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Original Article

Impact of tumor size, number of tumors and neutrophil-to-lymphocyte ratio in liver transplantation for recurrent hepatocellular carcinoma

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Aim: Hepatocellular carcinoma (HCC) is primarily treated with hepatic resection and/or locoregional therapy. When HCC recurs and further treatment is no longer possible owing to poor liver function, liver transplantation (LT) or living-donor LT (LDLT) is considered. The aim of this study was to clarify risk factors for tumor recurrence after LDLT in patients with recurrent HCC.

Methods: The study comprised 104 patients who had undergone LDLT because of end-stage liver disease with recurrent HCC. The recurrence-free survival rates after the LDLT were calculated. Risk factors for tumor recurrence were identified.

Results: The 1-, 3- and 5-year recurrence-free survival rates were 89.6%, 80.3% and 78.4%, respectively. By univariate analysis, the factors affecting recurrence-free survival were the sum of the largest tumor size and number of tumors of 8 or more ($P < 0.0001$), des-γ-carboxy prothrombin of more than 300 mAU/mL ($P = 0.0001$), and a neutrophil-to-lymphocyte ratio (NLR) of 4 or more ($P = 0.0002$), α-fetoprotein of more than 400 ng/mL ($P = 0.0001$) and bilobar tumor distribution ($P = 0.046$). A multivariate analysis identified independent risk factors for post-LDLT tumor recurrence including the sum of tumor size and number of tumors of 8 or more ($P = 0.0004$) and an NLR of 4 or more ($P = 0.01$). The 1- and 3- year recurrence-free survival rates in the recipients who had both risk factors were 30.0% and 15.0%, respectively.

Conclusion: LDLT should not be performed for patients who have both independent risk factors after any treatments for HCC.

Key words: hepatocellular carcinoma, living-donor liver transplantation, neutrophil-to-lymphocyte ratio, number of tumors, tumor size

INTRODUCTION

A SHORTAGE OF cadaveric organs for transplantation continues to impair our ability to provide liver transplantation (LT) despite progress in surgical techniques and immunosuppression.[1,2] Currently, there is no consensus on how to manage patients with hepatocellular carcinoma (HCC) while awaiting LT. Guidelines published in the UK state that locoregional therapy, such as transarterial chemoembolization (TACE), radiofrequency ablation (RFA), ethanol injection therapy and microwave coagulation therapy (MCT), should be considered for all listed patients with HCC.[3] In Asian countries, religious, cultural and political ideologies have created significant obstacles to the transplantation of organs from cadavers. As a result, HCC is primarily treated with hepatic resection and/or locoregional therapy.[4,5] However, when HCC recurs and further treatment is no longer possible owing to poor liver function, LT is considered.[4] Organ shortages have forced patients with recurrent HCC to endure long waiting periods that are associated with tumor development. Thus, living-donor LT (LDLT) is a potential choice for treating recurrent HCC patients after the use of other

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treatments.[4] Since the 1994 report demonstrating successful LDLT, living donors have been increasingly used because of the disparity between demand and supply, even in Western countries.[2,6] Moreover, a blood relationship between the donor and the recipient in LDLT may give the recipient a chance to receive a transplant even during the suboptimal conditions of HCC.[7–9]

Thus, it is important to focus on factors that affect tumor recurrence after LDLT in patients with recurrent HCC.

The neutrophil-to-lymphocyte ratio (NLR) has recently emerged as a useful prognostic factor for the recurrence of several malignancies. An NLR of 5 or more was reported to be a marker of survival in colorectal cancer patients.[10] Halazun *et al.* reported that an NLR of five or more was an independent predictor of the recurrence and poor overall survival in patients with colorectal liver metastases.[11] Recently, it was demonstrated that a preoperative NLR of 5 or more was an adverse predictor of recurrence-free survival for patients undergoing hepatic resection for HCC.[12] Furthermore, an elevated NLR significantly increased the risk of HCC recurrence after LT[13] or LDLT.[14]

Mazzaferro *et al.* recently proposed the "up-to-seven criteria", with 7 being the result of the sum of the largest tumor size (in cm) and number of tumors, to predict patient survival after LT, based on a large sample size.[15] We have reported the outcome of LDLT for otherwise unresectable and/or untreatable HCC patients[7,16] and proposed two risk factors for recurrence-free survival: a tumor size greater than 5 cm and des-γ-carboxy prothrombin (DCP) levels greater than 300 mAU/mL (Kyushu University [KU] criteria).[7] Furthermore, we previously reported a series of 68 cases of LDLT for patients who had received pretransplant treatment for HCC.[4] DCP above 300 mAU/mL was shown to be an independent risk factor for tumor recurrence after LDLT in the published work. Since this report, LDLT has become a more common treatment for such patients, thus generating a larger cohort for study.

Therefore, the aim of the present study was to clarify the risk factors of tumor recurrence after LDLT in patients with recurrent HCC.

METHODS

Recipients

ONE HUNDRED AND sixty-seven recipients underwent LDLT because of end-stage liver disease with HCC at Kyushu University Hospital between April 1999 and August 2012. In this study, 104 adult patients (41 female and 63 male) were enrolled who had undergone LDLT because of end-stage liver disease with recurrent HCC after treatment. The pretransplant treatments for HCC, such as RFA, TACE, MCT and/or hepatic resection, were dependent upon the recipient's liver function and tumor status. Graft types included left lobe with caudate lobe graft ($n = 63$), right lobe graft without the middle hepatic vein ($n = 37$) and posterior segment graft ($n = 4$). The etiology of liver cirrhosis included hepatitis C ($n = 75$), hepatitis B ($n = 20$), cryptogenic disease ($n = 4$), alcohol abuse ($n = 3$) and primary biliary cirrhosis ($n = 2$) (Table 1). Our selection criteria to perform LDLT for HCC patients were as follows: (i) no modality except LDLT available to cure the patients with HCC; (ii) no extrahepatic metastasis; and (iii) no major vascular infiltration.[4,7] There were no restrictions on the tumor size, number of tumors or pretransplant treatment. Since defining the KU criteria, we have not performed LDLT for HCC patients with a tumor size greater than 5 cm and DCP levels greater than 300 mAU/mL.

Pretransplant imaging was used to estimate the maximum tumor size, number of tumors and up-to-seven criteria. α-Fetoprotein (AFP), DCP and NLR were measured before the LDLT. The histological grades obtained from the explanted livers were used for tumor differentiation.

Donor and graft selection

Donors were selected from among the candidates who hoped to be living donors.[1,8] Donors were required to be within the third degree of consanguinity with recipients or spouses, and to be between 20 and 65 years of age. For a donor who was not within the third degree of consanguinity, individual approval was obtained from the Ethics Committee of Kyushu University Hospital. Good Samaritan donations were not used.

Eligible donors proceeded to the imaging studies, including chest and abdominal X-rays and 3-mm-slice computed tomography (CT) scans for graft volumetric analysis. 3-D CT was introduced for volumetric analysis and delineation of vascular anatomy. The standard liver weight (SLW) of recipients was calculated according to the formula of Urata *et al.*[17] Graft weight (GW) was predicted by CT volumetric analysis. Decisions regarding the graft type for recipients were based on the preoperatively predicted GW to SLW (GW : SLW) ratio. The left lobe with caudate lobe graft was used when the preoperatively predicted GW : SLW ratio was more than 35%. A posterior segment graft was used when the donor's vascular variation was suitable to take the posterior segment.

