Table 2 Univariate analysis for clinicopathological parameters and mesothelin expression on overall survival of patients with gastric carcinoma

Factor	N	P	RR (95% CI)
1. *Histological classification*			
por2-sig	62	0.89	1
Others	48		0.954 (0.478–1.903)
2. *pT factor*			
pT1	62	<0.0001	1
pT2-4	48		13.354 (4.679–38.113)
3. *pN factor*			
Positive	73	<0.0001	1
Negative	37		9.301 (4.147–20.860)
4. *pStage*			
I, II	80	<0.0001	1
III, IV	30		18.837 (8.032–44.179)
5. *Lymphatic permeation*			
Positive	62	<0.0001	1
Negative	48		18.529 (5.637–60.534)
6. *Blood vessel permeation*			
Positive	69	<0.0001	1
Negative	41		11.493 (4.722–27.971)
7. *Mesothelin expression*			
No	61	<0.0001	1
Yes	49		1.749 (0.874–3.500)
8. *Luminal membrane expression*			
No	94	<0.0001	1
Yes	16		7.205 (3.489–14.877)
9. *Cytoplasmic expression*			
No	68	0.98	1
Yes	42		1.007 (0.493–2.055)

Abbreviation: CI = confidence interval. RR indicates relative risk/hazard ratio.

Table 3 Multivariate analysis for clinicopathological parameters and mesothelin expression on overall survival of patients with gastric carcinoma

Factor	P	RR (95% CI)
1. *pT factor*		
pT1 *vs* pT2–4	0.35	2.497 (0.374–16.660)
2. *pN factor*		
Positive *vs* Negative	0.060	3.532 (0.946–13.181)
3. *pStage*		
I, II *vs* III, IV	0.0003	12.336 (2.533–60.069)
4. *Lymphatic permeation*		
Positive *vs* Negative	0.0043	11.996 (2.180–65.996)
5. *Blood vessel permeation*		
Positive *vs* Negative	0.29	2.091 (0.533–8.195)
6. *Luminal membrane expression*		
No *vs* Yes	0.0073	2.969 (1.341–6.573)

Abbreviation: CI = confidence interval. RR indicates relative risk/hazard ratio.

physiologically cleaved by some furin-like proteases into a 40-kDa C-terminal fragment that remains membrane bound, and a 31-kDa N-terminal fragment, which is secreted into the blood (Chang and Pastan, 1996). The C-terminal 40-kDa fragment is referred to as mesothelin, which is attached to the cell membrane by a GPI anchor (Chang and Pastan, 1996; Hassan *et al*, 2004). The 5B2 anti-mesothelin antibody (Novocastra Laboratory Vision BioSystems, Boston, MA, USA), which we employed here for IHC, can detect the 71-kDa precursor protein and also the 40-kDa C-terminal fragment (Inami *et al*, 2008); therefore, we could not decide which form of mesothelin has a pivotal role in malignant behaviour of gastric cancer cells. Recent studies reported that mesothelin is not only associated with increased cell proliferation and with the migration of pancreatic cancer cells *in vitro* (Bharadwaj *et al*, 2008; Li *et al*, 2008), but also contributes to tumour progression *in vivo* (Li *et al*, 2008). Mesothelin inhibits paclitaxel-induced apoptosis through concomitant activation of phosphoinositide-3-kinase (PI3K) signalling in the regulation of Bcl-2 family expression (Chang *et al*, 2009), and induces the activation of signal transducer and activator of transcription (Stat) 3, which leads to increased expression of cyclin E and makes pancreatic cancer cells proliferate faster (Bharadwaj *et al*, 2008). In addition, mesothelin-activated nuclear factor-kappaB (NF-κB) induces elevated interleukin (IL)-6 expression, which acts as a growth factor to support pancreatic cancer cell survival/proliferation through a novel auto/paracrine IL-6/soluble IL-6R trans-signalling (Bharadwaj *et al*, 2011a, b). Our study provided a new aspect that luminal membrane expression of mesothelin is associated with the malignant behaviour of tumour cells, such as depth of tumour invasion and vascular invasion, although it remains necessary to clarify the biological function of the 71-kDa mesothelin precursor and/or 40-kDa mesothelin protein in *in-vitro* and *in-vivo* studies, including the processing system by furin-like proteases.

In terms of discovering the clinicopathological parameters for gastric cancer, there are many previous studies demonstrating the prognostic significance of various molecules, such as epidermal growth factor receptor and c-erB-2 (HER-2). These molecules also could be of unique significance as the indicators of eligibility to specific molecular targeting therapies, because most of them are located in the cell membrane as the useful targets for the molecular targeted drugs such as antibody drugs. We believe that the immunohistochemical evaluation for luminal membrane expression of mesothelin in gastric cancer would be of clinical benefit not only as a prognostic factor but also as a predictive factor for the eligibility to mesothelin-targeting therapies in the future (Hassan *et al*, 2004, 2007a, b, c, 2010; Hassan and Ho, 2008; Li *et al*, 2008; Inami *et al*, 2009).

In conclusion, we demonstrated the clinicopathological significance of the luminal membrane expression of mesothelin in gastric cancer as an independent prognostic factor, although additional studies to increase the number of the cases for luminal membrane expression ($n = 16$) might be required for further confirmation. The immunohistochemical examination of mesothelin expression in surgically resected tumour specimens should be clinically useful for prognostication and for decision making about further treatment procedures after surgical therapy in patients with gastric cancer.

ACKNOWLEDGEMENTS

This work was supported in part by a grant-in-aid from the foundation for the Department of General Surgery, Hokkaido University Alumni Association.

Supplementary Information accompanies the paper on British Journal of Cancer website (http://www.nature.com/bjc)

REFERENCES

Argani P, Iacobuzio-Donahue C, Ryu B, Rosty C, Goggins M, Wilentz RE, Murugesan SR, Leach SD, Jaffee E, Yeo CJ, Cameron JL, Kern SE, Hruban RH (2001) Mesothelin is overexpressed in the vast majority of ductal adenocarcinomas of the pancreas: identification of a new pancreatic cancer marker by serial analysis of gene expression (SAGE). *Clin Cancer Res* **7**: 3862–3868

Baba K, Ishigami S, Arigami T, Uenosono Y, Okumura H, Matsumoto M, Kurahara H, Uchikado Y, Kita Y, Kijima Y, Kitazono M, Shinchi H, Ueno S, Natsugoe S (2011) Mesothelin expression correlates with prolonged patient survival in gastric cancer. *J Surg Oncol* **105**: 195–199

Bharadwaj U, Li M, Chen C, Yao Q (2008) Mesothelin-induced pancreatic cancer cell proliferation involves alteration of cyclin E via activation of signal transducer and activator of transcription protein 3. *Mol Cancer Res* **6**: 1755–1765

Bharadwaj U, Marin-Muller C, Li M, Chen C, Yao Q (2011a) Mesothelin confers pancreatic cancer cell resistance to TNF-alpha-induced apoptosis through Akt/PI3K/NF-kappaB activation and IL-6/Mcl-1 overexpression. *Mol Cancer* **10**: 106

Bharadwaj U, Marin-Muller C, Li M, Chen C, Yao Q (2011b) Mesothelin overexpression promotes autocrine IL-6/sIL-6R trans-signaling to stimulate pancreatic cancer cell proliferation. *Carcinogenesis* **32**: 1013–1024

Chang K, Pastan I (1996) Molecular cloning of mesothelin, a differentiation antigen present on mesothelium, mesotheliomas, and ovarian cancers. *Proc Natl Acad Sci USA* **93**: 136–140

Chang K, Pastan I, Willingham MC (1992) Isolation and characterization of a monoclonal antibody, K1, reactive with ovarian cancers and normal mesothelium. *Int J Cancer* **50**: 373–381

Chang MC, Chen CA, Hsieh CY, Lee CN, Su YN, Hu YH, Cheng WF (2009) Mesothelin inhibits paclitaxel-induced apoptosis through the PI3K pathway. *Biochem J* **424**: 449–458

Cheng WF, Huang CY, Chang MC, Hu YH, Chiang YC, Chen YL, Hsieh CY, Chen CA (2009) High mesothelin correlates with chemoresistance and poor survival in epithelial ovarian carcinoma. *Br J Cancer* **100**: 1144–1153

Einama T, Kamachi H, Nishihara H, Homma S, Kanno H, Takahashi K, Sasaki A, Tahara M, Okada K, Muraoka S, Kamiyama T, Matsuno Y, Ozaki M, Todo S (2011) Co-expression of mesothelin and CA125 correlates with unfavorable patient outcome in pancreatic ductal adenocarcinoma. *Pancreas* **40**: 1276–1282

Hassan R, Bera T, Pastan I (2004) Mesothelin: a new target for immunotherapy. *Clin Cancer Res* **10**: 3937–3942

Hassan R, Broaddus VC, Wilson S, Liewehr DJ, Zhang J (2007a) Anti-mesothelin immunotoxin SS1P in combination with gemcitabine results in increased activity against mesothelin-expressing tumor xenografts. *Clin Cancer Res* **13**: 7166–7171

Hassan R, Bullock S, Premkumar A, Kreitman RJ, Kindler H, Willingham MC, Pastan I (2007b) Phase I study of SS1P, a recombinant anti-mesothelin immunotoxin given as a bolus I.V. infusion to patients with mesothelin-expressing mesothelioma, ovarian, and pancreatic cancers. *Clin Cancer Res* **13**: 5144–5149

Hassan R, Ebel W, Routhier EL, Patel R, Kline JB, Zhang J, Chao Q, Jacob S, Turchin H, Gibbs L, Phillips MD, Mudali S, Iacobuzio-Donahue C, Jaffee EM, Moreno M, Pastan I, Sass PM, Nicolaides NC, Grasso L (2007c) Preclinical evaluation of MORAb-009, a chimeric antibody targeting tumor-associated mesothelin. *Cancer Immun* **7**: 20

Hassan R, Ho M (2008) Mesothelin targeted cancer immunotherapy. *Eur J Cancer* **44**: 46–53

Hassan R, Kreitman RJ, Pastan I, Willingham MC (2005a) Localization of mesothelin in epithelial ovarian cancer. *Appl Immunohistochem Mol Morphol* **13**: 243–247

Hassan R, Laszik ZG, Lerner M, Raffeld M, Postier R, Brackett D (2005b) Mesothelin is overexpressed in pancreaticobiliary adenocarcinomas but not in normal pancreas and chronic pancreatitis. *Am J Clin Pathol* **124**: 838–845

