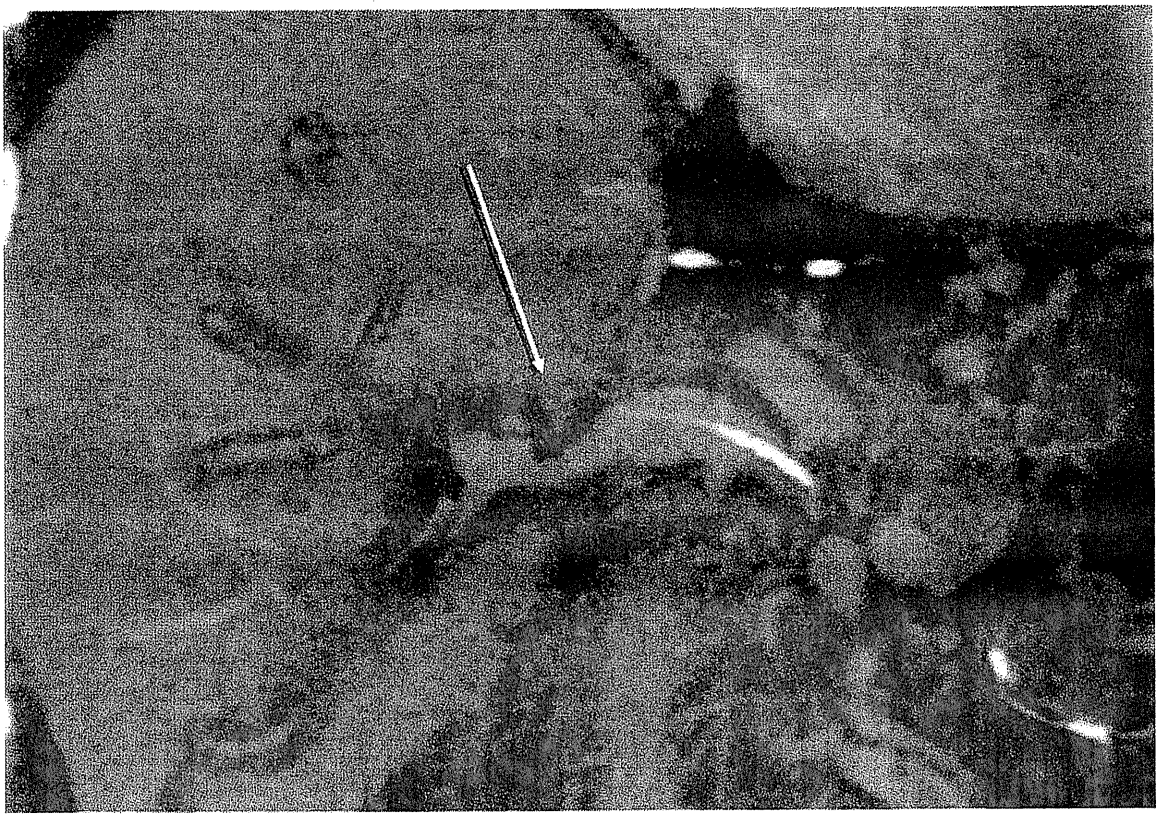

FIGURE 2. Patient 4. Computed tomography shows a portal vein mural thrombus at the tip of the catheter (arrow).

TABLE 1. Clinical characteristics of recipients and donors.

Case #	Recipients				Antibody for donor blood		Donors		
	Age/Gender	Blood type	Diseases	MELD		IgM / IgG	Age/Gender	Blood type	Graft
1	43/M	A	HBV	18	anti-B	x32 / x16	58/M	AB	Right lobe
2*	58/M	O	HBV+HCC	14	anti-A	x1024 / x8192	31/M	A	Right lobe
3	45/M	O	HCV+HCC	15	anti-A	x512 / x128	47/F	A	Right lobe
4	57/F	A	PBC	28	anti-B	x128 / x8	24/F	AB	Right lobe
5	67/F	B	Cryptogenic	37	anti-A	x128 / x8	36/M	A	Right posterior segment

*Case #2 was reported by Shimoda M, *et al.* (10).

TABLE 2. Complications and outcome.