Table 1 Characteristics of recipients and donors

Variables	*n*
Recipient	
Sex (male/female)	63/41
Age (years, range)	58.0 (41–72)
Etiology	
HCV	75
HBV	20
Cryptogenic	4
Alcohol	3
PBC	2
MELD score (range)	11.5 (4–31)
Diabetes mellitus (yes/no)	31/73
Splenectomy (yes/no)	60/44
CNI (TAC/CyA/None)	44/57/3
Donor	
Sex (male/female)	75/29
Age (years, range)	34.3 (20–63)
Graft (left/right/posterior)	63/37/4
GW : SLW ratio (%, range)	41.0 (23.6–67.6)
Tumor	
Maximum size (cm, range)	2.4 (0–7.0)
n (range)	17 (0–400)
Milan criteria (yes/no)	52/52
NLR (range)	3.1 (0.44–20.2)
AFP (ng/mL, range)	1516 (1–43 000)
DCP (mAU/mL, range)	349 (3–5934)
Duration between first Tx and LDLT (days, median, range)	1198 (61–4272)
Duration between last Tx and LDLT (days, median, range)	349 (30–2140)
Times of treatment (range)	3 (1–11)
Microvascular invasion (yes/no)	39/65
Pathological differentiation (well/moderate/poor)	7/63/34

AFP, α-fetoprotein; CNI, calcineurin inhibitor; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HBV, hepatitis B virus; HCV, hepatitis C virus; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus; Tx, pretransplant treatment.

Postoperative management

The graft retrieval technique, recipient surgery and perioperative management of the recipients, including immunosuppression regimens, have been described elsewhere.[9,18] Immunosuppression was initiated using a protocol based on either tacrolimus (Prograf; Astellas Pharma, Tokyo, Japan) or cyclosporin A (Neoral; Novartis Pharma, Tokyo, Japan) with steroid and/or mycophenolate mofetil (MMF; Chugai Pharmaceutical, Tokyo, Japan). Tacrolimus was used in 44 recipients and cyclosporin in 57 recipients. Three recipients did not receive calcineurin inhibitor owing to postoperative poor disease course. A target trough of tacrolimus was set at 10 ng/mL for 3 months after LDLT, followed by 5–10 ng/mL thereafter. A target trough level of cyclosporin A was set at 250 ng/mL for 3 months after LDLT, followed by 150–200 ng/mL thereafter. Methylprednisolone was initiated on the day of LDLT, tapered and converted to prednisolone 7 days after LDLT. Prednisolone treatment was tapered and discontinued 6 months after LDLT. MMF was used in 91 recipients and was started at 1000 mg/day on the day after LDLT, tapered and discontinued until 6 months after LDLT. A trough level was not measured for MMF.

All patients had monthly follow ups, and the median follow-up period was 1738 days, with 723 days and 2891 days as the 25th and 75th percentiles, respectively.

Post-LDLT tumor recurrence and risk factors

Hepatocellular carcinoma recurrence after the LDLT was set as the primary end-point of this study. All patients underwent abdominal CT scan every 3 months, and chest CT scan and bone scintigraphy every 6 months within 5 years after LDLT. Tumor recurrence was defined as when any imaging studies revealed the recurrence of HCC. Recurrence-free survival was defined as the time period between LDLT and tumor recurrence.

Univariate and multivariate analyses were performed to identify the factors associated with recurrence-free survival after the LDLT.

Statistical analysis

Recurrence-free survival rates were calculated by the Kaplan–Meier product-limited method. Data were expressed as means.

Cox regression analysis was applied to the multivariate analyses. Variables that were used for the analysis included recipient age, donor age, Model for End-Stage Liver Disease score, presence of hepatitis C virus, presence of diabetes mellitus, recipient sex, donor sex, GW : SLW ratio, the sum of the largest tumor size (in cm) and the number of tumors, pretransplant NLR, pretransplant AFP, pretransplant DCP, graft type, splenectomy, duration between first treatment for HCC and the LDLT, duration of last treatment for HCC and the LDLT, times of pretransplant treatment and type of calcineurin inhibitor. All statistical analyses were performed using JMP ver. 9.0 software (SAS, Cary, NC, USA). $P < 0.05$ was considered significant.

Approval of institutional review board

The Institutional Review Board of Kyushu University Hospital approved this study protocol (no. 23–58).

RESULTS

THE CHARACTERISTICS OF the recipients and donors from this study are shown in Table 1. Fifty-two of 104 patients (50.0%) exceeded the Milan criteria. Patients previously underwent at least one of the following treatments for primary or recurrent HCC: TACE ($n = 85$), RFA ($n = 54$), ethanol injection therapy ($n = 30$), MCT ($n = 17$), hepatic resection ($n = 11$) and hepatic arterial infusion chemotherapy ($n = 7$). Median times of treatment were 3.0 (1–11 times), median duration from first treatment to LDLT was 1199 days (61–4272 days) and median duration from last treatment to LDLT was 348 days (30–2140 days).

Receiver–operator curve (ROC) analysis for tumor recurrence after LDLT was used to detect the cut-off line of the sum of the largest tumor size (in cm) and number of tumors, and NLR. The area under the ROC (AUROC) of the sum of the largest tumor size (in cm) and number of tumors was 0.833. A cut-off value of the sum was set as 8.0, because ROC analysis revealed that a cut-off value of 8, which had 84.2% of the sensitivity and 80.0% of the specificity, was the most suitable value. Similarly, the AUROC of NLR was 0.700 and a cut-off value of NLR of 4 was set using the analysis.

The 1-, 3- and 5-year recurrence-free survival rates in enrolled recipients were 89.6%, 80.3% and 78.4%, respectively. Among the 104 recipients, 19 patients developed tumor recurrence after LDLT. A univariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more, had an NLR of 4 or more, AFP levels of more than 400 ng/mL, DCP levels of more than 300 mAU/mL and bilobar tumor distribution were risk factors for tumor recurrence after LDLT ($P < 0.0001$, $P = 0.0002$, $P < 0.0001$, $P < 0.0001$, and $P = 0.046$, respectively) (Table 2). Although the nodule size and number of nodules were risk factors of tumor recurrence by the univariate analysis, these factors statistically interfered with the sum of the largest tumor size (in cm) and number of tumors for performing multivariate analysis. The AUROC of the number of nodules was 0.790 and that of the largest nodule size was 0.753. Both data were less than that of the sum of the largest tumor size and number of tumors (0.833). Thus, we selected the sum of the largest tumor size and number of tumors for multivariate analysis. Multivariate analysis revealed that the sum of the largest tumor size (in cm) and number of tumors of 8 or more and an NLR of 4 or more were independent risk factors for tumor recurrence after LDLT in this study ($P = 0.0004$ and $P = 0.011$, respectively) (Table 3).

Table 4 shows the correlation between explant pathology and each risk factor. The frequency of microvascular invasion and poorly differentiated tumors increased among patients who had both independent risk factors of tumor recurrence.

The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an NLR of 4 or more were 100%, 81.8% and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0%, and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained (Fig. 1). The differences among the four groups were significantly different ($P < 0.0001$).

DISCUSSION

THIS IS THE largest study to investigate LDLT with recurrent HCC.[4] It is crucial to clarify when patients with poor liver function and HCC should be listed as candidates for LDLT. We chose recurrence-free survival rate as the end-point in this study because preliminary analysis revealed that 27 deaths occurred in the enrolled recipients, of which 14 causes of death were not tumor-related.