Hassan R, Schweizer C, Lu KF, Schuler B, Remaley AT, Weil SC, Pastan I (2010) Inhibition of mesothelin-CA-125 interaction in patients with mesothelioma by the anti-mesothelin monoclonal antibody MORAb-009: implications for cancer therapy. *Lung Cancer* **68**: 455–459

Inami K, Abe M, Takeda K, Hagiwara Y, Maeda M, Segawa T, Suyama M, Watanabe S, Hino O (2009) Antitumor activity of anti-C-ERC/mesothelin monoclonal antibody *in vivo*. *Cancer Sci* **101**: 969–974

Inami K, Kajino K, Abe M, Hagiwara Y, Maeda M, Suyama M, Watanabe S, Hino O (2008) Secretion of N-ERC/mesothelin and expression of C-ERC/mesothelin in human pancreatic ductal carcinoma. *Oncol Rep* **20**: 1375–1380

Sobin LH, Wittekind CW (ed) (2002) *TNM Classification of Malignant Tumors*. Wiley-Liss: New York

Li M, Bharadwaj U, Zhang R, Zhang S, Mu H, Fisher WE, Brunicardi FC, Chen C, Yao Q (2008) Mesothelin is a malignant factor and therapeutic vaccine target for pancreatic cancer. *Mol Cancer Ther* **7**: 286–296

Ordonez NG (2003a) Application of mesothelin immunostaining in tumor diagnosis. *Am J Surg Pathol* **27**: 1418–1428

Ordonez NG (2003b) Value of mesothelin immunostaining in the diagnosis of mesothelioma. *Mod Pathol* **16**: 192–197

Swierczynski SL, Maitra A, Abraham SC, Iacobuzio-Donahue CA, Ashfaq R, Cameron JL, Schulick RD, Yeo CJ, Rahman A, Hinkle DA, Hruban RH, Argani P (2004) Analysis of novel tumor markers in pancreatic and biliary carcinomas using tissue microarrays. *Hum Pathol* **35**: 357–366

Yen MJ, Hsu CY, Mao TL, Wu TC, Roden R, Wang TL, Shih Ie M (2006) Diffuse mesothelin expression correlates with prolonged patient survival in ovarian serous carcinoma. *Clin Cancer Res* **12**: 827–831

Surg Today (2012) 42:559–564
DOI 10.1007/s00595-011-0086-4

ORIGINAL ARTICLE

The long-term outcomes of patients with hepatocellular carcinoma after living donor liver transplantation: a comparison of right and left lobe grafts

Akinobu Taketomi · Ken Shirabe · Takeo Toshima · Kazutoyo Morita · Naotaka Hashimoto · Hiroto Kayashima · Tohru Ikegami · Tomoharu Yoshizumi · Yuji Soejima · Yoshihko Maehara

Received: 16 May 2011 / Accepted: 16 May 2011 / Published online: 14 January 2012

Abstract
Purpose The feasibility of living donor liver transplantation (LDLT) using left lobe (LL) grafts has been demonstrated. However, the long-term outcome of the hepatocellular carcinoma (HCC) patients with LL grafts has not been elucidated. The aim of this study was to analyze the long-term outcomes after LDLT for HCC according to the graft type.
Methods A retrospective analysis was performed evaluating the outcomes of LL graft recipients ($n = 82$) versus recipients of RL grafts ($n = 46$). The analysis endpoints were the overall and recurrence-free survival after LDLT. The demographics of both recipients and donors, and the tumor characteristics associated with the graft type were also analyzed.
Results The graft volume (436 ± 74 g), as well as the graft volume-standard liver volume rate (38.3 ± 6.2%) of the LL graft group were significantly decreased as compared to those of the RL graft group (569 ± 82 g, 46.3 ± 6.7%; $p < 0.01$). The 1-, 3-, 5- and 7-year overall survival rates of the LL graft group were 88.2, 80.2, 75.7 and 72.4%, respectively, which were not significantly different compared to those of the RL graft group (95.4, 87.3, 87.3 and 87.3%). The recurrence-free survival rates of the LL graft group (89.1% at 1 year, 78.8% at 3 years, 75.8% at 5 years and 70.3% at 7 years) were similar to those of the RL graft group (88.6, 88.6, 88.6 and 88.6%). The mean peak postoperative total bilirubin levels and duration of hospital stay after surgery for the LL grafting donors were significantly decreased as compared to those of the RL grafting donors ($p < 0.01$). The rate of severe complications (over Clavien's IIIa) associated with LL graft procurement was 6.2%, which was lower than that in the RL graft group (15.6%).
Conclusions The long-term outcomes in the HCC patients with LL grafts were similar to those of patients receiving RL grafts, and the outcomes of the donors of LL grafts were more favorable. Therefore, LL grafts should be considered when selecting LDLT for HCC to ensure donor safety.

Keywords Surgery · Hepatocellular carcinoma · Recurrence · Living donor liver transplantation · Graft type

Abbreviations

AFP	Alpha-fetoprotein
CL	Caudate lobe
CT	Computed tomography
DCP	Des-gamma-carboxy prothrombin
DDLT	Deceased donor liver transplantation
GRWR	Graft-recipient weight ratio
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
LDLT	Living donor liver transplantation
LL	Left lobe
LT	Liver transplantation
MMF	Mycophenolate mofetil
RL	Right lobe
RV	Remnant liver volume
SLV	Standard liver volume
TLV	Total liver volume

A. Taketomi (✉) · K. Shirabe · T. Toshima · K. Morita · N. Hashimoto · H. Kayashima · T. Ikegami · T. Yoshizumi · Y. Soejima · Y. Maehara
Department of Surgery and Science,
Graduate School of Medicine, Kyushu University,
3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan
e-mail: taketomi@surg2.med.kyushu-u.ac.jp

Introduction

Living donor liver transplantation (LDLT) is currently the treatment of choice for unresectable hepatocellular carcinoma (HCC; 1, 2). A large survey of 49 centers in Japan including a total of 653 patients with HCC has been reported [1]. A postoperative pathological study showed that the 5-year disease-free survival of those who met ($n = 325$) and exceeded ($n = 272$) the Milan criteria were 95.3 and 66.4%, respectively. Therefore, LDLT for patients with HCC within the Milan criteria can also achieve an acceptable outcome comparable to the outcome for deceased donor liver transplantation (DDLT) for HCC. Some DDLT centers have expanded the selection criteria, like up-to seven criteria, because of concerns that the Milan criteria are too stringent [2, 3]. The expanded criteria are also proposed by some LDLT high-volume centers in Japan [4–6]. Tumor markers, such as alpha-fetoprotein (AFP) or des-gamma-carboxy prothrombin (DCP), in addition to the tumor size and the number of tumors, are useful to properly rate the candidate without decreasing the long-term survival after liver transplantation (LT). Therefore, LDLT has been established as a treatment choice for HCC.

Living donor liver transplantation using right lobe (RL) grafts has gained widespread acceptance, whereas the use of left lobe (LL) grafts for adults has been severely limited because of their size limitation. On the other hand, some centers have limited LDLT between adults to LL grafting, arguing that RL donation involves unacceptable risks [7]. Donor selection criteria have been established based on graft liver volume (GV) and the remnant liver volume (RV) of the donor, as calculated using three-dimensional computed tomography (CT), and the LL is considered to be the first choice for the graft [8].

There is a potential risk for HCC after LDLT due to the rapid liver regeneration that occurs in the immediate post-LDLT period, which could lead to cancer progression in these patients, which in turn, could lead to early or multiple-site recurrence [9]. However, the long-term outcome of the HCC patients treated with LL grafts has not yet been elucidated.

The aim of this study was to compare the long-term and recurrence-free survival rates between recipients of LL and RL grafts.

Patients and methods

Three hundred forty-six LDLT procedures for HCC were performed at Kyushu University Hospital, Fukuoka, Japan, from July 1995 and November 2009, after prior approval from the Ethics and Indications Committee of Kyushu University. One hundred twenty-eight adult-to-adult LDLT for HCC were included in this study. The selection criteria for the HCC patients were [10] no modality except LDLT available to cure the patients with HCC and end-stage liver disease, [4] no extra-hepatic metastasis, [1] no major vascular infiltration, such as the portal vein or hepatic vein, which ensures that there was no restriction on the tumor size or the number of tumors.

The transplant procedures for both the donors and recipients have been described previously [11–13]. The immunosuppressive regimen was a combination of a calcineurin inhibitor (tacrolimus or cyclosporine) and steroids, with or without mycophenolate mofetil (MMF). Basilixiimab (20 mg) was given intravenously within 6 h after graft reperfusion and on postoperative day 4. A steroid injection was given intraoperatively (methylprednisolone 1 g) and tapered to zero by day 7. Maintenance immunosuppression therapy was conducted with low-dose tacrolimus or cyclosporine from postoperative day 7.

Donor evaluation and selection

The general selection criteria for grafts in adult-to-adult LDLT based on volumetric analysis have been described previously [8]. Briefly, the LL is initially considered for the graft and is generally used. The RL is chosen if the estimated LL with the caudate lobe (CL) volume of the donor is less than 35% of the SLV of the recipient. The person will be excluded as a donor candidate if the RV is less than 35% of the total liver volume (TLV). A biopsy of the donor liver is performed if the CT or ultrasonography study shows the possibility of steatosis, or if the donor's body mass index is greater than 25.

Patient follow-up

The clinical follow-up of patients who underwent HCC followed a strict protocol, which did not change during the study period. The patients were seen bi-weekly for the first month and then monthly for 6 months. The patients underwent ultrasound and enhanced CT examinations at 6 month intervals. Hepatic angiography, bone scintigraphy, or a thoracic CT examination was also performed if there was deterioration in the graft function, or an increase in the AFP or DCP level was noted. The mean follow-up period of the RL and LL graft groups was 3.63 and 3.52 years, respectively.

Statistical analysis

All statistical analyses were performed using the StatView® 5.0 software package (Abacus Concepts, Berkeley, CA, USA). The continuous variables were compared using the Mann–Whitney U test. All variables were expressed as

the means ± SD. The categorical data were compared using the Chi-square test. A logistic regression analysis was performed to identify the independent variables for postoperative complications. The differences were considered to be significant if $p < 0.05$.