Case#	Hemorrhage	HA	PVT	BT	ISS	CMV	ACR	AMR	Maximum IgM/IgG	Number of PE		POM	Recurrence of primary disease	Outcome
										Pre-LT	Post-LT			
1	-	aneurysm	mural	-	-	-	-	Yes	x32/x64	2	3	66	-	Alive
2	Yes	-	mural	-	-	-	-	-	x16/x64	6	12	62	HCC at 18 m	Alive
3	Yes	stenosis	mural	-	-	-	-	-	x8/x16	3	1	22	HCC at 10 m	Deceased (HCC)
4	-	stenosis	mural	-	-	-	-	-	x512/x16	2	0	45	-	Alive
5	Yes	-	-	-	Yes*	-	-	-	x256/x32	3	11	38	-	Alive

* Pancreatic fistula.

HA: hepatic artery, PVT: portal vein thrombi, BT: biliary tract complications, SSI: infection at site of surgery, ACR: acute cellular rejection, AMR: antibody-mediated rejection, PE: plasma exchange, LT: liver transplantation, POM: postoperative month.

Original Paper

Efficacy of Minimal Dosage of Calcineurin Inhibitor for Living Donor Liver Transplant Recipients with Preoperative Renal Dysfunction

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KEY WORDS: Live donor transplantation; Immunosuppressive regimens; Chronic renal failure; Anti-CD25 monoclonal antibody

ABBREVIATIONS: Acute Cellular Rejection (ACR); Calcineurin Inhibitor (CNI); Creatinine Clearance (CrCl); Micro-Emulsified Cyclosporin A (CsA); Tacrolimus (FK); Glomerular Filtration Rate (GFR); Living Donor Liver Transplantation (LDLT); Mycophenolate Mofetil (MMF)

ABSTRACT

Background/Aims: There is no standard protocol for immunosuppression for patients with preoperative chronic renal dysfunction (PCRD) scheduled for living donor liver transplantation (LDLT). In this prospective study, we evaluated the efficacy of low-dose calcineurin inhibitor (CNI) protocol for such patients.

Methodology: We studied 17 consecutive LDLT recipients with PCRD (creatinine clearance <50mL/min). Six patients (LD-B group) received combination of low-dose CNI (LD-CNI), mycophenolate mofetil, corticosteroids, and anti-CD25 monoclonal antibody (mAb). Their clinical data were compared with conventional CNI group (N group, n=8) and LD-CNI without CD25 mAb group (LD group, n=3).

Results: Preoperative characteristics and incidence of acute rejection were similar in the three groups. None of the LD-B group recipients developed renal failure, while one (9%) did in the N group. Patient survival was better in the LD-B group than control groups.

Conclusion: Our renal sparing protocol is feasible and effective for LDLT recipients with PCRD.

INTRODUCTION

Calcineurin inhibitors (CNI) are immunosuppressants used extensively in liver transplantation, although their renal toxicity is a major concern after liver transplantation especially in patients with preoperative chronic renal dysfunction (1, 2). Several studies stressed the need for use of renal sparing immunosuppression protocols in recipients with preserved renal function, but not in patients with preoperatively impaired renal function (3-9). Others recommended a combination of both liver and kidney transplantation for patients with preoperative renal impairment in deceased donor liver transplantation (10). However, it is quite difficult to perform a combination liver and kidney transplantation when living donor is the source of organ donation. Therefore, renal sparing immunosuppression protocols for patients with chronic renal dysfunction should be optimized and their feasibility and effectiveness analyzed carefully before liver transplantation.

The aim of this prospective non-randomized study was to evaluate the efficacy of CNI in liver recipients with preoperative renal dysfunction scheduled for living donor liver transplantation (LDLT), by comparing the outcome of such patients to control group with normal renal function.

METHODOLOGY

The study protocol was approved by the Human Ethics Review Committee of Osaka University Graduate School of Medicine and a signed consent form was obtained from each subject. The inclusion criteria were patients with end-stage liver disease scheduled for LDLT and preoperative creatinine clearance (CrCl) of <50mL/min. We excluded patients with ABO incompatibility for liver transplantation, fulminant hepatic failure with/without hepatorenal syndrome, and those on dialysis for chronic renal failure prior to LDLT. Among 67 patients consecutive with end-stage liver disease who underwent adult-to-adult LDLT between 1999 and 2006 in our hospital, 17 patients (25.3%) were enrolled in this study.

Preoperative CrCl was calculated as the conventional creatinine clearance measured in 24-h urine collection. The glomerular filtration rate (GFR) was estimated using the Modification of Diet in Renal Disease (MDRD) study equation modified for Japanese patients [Estimated GFR=0.881x186.3xserum Cr-1.154xage-0.203x(male: 1, female: 0.742)] (11). The selection of immunosuppressive regimen was based on the availability of anti-lymphocyte (anti-CD25) monoclonal antibody (mAb) (basiliximab, Novartis Pharma, Tokyo, Japan).