To date, several studies have attempted to extend the Milan criteria to encompass HCC patients with potentially curable tumors.[7,14,19–22] The up-to-seven criteria may predict patient survival even after LDLT.[4,14] The ROC analysis for tumor recurrence after LDLT revealed that the sensitivity of the cut-off value of 7 was 89.4% and the specificity was 71.7%. It meant that a cut-off value of 7 was less suitable than that of 8 in this study. Although we previously proposed that the number of tumors did not affect tumor recurrence after LDLT,[4,7,16] the results obtained from the present study suggest that the number of tumors as well as largest tumor size should be taken into consideration to select HCC patients for LDLT.

The precise mechanism of how NLR affects tumor recurrence is still unclear. Infiltration of pro-inflammatory macrophages, cytokines and chemokines in the tumor microenvironment can boost tumor

Table 2 Risk factors for tumor recurrence: univariate analysis

Variables	*n*	Recurrence-free survival (%)			*P*
		1 year	3 years	5 years	
Recipient variables					
Sex					
Male	63	84.5	82.7	79.5	0.81
Female	41	97.4	75.7	75.7	
Age (years)					
>60	46	88.1	82.3	82.3	0.67
≤60	58	90.8	79.1	76.1	
Etiology					
HCV	75	88.8	79.6	77.2	0.64
Others	29	91.4	82.0	82.0	
Pretransplant MELD					
<15	84	91.2	80.1	78.0	0.99
≥15	20	82.1	82.1	82.1	
Diabetes mellitus					
Yes	31	89.1	84.4	78.8	0.75
No	73	89.7	78.5	78.5	
NLR					
≥4	21	72.7	55.9	41.9	0.0002
<4	83	93.5	86.2	86.2	
Splenectomy					
Yes	60	90.9	79.9	79.9	0.82
No	44	87.8	80.2	77.4	
Calcineurin inhibitor					
TAC	44	90.0	80.9	80.9	0.78
CyA	57	89.4	80.1	77.3	
Donor variables					
Sex					
Male	75	92.7	82.9	80.4	0.34
Female	29	82.1	74.1	74.1	
Donor age (years)					
>40	25	95.2	89.6	89.6	0.19
≤40	79	88.0	77.6	75.3	
Graft type					
Others	67	90.2	75.4	72.5	0.13
Right	37	88.6	88.6	88.6	
GW : SLW ratio					
<35	24	86.1	76.0	76.0	0.62
≥35	80	90.5	81.5	79.1	
Tumor variables					
Nodule size (cm)					
≥5	6	50.0	33.3	33.3	0.0004
<5	98	92.2	83.5	81.4	
No. of nodules					
≥5	34	75.2	58.0	58.0	0.0002
<5	70	96.8	91.6	88.7	
Nodule size + number					
≥8.0	33	67.9	46.4	46.4	<0.0001
<8.0	71	100	96.5	93.8	

Table 2 *Continued*

Variables	*n*	Recurrence-free survival (%)			*P*
		1 year	3 years	5 years	
DCP (mAU/mL)†					
>300	19	51.6	38.7	38.7	<0.0001
≤300	84	97.3	89.5	87.1	
AFP (ng/mL)					
>400	22	75.8	53.1	44.3	<0.0001
≤400	82	93.3	87.5	87.5	
Tumor distribution					
Bilobar	65	85.3	74.7	72.1	0.046
Unilobar	39	97.0	90.4	90.4	
Duration between the first treatment and the LDLT					
<1 year	21	80.0	68.7	68.7	0.20
≥1 year	83	92.1	83.3	80.7	
Duration between the last treatment and the LDLT					
<1 year	72	86.5	76.5	76.5	0.26
≥1 year	32	96.6	89.1	82.3	
Times of treatment					
≥4	36	85.0	67.9	67.9	0.06
<4	68	91.9	86.7	83.9	

†Data of one case was lacking because of warfarin intake.
AFP, α-fetoprotein; CyA, cyclosporin A; DCP, des-γ-carboxy prothrombin; GW, graft weight; HCV, hepatitis C virus; KU, Kyushu University; LDLT, living-donor liver transplantation; MELD, Model for End-Stage Liver Disease; NLR, neutrophil-to-lymphocyte ratio; SLW, standard liver weight; TAC, tacrolimus.

growth, invasion and metastases.[23,24] Recently, Motomura *et al.* reported that interleukin (IL)-17-producing T cells are thought to release CXC chemokines that recruit neutrophils, leading to elevated NLR, and promote the differentiation of tissue macrophages in peritumoral regions into tumor-associated macrophages (TAM).[14] Both IL-17-producing T cells and TAM may accelerate tumor progression and antitumor T-cell exhaustion. As shown in Table 4, pathological examination revealed poorly differentiated HCC and microvascular invasion in the explanted liver in seven of eight recipients who had both independent risk factors of tumor recurrence. The use of routine biopsy to identify tumor grading has been abandoned owing to concerns of tumor seeding, leading to an extensive search for suitable surrogate markers to predict tumor differentiation or vascular invasion. Halazun *et al.* showed that elevated NLR correlated with microvascular invasion and poorly differentiated tumors.[13] The results from our study are consistent with this previous report. The interpretation

Table 3 Risk factors for tumor recurrence: multivariate analysis

Variables	Odds ratio	95% CI	*P*
Nodule size + number ≥8.0	15.2	3.34–68.9	0.0004
NLR ≥4	4.02	1.38–11.6	0.011
DCP >300 mAU/mL	3.09	0.87–11.0	0.082
AFP >400 ng/mL	1.23	0.37–4.08	0.73
Bilobar distribution	1.12	0.24–5.21	0.88

AFP, α-fetoprotein; CI, confidence interval; DCP, des-γ-carboxy prothrombin; NLR, neutrophil-to-lymphocyte ratio.

Table 4 Correlation between explant pathology and risk factors

Variables	No risk factor ($n = 58$)	NLR ≥4 ($n = 13$)	Tumor size and number of tumors ≥8 ($n = 25$)	Both risk factors ($n = 8$)	*P*
Microvascular invasion	12 (20.7%)	4 (30.8%)	16 (64.0%)	7 (87.5%)	<0.0001
Poorly differentiated tumor	12 (20.7%)	3 (23.1%)	12 (48.0%)	7 (87.5%)	0.0005

NLR, neutrophil-lymphocyte ratio.