Results

Recipients and tumor characteristics according to the graft type

The clinical parameters of the recipients were compared between the two groups according to the graft type (Table 1). Males were predominant in the RL group. The mean age of the LL group was significantly higher than that in the RL group ($p < 0.05$). The rate of patients with a Child-Pugh classification of "C" in the RL group was significantly higher than that in the LL group ($p < 0.05$). The graft volume (436 ± 74 g), as well as the graft volume-standard liver volume rate (38.3 ± 6.2%), of the LL graft group was significantly lower than that in the RL graft group (569 ± 82 g, 46.3 ± 6.7%; $p < 0.01$). Although the duration of surgery of the RL graft group was significantly longer than that of the LL graft group, the intraoperative blood loss was similar in the two groups. The mean length of warm and cold ischemia in the RL group (44 ± 12, 125 ± 66 min) was significantly longer than those of the LL group (38 ± 12, 57 ± 26 min). No difference was found between the two groups with regard to the hepatitis C virus (HCV) infection rate, alpha-fetoprotein (AFP) and DCP levels, number of tumors, maximum tumor size, TNM stage, and Milan classification. There were also no differences in the histological tumor differentiation and vascular invasion between the groups.

Table 1 Recipient and tumor characteristics according to the graft type

Factors	RL graft ($n = 46$)	LL graft ($n = 82$)	p value
Gender			
male (%)	73.9	53.7	0.0242
Age (years)[a]	54.6 ± 6.8	58.7 ± 7.9	0.0034
Hepatitis			
HCV/non-HCV	32/14	63/19	0.3673
Child-Pugh classification			
A/B/C	1/14/31	8/37/37	0.0341
GV (g)[a]	569 ± 82	436 ± 74	<0.0001
GV/SLV (%)[a]	46.3 ± 6.7	38.3 ± 6.2	<0.0001
Length of operation (min)[a]	903 ± 199	772 ± 143	<0.0001
Intraoperative blood loss (ml)[a]	7027 ± 5541	8121 ± 25134	0.7717
Length of warm ischemia (min)[a]	44 ± 12	38 ± 12	0.0038
Length of cold ischemia (min)[a]	125 ± 66	57 ± 26	<0.0001
AFP (ng/ml)			
<300/≥300	40/6	66/16	0.3520
DCP (mAU/ml)			
<300/≥300	37/9	66/16	0.9942
Number of tumors			
≤3/>3	34/12	49/33	0.1075
Tumor size (cm)	2.2 ± 1.3	2.6 ± 1.3	0.1282
Type of HCC			
Initial/recurrent	31/15	54/28	0.8597
Stage			
I/II/III	8/14/21	8/25/45	0.3942
Milan criteria			
Yes/no	29/17	43/39	0.2459
Tumor differentiation (histological)			
Well/mod/por	3/28/14	8/48/26	0.8231
Vascular invasion (histological)			
Yes/no	20/26	33/49	0.7215

AFP alpha-fetoprotein, *DCP* des-gamma-carboxy prothrombin, *GV* graft volume, *GV/SLV* graft volume versus standard liver volume ratio, *HCV* hepatitis C virus

[a] Mean value ± standard deviation

Survival after LDLT according to the graft type

The 1-, 3-, 5- and 7-year overall survival rates of the LL graft group were 88.2, 80.2, 75.7 and 72.4%, respectively, which were not significantly different in comparison to those of the RL graft group (95.4, 87.3, 87.3 and 87.3%, Fig. 1a). The recurrence-free survival rates of the LL graft group (89.1% at 1 year, 78.8% at 3 years, 75.8% at 5 years and 70.3% at 7 years) were also similar to those of the RL graft group (88.6, 88.6, 88.6 and 88.6, Fig. 1b). Patients demonstrating scores beyond the Milan criteria tend to show worse outcomes with LDLT in comparison to DDLT [14], thus, the survival of patients classified beyond the Milan criteria in the current series were also compared based on the graft type. The 1-, 3-, and 5-year overall survival rates of the patients beyond the Milan criteria of the LL graft group ($n = 39$) were 81.7, 70.1 and 65.7%, respectively, which were not significantly different in comparison to those of the patients beyond the Milan criteria in the RL graft group ($n = 17$, 94.1, 73.9 and 73.9%, Fig. 2a). The recurrence-free survival rates of the patients beyond the Milan criteria in the LL graft group (78.7% at 1 year, 65.7% at 3 years and 61.0% at 5 years) were similar to those of the patients beyond the Milan criteria in the RL graft group (74.0, 74.0 and 74.0%, Fig. 2b).

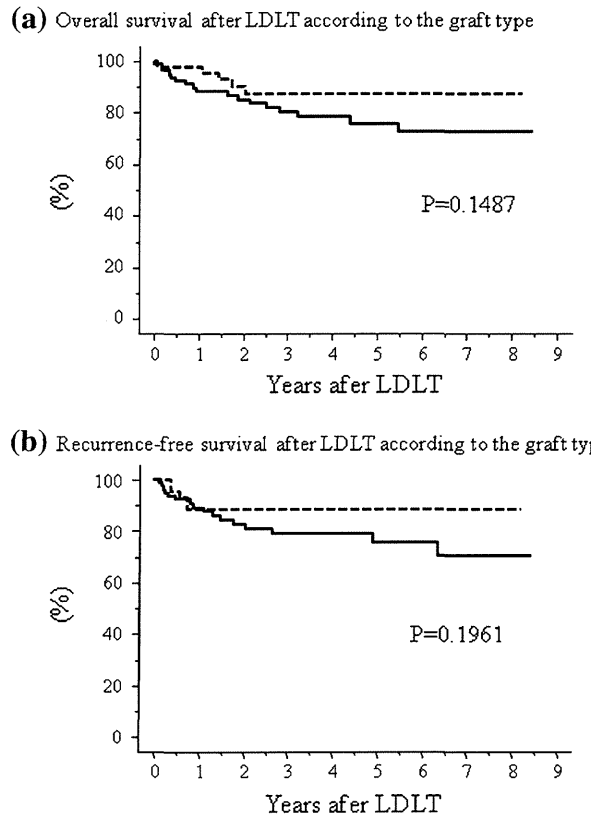


Fig. 1 The overall (**a**) or recurrence-free (**b**) survival after LDLT for 82 patients with LL grafts (*continuous line*) and 46 patients with RL grafts (*dotted line*)

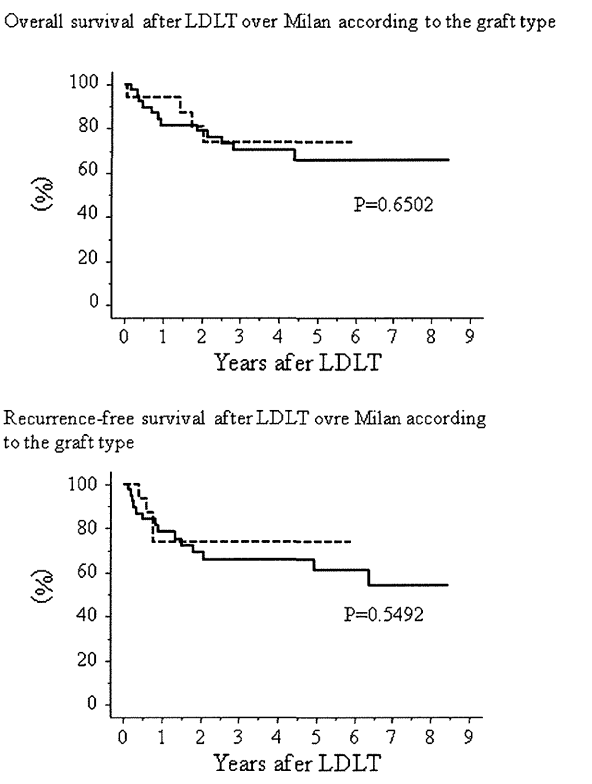


Fig. 2 The overall (**a**) or recurrence-free (**b**) survival after LDLT for 39 patients who were classified beyond the Milan criteria treated with LL grafts (*continuous line*) and 17 patients beyond the Milan criteria treated with RL grafts (*dotted line*)

Donor characteristics according to the graft type

The clinical parameters of the donors were compared between the two groups according to the graft type (Table 2). Males were predominant in the LL group. No difference was found in the duration of the operation and intraoperative blood loss between the groups. The RV ratio of the RL graft group (45.2 ± 6.3%) was significantly lower than that of the LL graft group (64.4 ± 6.2%, < 0.0001). The mean peak postoperative total bilirubin levels and duration of hospital stay after surgery of the LL grafting donors were significantly decreased as compared to those of the RL graft donors ($p < 0.01$). The rate of complications over Clavien's IIIa after the LL graft procurement was 6.2%, which was lower than that in the RL graft group (15.6%).

Table 2 The donor characteristics according to the graft type

Factors	RL graft ($n = 46$)	LL graft ($n = 82$)	p value
Gender (% male)	52.2	79.3	0.0014
Age (years)[a]	36.0 ± 11.0	32.4 ± 8.7	0.0448
Length of operation (min)[a]	445 ± 62	440 ± 73	0.7353
Intraoperative blood loss (ml)[a]	356 ± 305	370 ± 242	0.7807
RV ratio (%)[a]	45.2 ± 6.3	64.4 ± 6.2	<0.0001
Peak ALT (IU/l)[a]	679 ± 351	558 ± 246	0.0247
Peak TB (mg/dl)[a]	3.1 ± 1.6	2.3 ± 1.3	0.0015
Hospital stay (days)[a]	16.7 ± 9.3	11.1 ± 3.5	<0.0001
All complications	13 (28.3)	23 (28.0)	0.9796
Complications over Clavien's II	7 (15.6)	5 (6.2)	0.0856

ALT alanine aminotransferase, *LL* left lobe, *RL* right lobe, *RV* remnant liver volume, *TB* total bilirubin

[a] Mean ± standard deviation

Discussion

The factors involved in liver regeneration may stimulate the growth of occult tumors, thus leading to questions regarding the implications of the type of graft on the disease process and outcome. Donor selection criteria have been established based on the GV and the RV of the donor calculated using three-dimensional-CT, in which an LL graft is considered to be the first choice to ensure donor safety. However, the long-term outcome of the HCC

patients treated with LL grafts has not been elucidated. Thus, the aim of this study was to compare the long-term outcomes between the recipients of LL and RL grafts. Our results showed that the overall survival and recurrence-free survival rates of the LL graft group were similar to those of the RL graft group, although the graft volume of the LL graft group was significantly lower than that of the RL graft group. Furthermore, the overall survival and recurrence-free survival rates after LDLT of the patients beyond the Milan criteria were comparable between the two groups.