Renal sparing protocol (LD-B group)

Low-dose CNI immunosuppressive regimen consisted of micro-emulsified cyclosporine-A (CsA) (C0: 100-150ng/mL) or tacrolimus (FK) (trough: 4-6ng/mL),

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combined with mycophenolate mofetil (MMF) at an initial dose of 1,000mg b.i.d, and corticosteroids which was started with 1,000mg of methyl prednisolone during anhepatic period, 100mg methyl-prednisolone on day 1, tapered to 20mg on day 6, and 5mg on day 60. Anti-CD25 mAb (basiliximab) was administered twice on days 0 and 4.

Control group

Eleven patients of the control group received either normal dosage of CNI (CsA 250-350ng/mL, FK 10-15ng/mL, N group) or low-dose CNI (LD group), each without anti-CD25 mAb, which was not available at the time of the study. MMF and steroids were administered using the same doses as for LD-B group.

Postoperative renal function, incidence of acute cellular rejection (ACR), patient survival, and other clinical data were compared among these groups. De novo hypertension was defined as hypertension requiring anti-hypertensive drugs after LDLT without previous history of hypertension. De novo diabetes mellitus was defined as new-onset diabetes that required anti-diabetic drugs after LDLT without previous history of diabetes mellitus.

To evaluate CNI concentration, the trough level of tacrolimus was converted to 25x (FK trough) as a CsA equivalent of tacrolimus to compare with the C0 of CsA.

Continuous data were expressed as mean±SD. Group data sets were compared using student t-test or Mann-Whitney U test. Categorical data were presented as percentages, and differences between proportions were compared using the chi-square test. A p value less than 0.05 was considered significant.

RESULTS

Six patients received low-dose CNI immunosuppressive regimen with anti-CD25 mAb (LD-B group), whereas 8 patients received normal dosage of CNI with or without MMF (N group) and 3 patients received low-dose CNI without anti-CD25 mAb (LD group). Patients' characteristics were similar among the three groups, except that CNI was mainly CsA in the LD-B and LD group, whereas it was mainly FK in the N group. Preoperative CrCl was similar among the three groups (37.1±10.0, 44.9±2.5, and 34.4±10.6mL/min in LD-B, LD, and N group, respectively, **Table 1**). CNI concentrations were significantly higher in the N group than the LD-B group and LD group at days 7 and 14 after LDLT, but were similar after 1 month post-transplantation (**Figure 1A**). GFR was similar in the three groups preoperatively, and it tended to improve 1 month after LDLT in the LD group and LD-B group while it slightly deteriorated 1 month after LDLT in the N group, although the change was not statistically significant (**Figure 1B**). The incidence of ACR was similar among the three groups (40%, 33% and 35%, for the LD-B, L and N group, respectively, **Figure 2A**).

The morbidity and mortality rates in the early period after LDLT (less than 6 months) are shown in

TABLE 1 Patients' Characteristics

	LD-B (n=6)	LD (n=3)	N (n=8)
Recipient age	49.8±10.4	55.0±3.5	49.8±14.3
Gender (M/F)	5/1	2/1	6/2
Primary diagnosis			
Virus cirrhosis (HBV, HCV)	5	3	6
PSC	0	0	1
Laennecs	1	0	0
Cryptogenic	0	0	1
MELD score	21.8±6.5	22.3±3.1	26.5±12.8
PreOP Crn	1.44±0.46	1.15±0.07	1.48±0.62
PreoOP Ccr	37.1±10.0	44.9±2.5	34.4±10.6
Donor age	40.0±12.4	44.0±10.6	39.4±13.6
CNI (FK/CsA)	1/5	0/3	7/1
Graft (Left/Right)	1/5	2/1	1/7
GLV	633.7±91.0	598.0±29.7	666.3±89.3
GW/SLV (%)	48.8±4.1	49.6±2.1	53.5±8.5
WIT (min)	55.8±15.6	46.7±7.4	46.8±12.9
CIT (min)	83.2±29.5	80.0±44.2	71.4±57.1

Data are mean±SD or number of patients.
PSC: Primary sclerosing cholangitis, MELD: model for end-stage liver disease, PreOp Crn: preoperative serum creatinine, PreOP Ccr: preoperative creatinine clearance, CNI: calcineurin inhibitors, GLV: graft liver volume, GW/SLV%: graft weight/recipient standard liver volume (GW/SLV) ratio (%), WIT: warm ischemic time, CIT: cold ischemic time