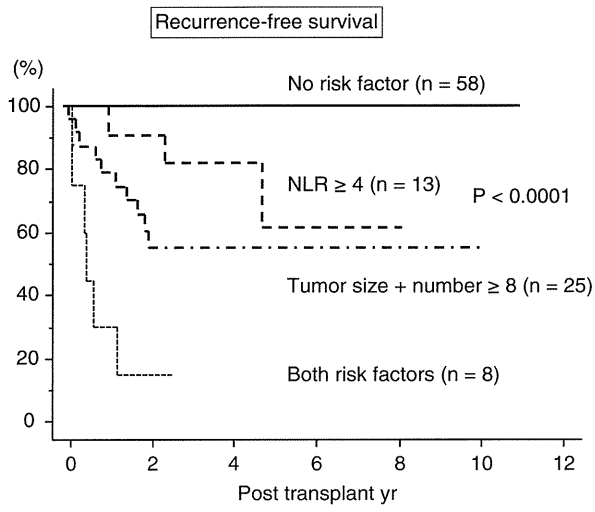

Figure 1 Recurrence-free recipient survival after living-donor liver transplantations for hepatocellular carcinoma. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had no risk factor ($n = 58$) were all 100%. The 1-, 3- and 5-year recurrence-free survival rates in recipients who had the sum of the largest tumor size (in cm) and number of tumors of 8 or more were 78.9%, 55.4% and 55.4%, respectively. Those in patients who had an neutrophil-to-lymphocyte ratio (NLR) of 4 or more were 100%, 81.8%, and 61.4%, respectively. The 1- and 3-year recurrence-free survival rates in recipients who had both risk factors were 30.0% and 15.0%, respectively. The 5-year recurrence-free survival rate could not be obtained. The differences among the four groups were significantly different ($P < 0.0001$). yr, years.

of NLR in patients with end-stage liver disease, often complicated with hypersplenism and pancytopenia, seems to require caution. Furthermore, patients with end-stage liver disease often develop specific bacterial peritonitis or other bacterial infections because of impaired immune system. There may be limitation for the evaluation of NLR in such patients.

Seventy-eight of 104 patients underwent pretransplant treatment more than twice in this study. Moreover, the times of pretransplant treatment, the interval between the first treatment and LDLT, and the interval between the last pretransplant treatment and LDLT did not affect the outcome of LDLT. Next, we focused on how to predict patients with a high risk of tumor recurrence after LDLT. For the univariate and multivariate analysis, we chose variables that had been obtained before transplantation. The 5-year recurrence-free survival rate after the LDLT was 100% for recipients who did not have both risk factors of tumor recurrence. Therefore, according to our results, HCC can be treated with any treatment modality whenever the patient's liver function is tolerable to such treatments. However, patients who have the sum of the largest tumor size (in cm) and the number of tumors of 8 or more and have an NLR of 4 or more should be excluded from LDLT. Further study is needed on whether LDLT can be performed for patients who have a single independent risk factor or not, because the 5-year recurrence-free survival rate for patients who had the sum of the largest tumor size (in cm) and the number of tumors of 8 or more was 55.4%, and for patients who had an NLR of 4 or more was 61.4%. A recent report recommended giving psychosocial considerations careful attention for both donor and recipient in LDLT.[25]

In conclusion, the type or duration of treatment for HCC did not affect the outcome of LDLT, but LDLT should not be performed for patients who have the sum of the largest tumor size (in cm) and number of tumors of 8 or more and with an NLR of 4 or more after any treatments for HCC to prevent tumor recurrence.

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Original Article

Early extensive viremia, but not rs8099917 genotype, is the only predictor for cholestatic hepatitis C after living-donor liver transplantation

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Aim: Cholestatic hepatitis C is one of the most serious but still unaddressed disorders after liver transplantation.

Methods: In this study, we analyzed 49 patients who underwent living-donor liver transplantation (LDLT) to treat hepatitis C virus (HCV) infection.

Results: Five patients developed cholestatic hepatitis C, with total bilirubin of 15.2 ± 3.1 mg/dL at diagnosis 6.2 ± 1.0 weeks after LDLT. Univariate analysis showed that larger graft to standard liver volume ratio, higher HCV RNA titer at 2 weeks, earlier peak HCV RNA titer and cytomegalovirus infection were the significant risk factors. The development of cholestatic hepatitis C was not significantly associated with interleukin-28B genotype (rs8099917); four out of five affected patients had the T/T genotype. Multivariate analysis showed that higher HCV RNA titer at 2 weeks was the only significant factor ($P = 0.026$) for the development of cholestatic hepatitis C. Receiver–operator curve analysis showed that that HCV RNA titer of more than 7.2 $\log_{10}$IU/mL was the optimal cut-off for characterizing cholestatic hepatitis C. All of the patients were serum HCV RNA negative after treatment with pegylated interferon and ribavirin and all the patients are alive.

Conclusion: Early extensive viremia, but not the rs8099917 genotype, was the only predictor for cholestatic hepatitis C after LDLT.

Key words: cholestatic hepatitis, hepatitis C, interleukin 28B, liver transplantation, living donor, splenectomy

INTRODUCTION

ALTHOUGH END-STAGE LIVER disease secondary to hepatitis C virus (HCV) is the leading indication for liver transplantation (LT), re-infection of HCV is a widespread, unaddressed and serious event.[1] It has been reported that approximately one-quarter of patients develops cirrhosis within 10 years after LT for HCV; therefore, graft outcomes after LT for HCV are inferior to those for other indications.[2]

Nevertheless, recurrent hepatitis C after LT is represented by a spectrum of disorders, including mild to severe inflammation with various degrees of fibrosis progression over several years.[1,2] Of note, HCV re-infection can result in very aggressive hepatitis in a small number of patients, and is usually characterized by rapid progression of cholestasis with fibrosis resulting in graft failure and death.[3,4] This outcome has been termed post-transplant cholestatic hepatitis C and its risk factors include higher donor age, HCV genotype 1, extremely high viral titers and bolus steroid administration for acute rejection.[3,4] More recently, two reports

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have shown that single nuclear polymorphism (SNP) in the interleukin (IL)-28B gene was a significant risk factor for the disease process.[5,6] To date, however, the pathogenesis of recurrent cholestatic hepatitis C after LT has not been elucidated.

Therefore, in the current study, we examined the clinical characteristics of patients who developed this rare type of recurrent cholestatic hepatitis C after living-donor liver transplantation (LDLT). We investigated whether its pathogenesis could be attributed to viral factors, host factors, including IL-28B genotypes or graft-related factors.

METHODS

Patients

LIVING-DONOR LIVER TRANSPLANTATION was performed in 54 patients positive for the HCV antibody at Kyushu University Hospital between February 2007 and July 2012. All procedures were approved by the Ethics and Indications Committee of Kyushu University. Forty-nine patients who were HCV RNA positive before LDLT were included in the current study. The mean follow-up time was 2.8 ± 1.1 years.

Transplantation and postoperative care

The surgical procedures for both the donors and the recipients are described in more detail elsewhere.[7,8] The graft type, either left or right lobe, was determined based on the need for a graft volume (GV) of more than 35% of the recipient's standard liver volume (SLV).[7] Splenectomy was performed for 47 (95.9%) recipients to prevent pancytopenia caused by interferon (IFN) therapy.[9] A biliary stent over the biliary anastomosis was placed during the surgery and was kept in place for 3–4 months after LDLT to prevent early stricture.[10]

The immunosuppression regimen consisted of tacrolimus or cyclosporin with mycophenolate mofetil and steroids as previously reported.[8] The immunosuppression level was maintained at a standard level to prevent acute rejection; unfortunately, this hinders the diagnosis and treatment of hepatitis C after LDLT. The tacrolimus level was maintained at 10–14 ng/mL for 1 month after LDLT and was then decreased to 7–10 ng/mL over the next few months. The cyclosporin level was maintained at 150–250 ng/mL for 1 month after LDLT and then decreased to 100–150 ng/mL over the next few months. Mycophenolate mofetil at the dose of 2 g/day, was then tapered down to 1 g daily over 1–3 months and tapered off at 6 months. All the patients received steroids during the study period. Methylprednisolone (1 g) was given after reperfusion, and titrated from 200 mg/day to 20 mg/day in a week, then switched to oral prednisolone, and tapered off by 6 months. The immunosuppression protocol for blood type-incompatible LDLT consisted of pretransplant rituximab and plasma exchanges with tacrolimus or cyclosporin and mycophenolate mofetil and steroids, as previously described.[11]

Antiviral treatment

Interferon was indicated for recurrent hepatitis C associated with serum HCV RNA positivity, abnormal liver function tests and histological evidence of recurrent hepatitis C. Preemptive antiviral treatment was not performed.