It has been unclear whether the rapid liver regeneration after LT can affect the recurrence of HCC. Interestingly, Shi et al. [15] reported experimental data about hepatectomy performed in rats with concomitant implantation of hepatoma cells in the remnant liver. The tumor volume and number increased significantly with the size of the partial hepatectomy, and the largest resections were also associated with increased hepatoma cell infiltration in the lungs. These findings suggest that the liver regeneration after partial hepatectomy may facilitate growth and malignant transformation of microscopic HCC. On the other hand, Hwang et al. [16] showed clinical data about the influence of the graft-recipient weight ratio (GRWR) to assess the risk of HCC recurrence during liver regeneration. The authors divided 181 LT recipients with HCC into four groups according to their GRWR: low GRWR (<0.8), mid GRWR (0.8–1.0), high GRWR (>1.0), and whole liver graft group (>1.5), and found no significant differences in the overall patient survival and recurrence-free survival among these four groups. Therefore, the question of whether liver regeneration influences HCC recurrence remains controversial.

There have been several reports comparing LDLT versus DDLT for HCC [14, 17, 18]. For example, Bhangui et al. [14] performed a comparative intention-to-treat analysis of the recurrence rates and survival outcomes after LDLT and DDLT in HCC patients. The authors reported that the recurrence rates in the two groups were similar (12.9 and 12.7%), and that there was a trend toward a longer time to recurrence after LDLT (38 ± 27 vs. 16 ± 13 months). Furthermore, the overall survival in the two groups was comparable on an intention-to-treat basis. In addition, the outcomes of the 312 HCC patients who underwent LT at 4 Korean institutions were evaluated [17]. A comparison of HCC recurrence curves did not reveal any statistically significant difference between LDLT and DDLT. The current study revealed that the overall survival and recurrence-free survival rates of the LL graft group were similar to those of the RL graft group. There were differences of about 10% in the GV/SLV and about 130 g for the graft volume between the LL and RL grafts, thus resulting in significant differences in the liver regeneration ratio after LDLT. Nonetheless, the type of graft did not affect the long-term outcome after LDLT for the HCC patients in our study. These findings might suggest that rapid liver regeneration does not affect the HCC recurrence after LT.

There have been at least 19 donor deaths associated with LDLT donation [19]. Extensive donor evaluation, detailed preoperative planning, and meticulous surgical technique are essential to minimize donor complications and to avoid donor death. LL grafting, which provides only 30–50% of the required liver volume, has been thought to be inadequate to sustain the metabolic demands of adult recipients. Furthermore, an at least 40–45% graft volume is usually required in the presence of severe portal hypertension. Subsequently, LDLT using RL grafts has gained widespread acceptance. However, the feasibility of LDLT using LL grafts between adults to ensure the safety of the donor has been demonstrated [11]. The current study showed that LL graft procurement was less invasive as compared to RL graft procurement, thus resulting in a lower rate of complications for the donors. LL grafts should therefore be considered more favorably when selecting donors in LDLT for HCC, since the long-term outcomes in the HCC patients with LL grafts were similar to those with RL grafts.

Acknowledgments We thank Professor Brian Quinn for his review of this manuscript.

Conflict of interest None of the authors has any conflict of interest.

References

1. Todo S, Furukawa H. Living donor liver transplantation for adult patients with hepatocellular carcinoma: experience in Japan. Ann Surg.2004;240:451–9 (discussion).
2. Mazzaferro V, Llovet JM, Miceli R, Bhoori S, Schiavo M, Mariani L, et al. Predicting survival after liver transplantation in patients with hepatocellular carcinoma beyond the Milan criteria: a retrospective, exploratory analysis. Lancet Oncol. 2009;10: 35–43.
3. Yao FY. Liver transplantation for hepatocellular carcinoma: beyond the Milan criteria. Am J Transplant. 2008;8:1982–9.
4. Taketomi A, Sanefuji K, Soejima Y, Yoshizumi T, Uhciyama H, Ikegami T, et al. Impact of des-gamma-carboxy prothrombin and tumor size on the recurrence of hepatocellular carcinoma after living donor liver transplantation. Transplantation. 2009;87: 531–7.
5. Fujiki M, Takada Y, Ogura Y, Oike F, Kaido T, Teramukai S, et al. Significance of des-gamma-carboxy prothrombin in selection criteria for living donor liver transplantation for hepatocellular carcinoma. Am J Transplant. 2009;9:2362–71.
6. Sugawara Y, Tamura S, Makuuchi M. Living donor liver transplantation for hepatocellular carcinoma: Tokyo University series. Dig Dis. 2007;25:310–2.
7. Konishi N, Ishizaki Y, Sugo H, Yoshimoto J, Miwa K, Kawasaki S. Impact of a left-lobe graft without modulation of portal flow in adult-to-adult living donor liver transplantation. Am J Transplant. 2008;8:170–4.

8. Yonemura Y, Taketomi A, Soejima Y, Yoshizumi T, Uchiyama H, Gion T, et al. Validity of preoperative volumetric analysis of congestion volume in living donor liver transplantation using three-dimensional computed tomography. Liver Transpl. 2005;11: 1556–62.
9. Fisher RA, Kulik LM, Freise CE, Lok AS, Shearon TH, Brown RS Jr, et al. Hepatocellular carcinoma recurrence and death following living and deceased donor liver transplantation. Am J Transplant. 2007;7:1601–8.
10. Taketomi A, Soejima Y, Yoshizumi T, Uchiyama H, Yamashita Y, Maehara Y. Liver transplantation for hepatocellular carcinoma. J Hepatobiliary Pancreat Surg. 2008;15:124–30.
11. Soejima Y, Taketomi A, Yoshizumi T, Uchiyama H, Harada N, Ijichi H, et al. Feasibility of left lobe living donor liver transplantation between adults: an 8-year, single-center experience of 107 cases. Am J Transplant. 2006;6:1004–11.
12. Taketomi A, Kayashima H, Soejima Y, Yoshizumi T, Uchiyama H, Ikegami T, et al. Donor risk in adult-to-adult living donor liver transplantation: impact of left lobe graft. Transplantation. 2009;87:445–50.
13. Taketomi A, Morita K, Toshima T, Takeishi K, Kayashima H, Ninomiya M, et al. Living donor hepatectomies with procedures to prevent biliary complications. J Am Coll Surg. 2010;211: 456–64.
14. Bhangui P, Vibert E, Majno P, Salloum C, Andreani P, Zocrato J, et al. Intention-to-treat analysis of liver transplantation for hepatocellular carcinoma: living versus deceased donor transplantation. Hepatology. 2011;53:1570–9.
15. Shi JH, Huitfeldt HS, Suo ZH, Line PD. Growth of hepatocellular carcinoma in the regenerating liver. Liver Transpl. 2011; doi:10.1002/lt.22325.
16. Hwang S, Lee SG, Ahn CS, Kim KH, Moon DB, Ha TY, et al. Small-sized liver graft does not increase the risk of hepatocellular carcinoma recurrence after living donor liver transplantation. Transplant Proc. 2007;39:1526–9.
17. Hwang S, Lee SG, Joh JW, Suh KS, Kim DG. Liver transplantation for adult patients with hepatocellular carcinoma in Korea: comparison between cadaveric donor and living donor liver transplantations. Liver Transpl. 2005;11:1265–72.
18. Li C, Wen TF, Yan LN, Li B, Yang JY, Wang WT, et al. Outcome of hepatocellular carcinoma treated by liver transplantation: comparison of living donor and deceased donor transplantation. Hepatobiliary Pancreat Dis Int. 2010;9:366–9.
19. Trotter JF, Adam R, Lo CM, Kenison J. Documented deaths of hepatic lobe donors for living donor liver transplantation. Liver Transpl. 2006;12:1485–8.

Liver Transplant From an ABO-Incompatible and Hepatitis C Antibody-Positive *but an HCV-RNA Negative* Living Donor in a Familial Amyloid Polyneuropathy Patient

Takayuki Takeichi, Katsuhiro Asonuma, Hidekazu Yamamoto, Yuki Ohya, Kenji Okumura, Kwang- Jong Lee, Yukihiro Inomata

Abstract

Familial amyloid polyneuropathy is a rare, progressively disabling, and ultimately fatal inherited disease. Liver transplant is currently the only available treatment proven to halt the progression of familial amyloid polyneuropathy. We report a 31-year-old woman with familial amyloid polyneuropathy who received a living-donor liver transplant from her husband who was hepatitis C virus antibody-positive *but HCV-RNA negative* and ABO incompatible. Six years after the transplant, both donor and recipient have normal liver biochemistry results; no hepatitis C viral load has been detectable in the recipient. This is the first report of a living ABO-incompatible liver transplant from an anti-hepatitis C virus antibody-positive *but an HCV-RNA negative* donor. This experience suggests that the use of an anti-hepatitis C virus antibody-positive hepatic graft is possible in select circumstances.

Key words: *Familial amyloid polyneuropathy, Hepatitis C virus, Living-donor liver transplant, ABO-incompatible transplant*

Introduction

Familial amyloid polyneuropathy (FAP) is an inherited disorder resulting in systemic deposition of amyloid fibrils containing mutant transthyretin variants.[1] The outcome of this disease is so poor that FAP has long been considered incurable. The first successful liver transplant in a patient with FAP was performed in 1990, and since then, liver transplant has become widely used for patients with FAP as a life-saving treatment.[2, 3] In Japan, there is little deceased-donor liver transplant, but living-donor liver transplant (LDLT) has been done in patients with FAP. The living donor is selected from among the patient's relatives. Because FAP is an inherited disorder, candidates for living donor can be difficult to find among the relatives. This may lead to an increased use of marginal living donors. We report the outcome of an ABO-incompatible (ABO-I) liver transplant from an anti-HCV-positive donor to a recipient with FAP.