Antiviral treatment consisted of pegylated (PEG) IFN-α-2b with ribavirin (Pegintron with Rebetol; Merck, Whitehouse Station, NJ, USA) or PEG IFN-α-2a with ribavirin (Pegasys with Copegus; Chugai Pharmaceutical, Tokyo, Japan) was used for antiviral treatment. Although PEG IFN-α-2b was primarily used for post-transplant induction of antiviral treatment, PEG IFN-α-2a could also be used for refractory or severe cases. The type of PEG IFN drug, regarding conversion between the products, was determined for individual cases. PEG IFN-α-2b and ribavirin were started at doses of 0.5–1.0 mcg/kg per week and 200–400 mg/day, respectively. The doses were escalated in a stepwise manner, in accordance with the individual's tolerability, to 1.5 mcg/kg per week and 800 mg/day, respectively. PEG IFN-α-2a and ribavirin were started at doses of 90–120 mcg/week and 200–400 mg/day, respectively, to 180 mcg/week and 800 mg/day respectively. The recommended duration of treatment was 48 weeks after achieving viral response (VR), defined as undetectable serum HCV RNA.

Measurement of the serum HCV RNA titer

The serum HCV RNA titer was determined by a real-time HCV assay (AccuGene HCV; Abbott Molecular, Des Plaines, IL, USA). The lower and higher limits of quantification for this assay are 1.08 log IU/mL and 8.0 log IU/mL, respectively. The serum HCV RNA titer was measured before LDLT, 2 weeks after LDLT and monthly thereafter.

IL-28B genotyping assay

DNA from the donors and the recipients was extracted from a biopsy or explanted liver tissue obtained during LDLT, and genotyping was performed using TaqMan

GTX press Master Mix (Life Technologies, Tokyo, Japan), in accordance with the manufacturer's instructions. The Custom TaqMan SNP Genotyping Assay (Life Technologies) was used to identify IL-28B genetic polymorphisms. We used rs8099917 as the representative SNP for IL-28B because of its higher sensitivity and specificity for IFN sensitivity in Asian individuals.[12] The T/T genotype of rs8099917 was defined as the major allele, while the T/G and G/G genotypes were regarded as the minor alleles.

Diagnosis of cholestatic hepatitis

Cholestatic hepatitis C was defined according to the factors as proposed by Wiesner *et al.*[13] with minor modifications: (i) total bilirubin of more than 6 mg/dl; (ii) elevated biliary enzymes with alkaline phosphatase (ALP) and/or γ-glutamyltransferase (GGT) of more than 5 times the upper limit of normal; (iii) very high serum HCV RNA titer of more than 6 log IU/mL; (iv) histological findings that include predominant ballooning of hepatocytes in the perivenular zone and limited inflammation; (v) occurring between 1 and 6 months after LT; and (vi) absence of surgical complications at the time of diagnosing cholestatic hepatitis C.

Percutaneous liver biopsy was obtained and evaluated for patients with abnormal liver function tests suggestive of recurrent hepatitis C or acute rejection. Biopsies were also obtained every year in accordance with the established protocol.

Statistical analysis

Values are expressed as the mean ± standard deviation. Variables were analyzed using the χ^2-test for categorical values or the Mann–Whitney *U*-test for continuous variables. Multivariate analyses were performed using the logistic regression model and odds ratios were calculated. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of patients with cholestatic hepatitis C

FIVE PATIENTS DEVELOPED cholestatic hepatitis C after LDLT (Table 1). The mean ages of the donors and the recipients were 58.2 ± 7.7 years and 29.2 ± 10.0 years, respectively. The mean GV/SLV ratio was 45.0 ± 7.3%. Donor age was less than 40 years old in all of the cases except for case 5. GV/SLV was more than 35% in all of the cases, except in case 3. Splenectomy was performed in all five cases.

Hepatitis C virus genotype was type 1b, except in case 4 (2a) and the mean HCV RNA titer before LDLT was 5.2 ± 0.7 $\log_{10}$IU/mL. The HCV RNA titer was more than 5 $\log_{10}$IU/mL in all the cases except case 5. The IL-28B (rs8099917) genotype was T/T in both the donors and recipients except in case 2, where the donor and recipient both had the T/G genotype.

The mean values of liver function parameters were 15.2 ± 3.1 mg/dL for total bilirubin, 357 ± 79 IU/L for aspartate aminotransferase (AST) and 859 ± 497 IU/L for GGT. The peak HCV RNA titer was 7.9 ± 0.1 $\log_{10}$IU/mL and more than 7.7 $\log_{10}$IU/mL in all five patients at diagnosis of cholestatic hepatitis C, 6.2 ± 1.0 weeks after LDLT. Although cases 1 and 5 had biliary anastomotic stenosis after LDLT, this complication occurred after treatment for cholestatic hepatitis C.

All of the five patients were treated with PEG IFN with ribavirin after histological confirmation of cholestatic hepatitis C. PEG IFN-α-2b was used in two patients and PEG IFN-α-2b was used in three patients. VR was observed in all of the patients. Among the patients who received IFN ($n = 41$) after LDLT, the total dosage of IFN was larger in patients with ($n = 5$) cholestatic hepatitis C (10.5 ± 3.0 vs 6.0 ± 4.6 mg, $P = 0.040$), compared with those without ($n = 36$). However, the total dosage of ribavirin (24.6 ± 26.1 vs 24.4 ± 20.7 g, $P = 0.981$) and the treatment period (90.0 ± 44.7 vs 62.2 ± 38.8 g, $P = 0.147$) was not different between the groups. Discontinued antiviral treatment was observed in no case in the patients with cholestatic hepatitis ($n = 5$) and 10 cases (27.8%) in the patients without ($n = 36$) due to intolerance and adverse reactions. Dose modification of IFN during the treatment course was observed in three patients (60%) and 18 patients (50.0%), respectively.

Risk factors for cholestatic hepatitis C

We next determined possible risk factors for cholestatic hepatitis C after LDLT. In univariate analyses, larger GV/SLV (45.0 ± 7.3% vs 39.2 ± 5.9%, $P = 0.049$), higher HCV RNA titer at 2 weeks after LDLT (7.7 ± 0.4 vs 5.8 ± 1.3 $\log_{10}$IU/mL, $P = 0.002$), earlier period for having peak HCV RNA titer (3.7 ± 2.3 vs 9.4 ± 5.6 weeks, $P = 0.031$) and cytomegalovirus infection (80.0% vs 27.2%, $P = 0.017$) were significantly associated with cholestatic hepatitis C after LDLT. By contrast, donor and recipient age, cold and warm ischemic time, HCV genotype, and donor and recipient IL-28B genotype were not associated with the occurrence of cholestatic hepatitis C (Table 2).

Table 1 Clinical characteristics of the five cases of cholestatic hepatitis C

Case	1	2	3	4	5
Recipient age, sex	54, F	62, F	52, M	53, F	70, F
MELD score	16	18	8	18	12
Hepatocellular carcinoma	Yes	Yes	Yes	No	Yes
Splenectomy	Yes	Yes	Yes	Yes	Yes
Donor age, sex	21, F	36, M	20, M	23, M	43, F
Immunosuppression regimen	FK-based	CyA-based	CyA-based	CyA-based	CyA-based
ABO incompatible	No	Yes	Yes	No	No
Graft type	Left	Left	Left	Right	Right
GV (g)	460	440	510	598	502
GV/SLV (%)	39.9	44.0	37.0	55.4	48.9
HCV genotype	1b	1b	1b	2a	1b
HCV RNA titer (log_{10}IU/mL)	5.7	5.7	5.3	5.5	3.9
Recipient IL-28B genotype	T/T	T/G	T/T	T/T	T/T
Donor IL-28B genotype	T/T	T/G	T/T	T/T	T/T
Peak liver function tests					
Total bilirubin (mg)	17.4	13.6	19.1	16.7	9.0
AST (IU/L)	354	382	486	163	399
GGT (IU/L)	519	1939	415	1023	401
HCV RNA (log_{10}IU/mL)	7.7	7.7	8.0	8.0	7.7
Weeks after LDLT	4	8	6	6	7
Histological findings					
Hepatocyte ballooning	++	++	++	+++	++
Cholestasis	+	–	–	–	–
Perivenulitis	+++	+	++	+	–
Portal infiltration	+	+	–	–	+
Ductular reaction	+	+	+	–	+
Interferon treatment					
Type and dose (μg/week)	α-2b (50)	α-2a (180)	α-2b (90)	α-2a (180)	α-2a (180)
Ribavirin dose (mg/day)	400	0	400	200	200
Response (weeks)	VR (130)	VR (17)	VR (15)	VR (49)	VR (23)
On treatment (weeks)	Yes (170)	Yes (74)	Yes (70)	Yes (69)	Yes (68)
Graft outcomes (years)	Alive (3.4)	Alive (1.6)	Alive (1.5)	Alive (1.5)	Alive (1.5)

AST, aspartate aminotransferase; CyA, cyclosporin; FK, tacrolimus; GGT, γ-glutamyltransferase; GV, graft volume; HCV, hepatitis C virus; IL, interleukin; LDLT, living-donor liver transplantation; MELD, Model for End-Stage Liver Disease; SLV, standard liver volume; VR, viral response.

In multivariate logistic regression analysis, higher HCV RNA titer at 2 weeks after LDLT ($P = 0.026$) was the only significant factor associated with having cholestatic hepatitis C. The other factors identified in univariate analyses, including earlier peak of HCV RNA titer ($P = 0.317$), larger GV/SLV ($P = 0.382$) and cytomegalovirus infection ($P = 0.936$) were not significantly associated with cholestatic hepatitis C after LDLT. Receiver–operator curve (ROC) analysis showed that HCV RNA titer of more than 7.2 log_{10}IU/mL at 2 weeks after LDLT was the optimal cut-off for discriminating cholestatic hepatitis C after LDLT. The area under the ROC for this value was 0.989 (Fig. 1).

Histological characteristics of cholestatic hepatitis C after LDLT

The histological characteristics of the five cases of cholestatic hepatitis C are summarized in Table 1. Although hepatocyte ballooning was prominent in all of the five patients (Fig. 2), portal infiltration and cholestasis were relatively minor or absent, despite the high serum bilirubin level. Perivenulitis was observed in four cases and was significantly more common in patients with recurrent cholestatic hepatitis C than in patients with recurrent non-cholestatic hepatitis C (80.0% vs 20.5%, $P = 0.004$, Table 2). Ductular reaction was observed in four cases.

Table 2 Factors associated with cholestatic hepatitis C

Factors	Cholestatic hepatitis		*P*-value
	No ($n = 44$)	Yes ($n = 5$)	
Recipient age (years)	57.4 ± 8.0	58.2 ± 7.7	0.839
Recipient sex, male	22 (50.0)	1 (20.0)	0.203
Hepatocellular carcinoma, yes	31 (70.5)	3 (60.0)	0.631
MELD score	14.8 ± 7.0	14.4 ± 4.3	0.908
History of IFN treatment, yes	34 (80.9)	3 (60.0)	0.602
Donor age (years)	34.5 ± 10.9	29.2 ± 10.0	0.302
Donor sex, male	31 (70.5)	3 (60.0)	0.631
ABO incompatible, yes	5 (11.4)	2 (40.0)	0.083
Graft type, left lobe	17 (38.6)	2 (40.0)	0.952
GV (g)	461 ± 91	502 ± 61	0.341
GV/SLV (%)	39.2 ± 5.9	45.0 ± 7.3	0.049
Splenectomy, yes	42 (95.5)	5 (100.0)	0.626
Cold ischemic time (min)	100 ± 62	83 ± 43	0.551
Warm ischemic time (min)	39 ± 10	37 ± 9	0.631
Operative time (min)	793 ± 136	740 ± 107	0.404
Blood loss (L)	4.5 ± 6.5	4.9 ± 3.2	0.894
Recipient IL-28B genotype, T/T	23 (60.5)	4 (80.0)	0.393
Donor IL-28B genotype, T/T	27 (64.3)	4 (80.0)	0.483
HCV genotype 1, yes	34 (80.9)	3 (60.0)	0.279
HCV RNA titer (log_{10}IU/mL)			
Before LDLT	5.4 ± 1.2	5.2 ± 0.7	0.813
At 2 weeks after LDLT	5.8 ± 1.3	7.7 ± 0.4	0.002
Peak titer	6.8 ± 1.3	7.9 ± 0.1	0.089
Time to peak HCV RNA titer (weeks)	9.4 ± 5.6	3.7 ± 2.3	0.031
Viral response (%)	22 (64.7)	5 (100.0)	0.110
Tacrolimus use, yes	22 (50.0)	1 (20.0)	0.202
Acute rejection, yes	1 (2.3)	0 (0.0)	0.733
Bile duct stenosis, yes	8 (18.2)	2 (40.0)	0.251
Cytomegalovirus infection, yes	12 (27.2)	4 (80.0)	0.017
Central perivenulitis on biopsy, yes	9 (20.5)	4 (80.0)	0.004

GV, graft volume; HCV, hepatitis C virus; IL, interleukin; LDLT, living-donor liver transplantations; MELD, Model for End-Stage Liver Disease; SLV, standard liver volume; SNP, single nuclear polymorphism; VR viral response.

DISCUSSION

IN THE CURRENT study, HCV RNA titer of more than 7.2 log_{10}IU/mL at 2 weeks after transplantation was the only predictive factor for recurrent cholestatic hepatitis C after LDLT. None of the other donor or recipient factors, including IL-28B (rs8099917) genotypes were associated with this severe disease in multiple regression analysis. Cholestatic hepatitis C was diagnosed in all five patients based on early extensive viremia and histological findings (e.g. pan-lobular hepatocyte ballooning). VR was achieved in all of the cases following immediate treatment with PEG IFN with ribavirin.

Although cholestatic hepatitis C is an uncommon (2–5%) form of HCV recurrence, it is usually associated with rapid progression of cholestasis with fibrosis, and often results in graft failure within 1 year after transplantation.[3–6] Early and accurate diagnosis of cholestatic hepatitis C and immediate treatment is essential to save the transplanted grafts, although diagnosis is often difficult.[14–16] The difficulties in diagnosis are mainly due to the differential diagnoses, including acute rejection, biliary stenosis or primary graft dysfunction, for which the treatments are opposite or are very different from those used for cholestatic hepatitis C.[3] We think that the combination of HCV RNA titer of more than 7.2 log_{10}IU/mL at 2 weeks after LDLT and pan-lobular ballooning of the hepatocytes are key factors for identifying cholestatic hepatitis C.