Case Report

A 31-year-old woman presented to us with no relevant history of disease during her childhood. Neurologic manifestations had appeared 5 years earlier, and she was diagnosed with FAP 3 years after that. She had a familial history of FAP, and her mother had died of FAP at 43 years of age, while her sister was a gene carrier (although no symptoms had developed). Her father had hepatitis C virus (HCV) cirrhosis. She was indicated for liver transplant, and the transplant had to be done quickly because of her 5-year history of FAP and its late diagnosis and far advanced nature. However, the possibility of deceased-donor liver transplant in Japan is not good. The only possible living-donor candidate was her 26-year-old husband, but he had an HCV infection and had received interferon therapy 5 years earlier. Furthermore, his blood type was A, and the recipient's blood type was O; thus, the blood types were incompatible. The results of his liver function tests were normal: total bilirubin, 0.8 mg/dL; aspartate aminotransferase,

From the Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto 860-8556, Japan
Acknowledgements: *No specific funding was provided for this study. The authors of this manuscript have no conflicts of interest to disclose.*
Corresponding author: *Takayuki Takeichi, MD, Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University Hospital 1-1-1 Honjo , Kumamoto 860-8556, Japan*
Phone: *+81 96 373 5618* ***E-mail:*** *ttakeichi@fc.kuh.kumamoto-u.ac.jp*

Experimental and Clinical Transplantation (2012): .....-

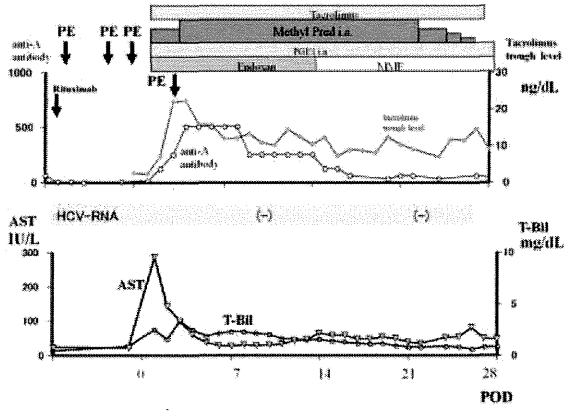
DOI: 10.6002/ect.2012.0158

20 IU/L; alanine aminotransferase, 26 IU/L; alkaline phosphatase, 250 U/L; gamma-glutamyl transpeptidase, 41 U/L; albumin, 4.2 g/dL; and prothrombin time, 12.5 seconds (90%). His viral profile was as follows: HBs antigen (-); HBs antibody (-); anti-HCV (+); and HCV-RNA (-). A needle liver biopsy was done, and the histologic findings showed only mild steatosis, no necrosis, no hepatitis, and no fibrosis. Despite the fact that the husband was anti-HCV-positive and ABO-I, we decided to proceed with an LDLT because her disease prognosis was poor and there was little chance of any other liver donor available. Furthermore, the donor was happy to donate his liver to his wife even though there is a risk to both the donor and the recipient with LDLT. Approval was obtained from the Ethics Committee of Kumamoto University Graduate School of Biomedical Sciences after an interview with the donor and the recipient.

We performed an LDLT using a left lobe graft without the caudate lobe. The surgical procedure for the donor and the recipient has been described elsewhere.[4] The donor's operative duration was 7 hours 32 minutes. The donor's operative blood loss was 470 mL, and no blood transfusion was performed. The total operative duration for the recipient was 10 hours 28 minutes. The actual graft weight was 470 grams, which was 1.04% of the recipient's body weight. The recipient's operative blood loss was 350 mL; thus, no transfusion was necessary.

Because of the ABO-I blood combination, the recipient was treated with an immunosuppression protocol consisting of preoperative rituximab, a plasma exchange, a triple immunosuppressive regimen, intra-arterial infusion therapy, and a splenectomy at surgery (Figure 1). She received 500 mg rituximab intravenously 2 weeks before the LDLT. Her anti-ABO IgM and IgG titers were ×512 and ×256 one week before the operation. Plasma exchange was performed 3 times within 1 week of the LDLT. Her anti-ABO IgM and IgG titers dropped to ×2 and ×4 just before the operation. For hepatic artery infusion, an intra-arterial catheter was placed during the operation, and continuous infusion of prostaglandin E1 (0.01 μg/kg/min on days 0 to 14) and methylprednisolone (125 mg/d on days 0 to 7, 50 mg/d on days 8 to 14; then we tapered the dosage and discontinued the drug on day 21). Endoxan (100 mg) was administered from postoperative days

Figure 1. Time Course After Living-Donor Liver Transplant



Abbreviations: IA, intra-arterial infusion; PE, plasma exchange; PGE1, prostaglandin E1; POD, postoperative day; T-Bil, total bilirubin

(POD) 1 to 14; this was followed by mycophenolate mofetil 500 mg twice a day from POD 15 onward.

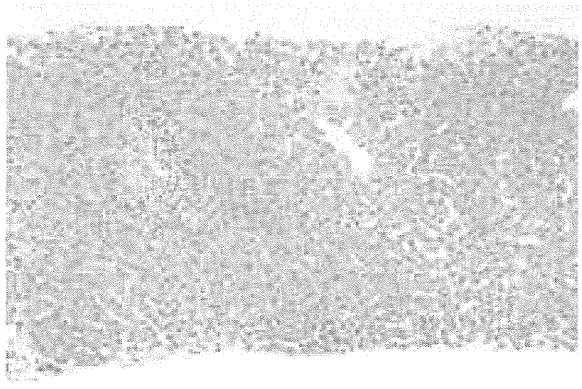
Posttransplant immunosuppression consisted of tacrolimus and steroids. The trough level of tacrolimus was maintained between 10 and 15 ng/mL during the first 2 weeks. Because the anti-ABO IgM and IgG titers rose markedly from the day after transplant, we performed a plasma exchange on POD 3. Although the titers did not decrease immediately, the patient's liver function recovered well. The quantity of steroids in the hepatic artery infusion was increased and the titer gradually decreased. The patient had prolific nausea after transplant probably because of the original disease, but her liver function results recovered to normal on POD 21. Her renal functions were normal before and after the transplant. Hepatitis C virus RNA was not detectable by polymerase chain reaction after the transplant. The hepatic artery catheter was removed on POD 31, and she was discharged from hospital with excellent graft condition 50 days after the operation.

At the time of this writing it has been 6 years after the transplant, and the patient has been well, with excellent graft function, unremarkable liver biochemistry, and has been HCV-RNA negative. Figure 2 shows a liver biopsy 6 years after the transplant, with no evidence of cellular rejection or fibrosis. Progression of FAP is controlled and she has an excellent quality of life.

The postoperative course of the donor also was uneventful. Although serum aspartate aminotransferase increased to 225 IU/L on POD 3, it returned to normal by POD 7. The maximum total

bilirubin level was 2.5 mg/dL on POD3. He left hospital on POD 17. He returned to work 3 months after the operation. At the time of this writing, after 6 years, his liver function test results are normal, and HCV-RNA is negative.

Figure 2. Graft Liver Biopsy 6 Years After Living-Donor Liver Transplant



There was no evidence of fibrosis, hepatitis, or cellular rejection.

Discussion

Liver transplant is the only effective treatment for FAP. More than 65 patients in Japan with FAP have undergone a liver transplant, with living donors consisting of parents, siblings, or husbands; there has been 1 deceased donor.[6] In Japan, organs from deceased donors remain scarce, so that living-related liver transplant is more common. Because FAP is an autosomal dominant inherited disease, potential living donors are restricted. In the present case, there were potentially serious problems for the donor and recipient, such as a risk of flare-up of the HCV infection in the donor, and HCV transmission under strong immunosuppression due to ABO-I matching in the recipient.

ABO-I living-related liver transplant increasingly has been performed in Japan to overcome the shortage of donor organs. Initially, the outcome was poor because of antibody-mediated rejection; however, it has dramatically improved with the use of local steroid infusion and rituximab prophylaxis.[7] In the present case, the patient had no antibody-mediated rejection after receiving a living-related liver transplant.

Several single-center studies have shown no significant differences in survival among HCV-positive recipients transplanted with anti-HCV-positive grafts compared with recipients transplanted with anti-HCV-negative donor organs.[8-10] Saab and associates reported that the use of HCV-positive grafts in recipients with HCV infection does not appear to affect patient survival, graft survival, or recurrence of HCV infection when compared with using anti-HCV-negative grafts.[11] There are several reports of HCV flare-up after chemotherapy and bone marrow transplant in patients with anti-HCV-positive/HCV-RNA-positive grafts.[12-14] The persistence of HCV in patients with previously cleared HCV remains controversial. However, we could not find and research reporting on the use of anti-HCV-positive/HCV-RNA-negative allografts in non-HCV recipients.

In kidney transplant, Nicot and associates have reported the persistence of HCV in immunocompromised transplant patients who were cleared of the virus while on dialysis, but there was no relapse of HCV infection after long-term follow-up despite intensive immunosuppressive therapy.[15] In the current study, although we were concerned about a transmission of HCV virus and de novo HCV hepatitis in the recipient under strong immunosuppression, the patient had a successful posttransplant outcome, with normal liver biochemistry and undetectable HCV in the allograft and serum at 6 years' follow-up.

Conversely, living donor safety is mandatory. In the current case, we also were concerned about an HCV flare-up in the donor after surgery because of the stress of the invasive surgery and liver regeneration, but we could find no reports of an HCV flare-up after hepatectomy. Six years after surgery, the results of the donor's liver function tests are normal and his HCV-RNA remained negative. Although this is a special case of using a marginal donor, an anti-HCV-positive patient with an HCV-RNA negative donor can be taken into consideration for a donor candidate in a special occasion.

In conclusion, we describe the successful transplant of an FAP patient who underwent ABO-I LDLT using a graft from an anti-HCV-positive donor. When the donor is anti-HCV-positive and HCV-RNA-negative with normal liver histology, transplant may be considered in some situations. Long-term follow-up is required for donor and recipient.

References

1. Ikeda S, Hanyu N, Hongo M, et al. Hereditary generalized amyloidosis with polyneuropathy. Clinicopathological study of 65 Japanese patients. *Brain.* 1987;110 (Pt 2):315-337.
2. Takei Y, Ikeda S, Ikegami T, et al. Ten years of experience with liver transplantation for familial amyloid polyneuropathy in Japan: outcomes of living donor liver transplantations. *Intern Med.* 2005;44(11):1151-1156.
3. Ando Y. Liver transplantation and new therapeutic approaches for familial amyloidotic polyneuropathy (FAP). *Med Mol Morphol.* 2005;38(3):142-154.
4. Tanaka K, Uemoto S, Tokunaga Y, et al. Surgical techniques and innovations in living related liver transplantation. *Ann Surg.* 1993;217(1):82-91.
5. Inomata Y, Uemoto S, Asonuma K, Egawa H. Right lobe graft in living donor liver transplantation. *Transplantation.* 2000;69(2):258-264.
6. The Japanese Liver Transplantation Society. Liver Transplantation in Japan —Registry by the Japanese Liver Transplantation Society—. *Isyoku* 2010;44:621-632.
7. 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(1):143-152.
8. Velidedeoglu E, Desai NM, Campos L, et al. The outcome of liver grafts procured from hepatitis C-positive donors. *Transplantation.* 2002;73(4):582-587.
9. Marroquin CE, Marino G, Kuo PC, et al. Transplantation of hepatitis C-positive livers in hepatitis C-positive patients is equivalent to transplanting hepatitis C-negative livers. *Liver Transpl.* 2001;7(9):762-768.
10. Busuttil RW, Tanaka K. The utility of marginal donors in liver transplantation. *Liver Transpl.* 2003;9(7):651-663.
11. Saab S, Ghobrial RM, Ibrahim AB, et al. Hepatitis C positive grafts may be used in orthotopic liver transplantation: a matched analysis. *Am J Transplant.* 2003;3(9):1167-1172.
12. Vento S, Cainelli F, Mirandola F, et al. Fulminant hepatitis on withdrawal of chemotherapy in carriers of hepatitis C virus. *Lancet.* 1996;347(8994):92-93.
13. Aksoy S, Abali H, Kilickap S, Erman M, Kars A. Accelerated hepatitis C virus replication with rituximab treatment in a non-Hodgkin's lymphoma patient. *Clin Lab Haematol.* 2006;28(3):211-214.
14. Zekri AR, Mohamed WS, Samra MA, Sherif GM, El-Shehaby AM, El-Sayed MH. Risk factors for cytomegalovirus, hepatitis B and C virus reactivation after bone marrow transplantation. *Transpl Immunol.* 2004;13(4):305-311.
15. Nicot F, Kamar N, Mariamé B, Rostaing L, Pasquier C, Izopet J. No evidence of occult hepatitis C virus (HCV) infection in serum of HCV antibody-positive HCV RNA-negative kidney-transplant patients. *Transpl Int.* 2010;23(6):594-601.

Surg Today
DOI 10.1007/s00595-012-0368-5

CASE REPORT

Sclerosing encapsulating peritonitis after living donor liver transplantation: a case successfully treated with tamoxifen: report of a case

Takayuki Takeichi · Yasuko Narita · Kwang-Jong Lee · Hidekazu Yamamoto · Katsuhiro Asonuma · Yukihiro Inomata

Received: 21 February 2012 / Accepted: 17 May 2012

Abstract Sclerosing encapsulating peritonitis (SEP) is a rare cause of bowel obstruction. It is difficult to diagnose and the prognosis is poor. This report describes a case of SEP after living donor liver transplantation that was successfully treated with tamoxifen. A 56-year-old male, that had received a liver transplant for hepatitis C virus-related hepatocellular carcinoma 5 years earlier, was admitted with continuous abdominal pain and nausea. He had increased C-reactive protein levels and white blood cell count, and underwent laparotomy 5 days after hospitalization. The surgical findings showed ascites and SEP of the small bowel. An attempt to peel off the adhesions was stopped because there was a strong risk of intestinal tract damage. Tamoxifen treatment was initiated for SEP after surgery. The patient's symptoms gradually improved and he was able to resume feeding. He had been symptom-free for over 3 years at the last follow-up.

Keywords Sclerosing encapsulating peritonitis · Living donors · Liver transplantation · Liver cirrhosis · Tamoxifen

Introduction

Sclerosing encapsulating peritonitis (SEP) is a rare cause of bowel obstruction and stenosis [1], and is characterized by thick, fibrous membrane formation in the peritoneum. It is difficult to diagnose prior to surgery and the outcome of SEP is poor with a high mortality [2]. Surgery is the primary treatment option, but this is still associated with significant mortality, and there is a high rate of recurrence [3]. SEP is an uncommon but serious complication of long-term peritoneal dialysis. Liver transplantation is the established treatment for the patients of end-stage liver disease [4]. There are few reports of SEP after liver transplantation. This report presents a case of SEP after living donor liver transplantation that was successfully treated with tamoxifen, a drug reported to have efficacy in SEP [5, 6].

Case report

A 58-year-old male received a liver transplant 5 years previously because of decompensated end-stage liver disease caused by hepatitis C virus-related liver cirrhosis and hepatocellular carcinoma that fulfilled the Milano criteria. He had ascites prior to liver transplantation, but no history of ascites drainage. He underwent living donor liver transplantation with a right lobe graft from his wife. The parietal peritoneum and retroperitoneal liver were observed to be normal during surgery. The patient started immunosuppressive therapy after liver transplantation, with tacrolimus, steroids, and mycophenolate mofetil. He had a splenectomy after a decrease in the platelet count 1 year after liver transplantation, as a result of treatment for recurrence of hepatitis C. He was then started on interferon therapy for hepatitis C but this was stopped 9 months later because of the occurrence of pancytopenia. His general condition continued to be relatively stable. He was admitted with abdominal pain and nausea 5 years after liver transplantation. Laboratory tests found an increase in C-reactive protein (2.5 mg/dL) and white blood cell count (12000/mm^3). He had colicky abdominal pain, but no

T. Takeichi (✉) · Y. Narita · K.-J. Lee · H. Yamamoto · K. Asonuma · Y. Inomata
Department of Transplantation and Pediatric Surgery, Postgraduate School of Medical Science, Kumamoto University, 1-1-1 Honjo, Kumamoto 860-8556, Japan
e-mail: ttakeichi@fc.kuh.kumamoto-u.ac.jp

Published online: 26 October 2012

rebounding pain was found on physical examination. Contrast abdominal computed tomography (CT) revealed no obstruction of the small intestine, but it did show a dilation of the loops of the small bowel (Fig. 1). He received conservative treatment, but little improvement occurred, with continued abdominal pain and inability to tolerate oral feeding. The patient's abdominal distension increased 5 days later, so he underwent emergency surgery, because an adhering ileus was suspected. Laparotomy revealed dense, thick, adhesive sheaths wrapped around the loops of the small bowel (Fig. 2). The sheath extended from 50 cm from the ligament of Treitz to 50 cm from the terminal ileum. The remaining normal small bowel was only about 100 cm long. The surgeons attempted to release the adhesions of the small bowel, but it was difficult to remove the tight fibrous sheath. These attempts were stopped because of concerns of intestinal perforation, and the abdomen was closed. Medical treatment was initiated surgery, with 20 mg tamoxifen daily in addition to immunosuppressive therapy (tacrolimus and prednisone),

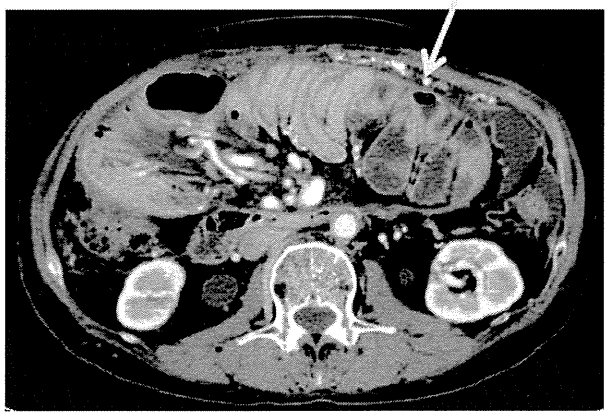


Fig. 1 Computed tomography of the abdomen showing dilated loops of the small bowel with a small amount of ascites

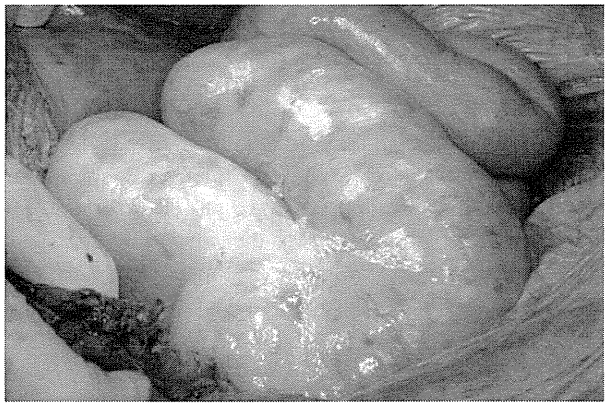


Fig. 2 Surgical findings show dense thick adhesive sheaths wrapped around the loops of the small bowel

which had been administered since transplantation. His condition improved and he was able to start oral feeding 7 days postoperatively. The postoperative recovery continued uneventfully, and he was discharged 3 weeks later. A CT scan showed improvement in the dilatation of the small bowel. He had been symptom-free for over 3 years with the continued administration of tamoxifen when this report was submitted.

Discussion

Sclerosing encapsulating peritonitis is a rare complication recognized as a cause of intestinal obstruction. It was first described in 1907 as peritonitis chronica fibrosa incapsulata by Owtschinnikow [7]. Foo et al. [8] were the first to describe the "abdominal cocoon" in adolescent girls. The causes of SEP are unclear, but the major possible causes are chronic peritoneal dialysis, ventriculoperitoneal shunt and end-stage liver disease complicated by ascites and spontaneous bacterial peritonitis [9–11]. The current patient had no episode of massive ascites or peritonitis after liver transplantation, thus the cause of SEP was not determined. The mortality rate of SEP is high, usually as a result of recurrence and sepsis [3, 12]. In particular, SEP is a dangerous complication arising from long-term dialysis [13]. Dialysis may predispose patients to peritoneal irritation and inflammation, with resulting fibrosis. Although surgery is an essential treatment, with the goal of enterolysis of the membranes and adhesions, the outcome is influenced by the grade of the inflammation and the thickness of the adhesions. The prognosis is particularly poor in cases of long-term SEP, and in cases with strong adhesion. The surgeons could not peel the strong adhesions of the thickened membranes because of the danger of perforating the intestine. There are several reports of effective medical treatment for SEP, which includes treatment with corticosteroid, immunosuppression and tamoxifen [5, 6]. Tamoxifen has been used in the treatment of fibrosing diseases, such as desmoid tumors and idiopathic retroperitoneal fibrosis [14, 15], and in the treatment of breast cancer [16]. Tamoxifen stimulates the production of transforming growth factor $\beta 1$ (TGF-$\beta 1$), which in turn stimulates the expression of metalloproteinase (MMP)-2 and MMP-9 [17]. Therefore, TGF-$\beta 1$ may promote mesothelial healing by facilitating the removal of denatured collagen, since MMP-9 degrades type IV and denatured collagens. However, the detailed mechanism of the action of tamoxifen on SEP has not been proven. The patient had been receiving immunosuppressive treatment with a steroid and tacrolimus since his liver transplant. The addition of tamoxifen to the immunosuppressive regimen was very effective in this patient.