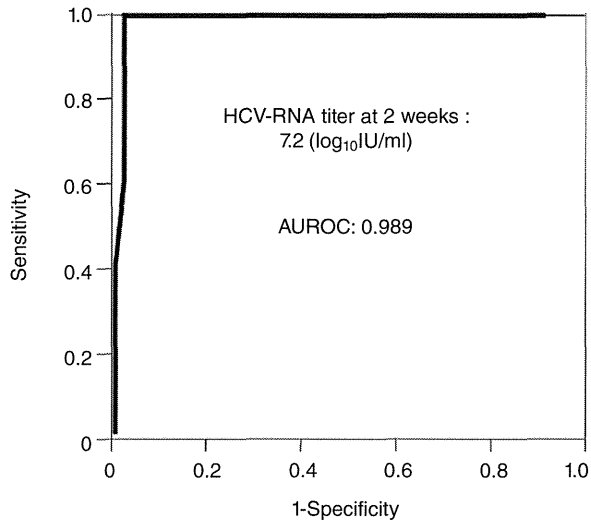

Figure 1 Receiver–operator curve analysis showed that HCV RNA titer of more than 7.2 $\log_{10}$IU/mL at 2 weeks after LDLT was the optimal cut-off for discriminating cholestatic hepatitis C. AUROC, area under the receiver–operator curve; HCV, hepatitis C virus; LDLT, living-donor liver transplantations.

Extensive HCV infection in hepatocytes and the direct cytopathological effects of HCV, together with a relative absence of inflammation, are thought to be the major mechanisms involved in the development of cholestatic hepatitis C.[17] Therefore, a very high HCV RNA titer was proposed as one of the diagnostic criteria for cholestatic hepatitis after LT in a consensus statement published in 2003.[13] However, the cut-off level for a very high HCV RNA titer was not reported in that consensus statement. More recently, Shackel *et al.*[18] reported that a peak HCV RNA titer of more than 7.0 $\log_{10}$IU/mL within 1 year of LT was a predictor of HCV-associated graft failure. Moreover, Granziadei *et al.*[5] showed that HCV RNA titer of more than 6.0 $\log_{10}$IU/mL 2 weeks after transplantation is the most significant risk factor for the development of cholestatic hepatitis. However, they did not report how they selected this value. We used ROC analysis and found that a HCV RNA titer of more than 7.2 $\log_{10}$IU/mL at 2 weeks after LDLT was the optimal cut-off for predicting cholestatic hepatitis C after transplantation.

Histological features are also important for the diagnosis of cholestatic hepatitis C.[3,14] Hepatocyte ballooning with limited inflammation is considered to be a typical finding, and it was observed in all of our cases with pan-lobular distribution. However, the international consensus criteria stated that ballooning predominantly occurred in the perivenular zone.[14] In LDLT, perivenular hepatocyte ballooning with cholestasis is often observed in dysfunctional grafts associated with small graft size, older donor or systemic inflammation.[19] Hepatocyte cholestasis was apparent in just one case (20%) in our series, and it might be attributed to the early biopsy before becoming fully established and irreversible.

Perivenulitis with centrilobular hepatocyte dropouts is a distinct histopathological process that could occur after LT, and is associated with post-transplant processes, including cytotoxic drugs, acute or chronic rejection, recurrent or de novo autoimmune hepatitis, and viral hepatitis.[20] Recent research focused on its immunological significance with significant graft injuries.[21] In hepatitis C after LT, Khettry *et al.*[22] reported that perivenulitis was significantly recognized in cases with severe recurrent hepatitis C associated with other pathological features with autoimmune hepatitis. Antonini *et al.*[23] reported that this phenomenon was more common in cholestatic patients than in non-cholestatic patients (36% vs 4%). Taking into account that cholestatic type recurrent hepatitis C causes significant hepatocyte injuries with vigorous cytokine production with unspecified immune reactions,[20–23] perivenulitis could be a significant pathological marker in cholestatic hepatitis C.

Interleukin-28B genotyping is an important predictor for the viral response to IFN. We previously reported that the T/T genotype of rs8099917 in donors and recipients is a positive predictor of the response to IFN after LDLT for hepatitis C.[12] In the current series, however, the T/T genotype was not associated with the recurrence of cholestatic hepatitis C. By contrast, Graziadei *et al.*[5] reported that rs12979860 genotypes, other than the favorable C/C genotype, in the recipients were significantly associated with cholestatic hepatitis C after LT, although the relevance of rs12979860 in donors has not been exclusively investigated. Hanouneh *et al.*[6] reported that the favorable T/T genotype of rs8099917 in the donor was associated with cholestatic recurrence. Based on these results, no consensus can be reached regarding the impact of IL-28B genotype on recurrence of cholestatic recurrent hepatitis C. Additionally, because there is a discrepancy between the IL-28B genotype, IL-28B transcription and the expression of IFN-stimulated genes,[24] further studies are needed to clarify the role of IL-28B in anti-HCV therapy.

It is still unclear why HCV can infect and replicate so vigorously, and cause cholestatic recurrence in a small number of patients after LT. We consider that

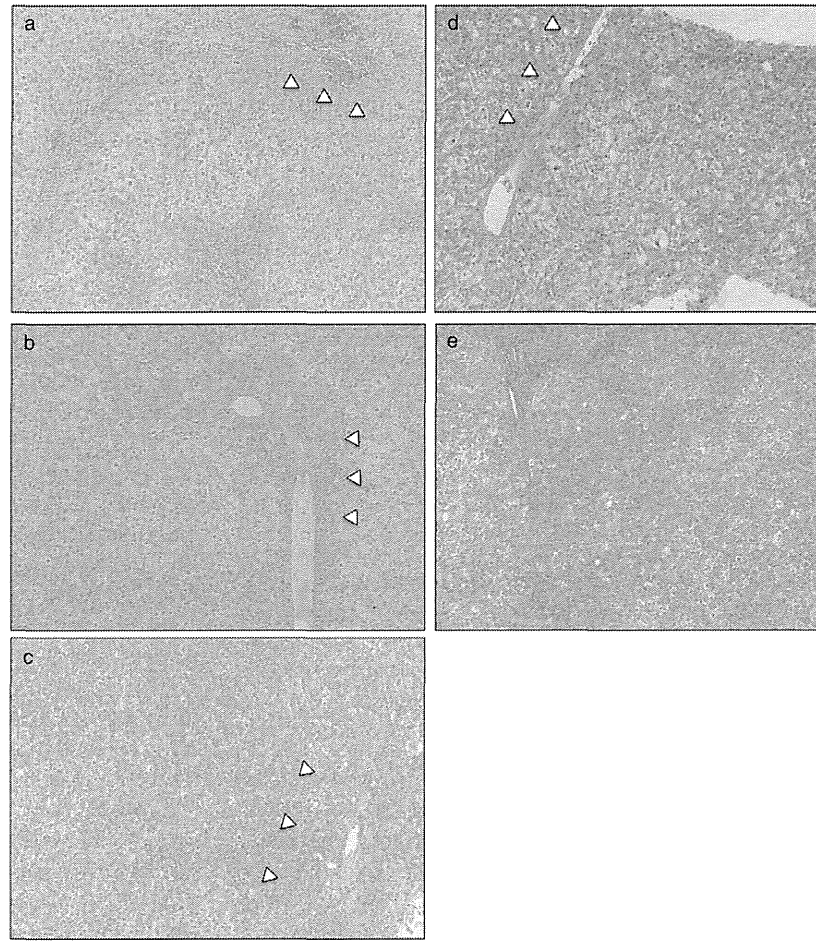

Figure 2 Histological findings of cases 1–5 (a–e, respectively) with recurrent cholestatic hepatitis C. Pan-lobular hepatocyte ballooning was prominent in all of the five patients. Perivenulitis was observed in cases 1–4 (a–d, white arrowheads) (hematoxylin–eosin, original magnification ×100).