Table 1 The demographic data of SEP after LT

References	Age	Gender	Original disease	Pre-LT history	Post-LT history	Diagnosis after LT	Procedure	Maintenance immunosuppression	Post-medical treatment	Outcome
Abuls et al. [16]	52	M	HCV-LC	SBP	Abdominal bleeding	8 months	Adhesiolysis	Prograf	Aldactone	Alive (6 months)
Mekeel et al. [17]	42	M	HCV-LC	SBP, massive ascites	re-LTx for HCV recurrence	2 years[a]	Adhesiolysis	Prograf, corticosteroid	None	Died
	62	M	HCV-LC,HCC	SBP,ascites	HV stenosis, BD stenosis	At LT	Adhesiolysis and intestine resection	Prograf, corticosteroid	None	Died
	59	M	ETOH-LC	SBP, ascites	CMV infection	5 months	Adhesiolysis	Prograf	None	Died (9 months)
Yamamoto et al. [8]	57	M	HBV-LC	Hemodialysis, SBP		At LT	Adhesiolysis	Unexplained	None	Died (17 days)
Lin et al. [18]	64	M	HBV-LC,HCC	PVS for asites		2 weeks	Adhesiolysis	Prograf,Cellcept	None	Alive
Maguire et al. [19]	16–57 years	4 M and 1 F	Unexplained	Unexplained	Unexplained	58 ± 22 days	Adhesiolysis	Unexplained	Unexplained	Mortality 20 %
This case	58	M	HCV-LC,HCC	Ascites	Splenectomy, PVstenosis	5 years	Lapalotomy	Prograf, corticosteroid	Tamoxifen	Alive (3 years)

PVS peritoneal venous shunt

SBP spontaneous bacterial peritonitis

[a] From second LTx

There have so far been few previous reports of SEP associated with liver transplantation. There are four case reports and one series report, for a total of 12 patients, including the present case (Table 1) [11, 18–21]. Five cases had experienced spontaneous bacterial peritonitis before transplantation, with major ascites and a peritoneal venous shunt was performed for refractory ascites before liver transplantation in most cases. The time of SEP diagnosis was up to 5 years from the time of liver transplantation. All patients underwent laparotomy and all of the cases, excluding the current case, underwent enterolysis. There was no other medical treatment apart from immunosuppressive drugs. The addition of tamoxifen to tacrolimus and prednisone was effective for SEP after laparotomy in the current case. It is possible that the drugs had a synergistic effect against SEP. The outcome of SEP is generally considered to be poor and Mekeel et al. reported three patients that developed SEP after liver transplantation, all of whom died. Five of the 12 reported cases of SEP have died.

In conclusion, this report described a case of SEP associated with living donor liver transplantation, and successful treatment using tamoxifen. SEP is difficult to treat and has a poor prognosis. Treatment with tamoxifen may be beneficial for patients with SEP that cannot be treated surgically.

Conflict of interest Takeichi and other co-authors have no conflicts of interest to declare.

References

1. Wei B, Wei HB, Guo WP, Zheng ZH, Huang Y, Hu BG, Huang JL. Diagnosis and treatment of abdominal cocoon: a report of 24 cases. Am J Surg. 2009;198:348–53.
2. Célicout B, Levard H, Hay J, Msika S, Fingerhut A, French Associations for Surgical Research. Sclerosing encapsulating peritonitis: early and late results of surgical management in 32 cases. Dig Surg. 1998;15:697–702.
3. Kawanishi H, Moriishi M, Tsuchiya S. Experience of 100 surgical cases of encapsulating peritoneal sclerosis: investigation of recurrent cases after surgery. Adv Perit Dial. 2006;22:60–4.
4. Raut V, Uemoto S. Management of ABO-incompatible living-donor liver transplantation: past and present trends. Surg Today. 2011;41:317–22.
5. Eltoum MA, Wright S, Atchley J, Mason JC. Four consecutive cases of peritoneal dialysis-related encapsulating peritoneal sclerosis treated successfully with tamoxifen. Perit Dial Int. 2006;26(2):203–6.
6. Evrenkaya TR, Atasoyu EM, Unver S, Basekim C, Baloglu H, Tulbek MY. Corticosteroid and tamoxifen therapy in sclerosing encapsulating peritonitis in a patient on continuous ambulatory peritoneal dialysis. Nephrol Dial Transpl. 2004;19(9):2423–4.
7. Owtschinnikow PJ. Peritonitis chronica fibrosa incapsulata. Arch Klin Chir. 1907;83:623–34.
8. Foo KT, Ng KC, Rauff A, Foong WC, Sinniah R. Unusual small intestinal obstruction in adolescent girls: the abdominal cocoon. Br J Surg. 1978;65:427–30.

9. Summers AM, Clancy MJ, Syed F, Harwood N, Brenchley PE, Augustine T, Riad H, Hutchison AJ, Taylor P, Pearson R, Gokal R. Single-center experience of encapsulating peritoneal sclerosis in patients on peritoneal dialysis for end-stage renal failure. Kidney Int. 2005;68:2381–8.
10. Sigaroudinia MO, Baillie C, Ahmed S, Mallucci C. Sclerosing encapsulating peritonitis–a rare complication of ventriculoperitoneal shunts. J Pediatr Surg. 2008;43:E31–3.
11. Yamamoto S, Sato Y, Takeishi T, Kobayashi T, Hatakeyama K. Sclerosing encapsulating peritonitis in two patients with liver cirrhosis. J Gastroenterol. 2004;39:72–175.
12. Balasubramaniam G, Brown EA, Davenport A, Cairns H, Cooper B, Fan SL, et al. The Pan-Thames EPS study: treatment and outcomes of encapsulating peritoneal sclerosis. Nephrol Dial Transpl. 2009;24(10):3209–15.
13. Trigka K, Dousdampanis P, Chu M, Khan S, Ahmad M, Bargman JM, Oreopoulos DG. Encapsulating peritoneal sclerosis: a single-center experience and review of the literature. Int Urol Nephrol. 2011;43:519–26.
14. Kinzbrunner B, Ritter S, Domingo J, Rosenthal CJ. Remission of rapidly growing desmoid tumors after tamoxifen therapy. Cancer. 1983;52:2201–4.
15. Clark CP, Vanderpool D, Preskitt JT. The response of retroperitoneal fibrosis to tamoxifen. Surgery. 1991;109:502–6.
16. Mathew J, Asgeirsson KS, Jackson LR, Cheung KL, Robertson JF. Neoadjuvant endocrine treatment in primary breast cancer—review of literature. Breast. 2009;18:339–44.
17. Overall CM, Wrana JL, Sodek J. Independent regulation of collagenase, 72kD progelatinase and metalloendoproteinase inhibitor expression in human fibroblasts by transforming growth factor-b. J Biol Chem. 1989;264:1860–9.
18. Abul S, Al-Oazweni H, Zalat S, Al-Sumait B, Asfar S. Cocoon abdomen in a liver transplant patient. J R Coll Surg Edinb. 2002;47:579–81.
19. Mekeel K, Moss A, Reddy KS, Douglas D, Mulligan D. Sclerosing peritonitis and mortality after liver transplantation. Liver Transpl. 2009;15:435–9.
20. Lin CH, Yu JC, Chen TW, Chan DC, Chen CJ, Hsieh CB. Sclerosing encapsulating peritonitis in a liver transplant patient: a case report. World J Gastroenterol. 2005;11:5412–3.
21. Maguire D, Srinivasan P, O'Grady J, Rela M, Heaton ND. Sclerosing encapsulating peritonitis after orthotopic liver transplantation. Am J Surg. 2001;182:151–4.

Transplant Infectious Disease, ISSN 1398-2273

Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation?

S. Eguchi, M. Hidaka, A. Soyama, M. Takatsuki, H. Miyaaki, T. Ichikawa, K. Nakao, T. Kanematsu. Is liver-targeted FOXp3 staining beneficial after living-donor liver transplantation? Transpl Infect Dis 2012: **14**: 156–162. All rights reserved.

Abstract: As treatments for acute cellular rejection (ACR) and recurrent hepatitis caused by hepatitis C virus (HCV) are dramatically different, making a precise diagnosis is considered to be essential in patients after liver transplantation. Therefore, we investigated whether immunohistochemical detection of FOXp3, a marker for regulatory T cells (CD4+ CD25+), could be used to differentiate between recurrent hepatitis C and ACR. From a group of 103 cases of living-donor liver transplantation (LDLT), 48 samples were taken via liver biopsy from 20 patients with HCV infection. An initial diagnosis was made based on hematoxylin and eosin staining, which was scored with the hepatitis activity index (HAI) grading, whereas ARC was scored with the rejection activity index (RAI). The FOXp3 immunohistochemical staining on serial specimens was retrospectively analyzed, scoring from 0 to III. The time after LDLT was a median of 270 (range: 14–2000) days, whereas the median number of biopsies per patient was 3 (range: 1–8). The HAI was significantly different between 0 vs. I, and II vs. III, in terms of the FOXp3 score. On the other hand, a significant difference in the RAI was only found between 0 vs. I. In conclusion, FOXp3 may represent a surrogate marker for recurrent HCV infection after LDLT.

S. Eguchi[1], M. Hidaka[1], A. Soyama[1], M. Takatsuki[1], H. Miyaaki[2], T. Ichikawa[2], K. Nakao[2], T. Kanematsu[1]

[1]Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, [2]Gastroenterology and Hepatology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Key words: FOXp3; recurrent hepatitis C; acute cellular rejection; living-donor liver transplantation; CD25

Correspondence to:
S. Eguchi, MD,
Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, 1-7-1 Sakamoto, Nagasaki
852-8501, Japan
Tel: 81 95 819 7316
Fax: 81 95 819 7319
E-mail: sueguchi@nagasaki-u.ac.jp

Received 24 May 2011, revised 19 July 2011, accepted for publication 26 August 2011

DOI: 10.1111/j.1399-3062.2011.00690.x
Transpl Infect Dis 2012: **14**: 156–162

Recurrent hepatitis C virus (HCV) infection after liver transplantation (LT) remains a therapeutic challenge, especially in a setting with living donors, where the possibility of retransplantation is limited. To date, the best treatment for chronic HCV infection is interferon (IFN) combined with ribavirin, with a 50% sustained virologic response rate (1). In fact, diagnosis of recurrent hepatitis C is sometimes difficult to make, because the presentation, in the histologic view, has many similarities to rejection (2).

Regulatory T cells (Tregs) are supposed to regulate an over-reactive autoimmune response, and were detected to be CD4+ CD25+. Tregs are engaged in the maintenance of self-tolerance by suppressing the activation and expansion of self-reactive lymphocytes (3–5). Loss of this suppressing function may lead to chronic inflammation and/or autoimmunity (6–12). Currently, the best indicator of Tregs function is thought to be the intracellular expression of forkhead box P3 (FOXp3), which is also crucial for Tregs development (13). One of our co-authors (H.M.) previously reported the usefulness of examining liver-targeted Tregs as indicators of chronic hepatitis B virus and HCV infection (14).

In the setting of LT, it was also reported that needle biopsy could provide a source for determining FOXp3 messenger RNA (mRNA) expression after LT (15). In addition, it was reported that FOXp3 mRNA in peripheral blood is a useful marker for acute cellular rejection (ACR), whereas CD4+ CD25+ numbers in peripheral blood may be a marker to predict recurrence of HCV after LT (16, 17).

However, to the best of our knowledge, studies have not previously examined the use of FOXp3 in

Characteristics of liver transplant patients with HCV

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis		FOXp3	IFN
				HAI	RAI		
1	58/M	1b	1800	5	4	0	+
			2000	5	3	1	+
2	57/F	1b	540	0	0	0	+
			720	3	0	2	+
3	53/F	1b	30	3	3	0	–
			180	6	6	1	–
			540	3	7	0	+
			1020	3	0	2	+
4	52/M	II	20	1	8	1	–
			35	6	4	2	–
5	63/F	1b	150	3	4	1	+
			270	4	0	2	+
			510	5	0	2	+
			630	0	0	1	– (SVR)
6	57/M	1b	14	1	4	0	–
			180	6	2	2	+
			540	6	0	0	+
7	55/F	1b	180	3	6	1	–
			194	7	5	3	–
			208	6	4	2	–
			360	7	3	1	+
			1080	7	3	1	+
8	64/F	1b	21	2	3	2	–
			74	2	3	2	–
			180	6	3	3	+
9	61/F	1b	30	3	0	3	–
			720	4	2	1	–
			780	4	2	1	–
10	62/M	II	60	6	4	3	+
11	67/M	1b	14	7	7	1	–
			400	7	3	2	+
			720	3	5	1	–
12	58/M	1b	90	2	1	0	–
			360	3	6	1	+
			720	2	4	2	+ (SVR)
13	51/F	1b	450	10	5	3	–
			900	10	6	3	–
			1380	10	5	3	+
			1835	10	4	3	+
14	59/M	1b	360	2	0	0	+ (SVR)
15	54/M	1b	390	0	0	0	– (SVR)
16	68/F	1b	150	3	3	1	–
			300	6	3	1	+
17	59/F	1b	180	4	0	0	–
			360	5	0	0	+
			480	2	1	0	+
18	65/F	1b	30	4	4	1	–
			120	9	6	3	+
			135	3	3	2	+

Table 1 continued

Pt no.	Age/gender	Genotype	Days after LDLT	Histologic diagnosis		FOXp3	IFN
				HAI	RAI		
19	59/M	1b	21	4	3	0	–
			41	1	3	0	–
20	65/M	1b	480	4	0	2	+
			570	3	0	1	+

HCV, hepatitis C virus; Pt no., patient number; LDLT, living-donor liver transplantation; HAI, hepatitis activity index; RAI, rejection activity index; FOXp3, marker for regulatory T cells; IFN, interferon; M, male; F, female; SVR, sustained virologic response.

Table 1

liver infiltrating lymphocytes to differentiate recurrent HCV infection from ACR.

Patients and methods

Patients

Of 103 cases of living-donor LT (LDLT), 29 patients (mean age: 57.8 ± 10.6, male:female ratio: 17:12) were positive for anti-HCV antibodies. Fifty-eight samples were taken via liver biopsy from 20 patients (Table 1). Liver biopsy tissue specimens were taken by a needle puncture for diagnostic purposes. HCV serotype was type I in 18 of those patients, whereas it was type II in 2 patients. In all patients, IFN therapy was eventually attempted. Immunosuppression was based on our protocol using cyclosporine as previously reported (1).

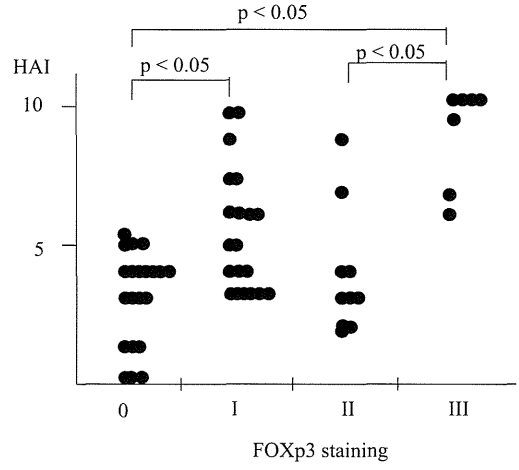


Fig. 1. The relationship between hepatitis activity index (HAI) grading and FOXp3 staining. Significant differences were seen between 0 and I, II and III, and 0 and III with regard to the FOXp3 staining.

Methods

All tissues were fixed in 10% neutral buffered formalin and were then embedded in paraffin, and 4-mm-thick serial sections were cut from each paraffin block. T cells were examined immunohistochemically using an anti-CD4 antibody (Novocastra, Newcastle, UK).

Initial diagnosis was made based on hematoxylin and eosin (H&E) staining, followed by FOXp3 immunohistochemical staining (eBioscience, San Diego, California, USA) on serial specimens. Among aggregated lymphocytes, the number of FOXp3-positive CD4+ lymphocytes was scored as 0 = none, I = 1–9 cells, II = 10–19 cells, and III = >20 cells, as in our previous report (14). The association of FOXp3 with hepatitis activity index (HAI) and/or rejection activity index (RAI) (median 3, range: 0–8) was investigated.

To classify the degree of hepatic inflammation (hepatic activity), we used the HAI score as described by Knodell et al. (18). Based on their criteria, the H&E-stained specimens of the non-cancerous liver tissues were examined and classified into 4 categories. ACR was scored based on the RAI according to the Banff schema (19, 20).

All data are expressed as the median values with ranges. The statistical analysis was performed using the Mann–Whitney *U*-test for continuous values, and the chi-squared test for categorical values. A significant difference was defined as a *P*-value of <0.05. The StatView 5.0 statistical software package (Abacus Concepts, Berkeley, California, USA) was used for all statistical analyses.

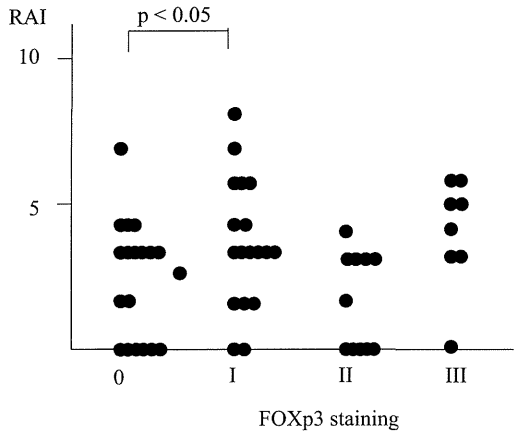


Fig. 2. The relationship between the rejection activity index (RAI) and FOXp3 staining. A significant difference was only seen between 0 and I in terms of FOXp3 staining with regard to the RAI.

Results

Table 1 showed characteristics of the patients and a summary of the histologic findings. The median days from the time of liver biopsy was 270 days (range: 14–2000 days) after LT. The median grade, based on the HAI, was 4 (0–10). The median degree of rejection, based on the RAI, was 3 (0–8). The difference in HAI was significant between 0 vs. I, as well as II vs. III, based on the number of FOXp3-positive cells (Fig. 1). On the other hand, a significant difference in the RAI was only seen for 0 vs. I (Fig. 2).

Figure 3 shows a representative liver biopsy specimen of a patient with recurrent HCV infection after LT. The patient was a 58-year-old woman who had undergone LT 4 years earlier. Her aspartate aminotransferase (AST)/alanine aminotransferase (ALT) levels were elevated at 107/80. H&E staining revealed an HAI grade of 10 and RAI of 5.

Figure 4 shows another representative liver biopsy specimen. This patient was a 62-year-old woman who underwent LT 4 months before the biopsy. Her AST/ALT levels were elevated at 68/65. H&E staining revealed an HAI grade of 9 and RAI of 6.

On the other hand, Figure 5 shows a representative liver biopsy specimen from a patient with ACR. The patient was 58-year-old woman who had undergone LT 4 years before the biopsy samples were taken. Her AST/ALT levels were elevated to 120/108. H&E staining revealed HAI grade of 3 and RAI of 6.

Discussion

This study examined the distribution and frequency of the appearance of Tregs in the liver after LT. After LT, in patients with hepatitis C, both ACR and recurrent HCV can lead to elevation of transaminases, in

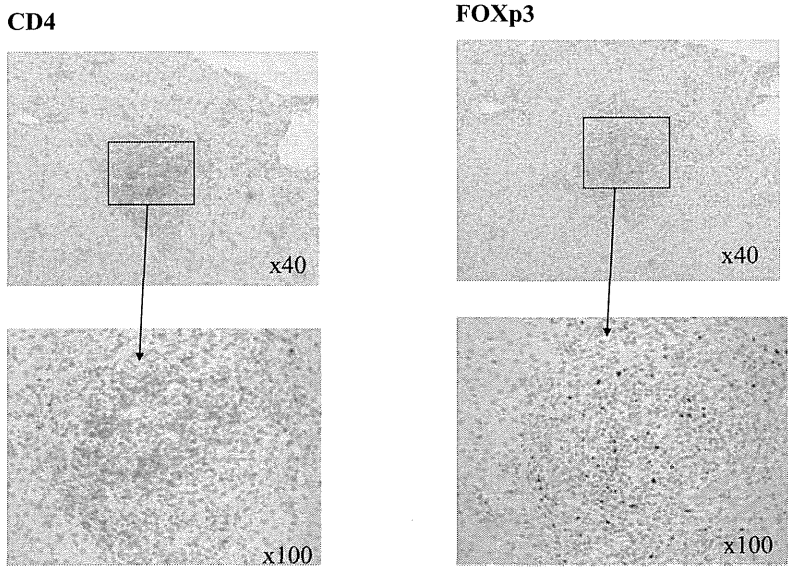


Fig. 3. Case 13. Representative findings in a liver with recurrent hepatitis C. Many liver infiltrating lymphocytes were positive for CD4 and FOXp3. HAI, hepatitis activity index; RAI, rejection activity index.