quasispecies of HCV may play some role in this process. Previous studies showed that the number of quasispecies increased following transplantation and onset of mild recurrence, but the species distribution was more homogenous in patients with severe recurrence.[25,26] It was also reported that HCV infection becomes more severe in patients infected with HIV type 1 with decreased or homogenous quasispecies.[23,27] Because an increased number of quasispecies is thought to represent the response of HCV to a strong immune pressure, induction of the local non-specific histocompatibility independent immune system may also mediate the disease process. Although viral mutations with increased capability of antiviral drug resistance as observed in cholestatic hepatitis B may have roles,[28] we regard it as doing little in cholestatic recurrent hepatitis C after LT because it becomes evident very early after transplantation before antiviral treatment is initiated. Therefore, we regard mechanisms in higher replication property against natural immune pressure including quasispecies as playing an important role.[23–27]

In terms of treatment, we think that PEG IFN with ribavirin should be the first choice of regimen for cholestatic hepatitis C, considering its clinically relevant outcomes. Nevertheless, the important point is that antiviral treatment should only be initiated once clinical cholestasis is evident, and histological cholestasis and fibrosis are established.[4–6,14] If started too late, the tolerability of IFN may become a major problem for decompensated liver grafts. Satapathy *et al.*[4] reported that seven out of eight patients (88%) with cholestatic hepatitis discontinued IFN because of decompensation or complications. The important key step to initiate early antiviral treatment for cholestatic hepatitis C is the accurate

pathological diagnosis differentiating acute rejection, although it is not an easy task. Bolus steroids for severe hepatitis C could terminate a transplanted graft.[29] Therefore, we maintain an appropriate immunosuppression level for the first 3 months after LT for HCV-associated liver diseases and never perform rapid tapering, making pathological interpretation easier. If treatment is started early, routine splenectomy of HCV patients during LDLT is reported to increase their tolerability of intense antiviral therapies.[9]

In conclusion, HCV viremia of more than 7.2 $\log_{10}$IU/mL at 2 weeks after transplantation was the predictor of recurrent cholestatic hepatitis C after LDLT in this study. IL-28B (rs8099917) genotype and other donor and recipient factors were not associated with its recurrence. Early diagnosis followed by antiviral treatment using PEG IFN with ribavirin is important to achieve VR and graft survival.

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Original Article

Clinical usefulness of ^{18}F-fluorodeoxyglucose positron emission tomography/computed tomography for patients with primary liver cancer with special reference to rare histological types, hepatocellular carcinoma with sarcomatous change and combined hepatocellular and cholangiocarcinoma

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Aim: The role of ^{18}F-fluorodeoxyglucose positron emission tomography (FDG-PET) in the diagnosis and staging of primary liver cancer has been demonstrated in several reports. However, no preoperative evaluations of sarcomatous hepatocellular carcinoma (HCC) and combined hepatocellular and cholangiocarcinoma (cHCC-CC) with FDG-PET have been reported so far.

Methods: Fifty-three HCC patients and three cHCC-CC patients who received liver resection or living-donor liver transplantation were enrolled in this study. All 56 patients had undergone preoperative FDG-PET, and a total of 67 HCC and three cHCC-CC were analyzed histologically. The relationship between clinicopathological features and the maximum standardized uptake value (SUVmax) of tumors were evaluated.

Results: The detection rate of HCC by FDG-PET was 43.3 %, and the sensitivity of FDG-PET for the detection of HCC was significantly associated with tumor differentiation, tumor size and microvascular invasion. All three cHCC-CC were detected by FDG-PET. The SUVmax values of the three sarcomatous HCC (SUVmax 14.1, 18.6 and 25.0) and the three cHCC-CC (SUVmax 9.9, 12.0 and 13.0) were higher than that of the poorly differentiated HCC (mean SUVmax 5.7 ± 2.3).

Conclusion: SUVmax may be a useful diagnostic tool for the preoperative evaluation of the aggressiveness of primary liver cancers such as sarcomatous HCC and cHCC-CC.

Key words: ^{18}F-fluorodeoxyglucose positron emission tomography, combined hepatocellular and cholangiocarcinoma, hepatocellular carcinoma, sarcomatous hepatocellular carcinoma

INTRODUCTION

POSITRON EMISSION TOMOGRAPHY (PET) using ^{18}F-fluorodeoxyglucose (FDG) has become standard procedure for the detection of a variety of malignant tumors.[1] It is considered a useful diagnostic tool for tumor characterization and assessing therapy response.[2] For hepatocellular carcinoma (HCC), however, several reports suggest that the sensitivity of FDG-PET (50–55%) is insufficient.[3,4] Because the enzymatic activity of well-differentiated HCC cells is similar to that of the surrounding normal liver, the accumulation of FDG in these tumors is low, and the role of FDG-PET imaging in the early detection of HCC is limited.[5] On the other hand, previous studies have demonstrated that FDG accumulation is increased in undifferentiated HCC, and recently, preoperative FDG-PET has been shown to be closely associated with tumor differentiation and prognosis in HCC patients.[6,7]

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The histological differentiation grade is an important prognostic factor for HCC.[8] Once cancer is established, HCC dedifferentiates to a more malignant histology in a multistep fashion, from well- and moderately to poorly differentiated tumors.[9] Although the prognosis of well-differentiated HCC is good following resection, poorly differentiated HCC have a poor prognosis due to a high rate of vascular invasion and metastasis.[10,11] The basic histological pattern of HCC is trabecular; however, a sarcomatous appearance has been sporadically reported as one of the histological features of HCC.[12] Approximately 1.8% of all resected HCC have a sarcomatous feature, usually associated with a very poor prognosis because of its rapid growth, low resectability and frequent recurrence after resection.[13,14]

Combined hepatocellular and cholangiocarcinoma (cHCC-CC) is a rare primary liver cancer that contains the histological features of both HCC and CC.[15] cHCC-CC has been reported to show frequent vascular invasion and lymph nodes metastasis, and has a poorer prognosis than HCC.[16,17] It is difficult for patients with cHCC-CC to get a correct preoperative diagnosis because of the lack of a sensitive diagnosis procedure.[18]

Although previous studies have shown that FDG-PET is useful for evaluating various liver tumors, there have been no reports regarding preoperative FDG uptake in resectable sarcomatous HCC and cHCC-CC. In the present study, we retrospectively investigated the feasibility of FDG-PET for the detection of different types of primary liver cancer including sarcomatous HCC and cHCC-CC.

METHODS

Patients

IN THIS STUDY, we retrospectively reviewed 53 HCC patients and three cHCC-CC patients who received liver resection (LR) or living-donor liver transplantation (LDLT) at Kyushu University Hospital between April 2010 and August 2011. There were 35 male and 21 female patients, and the mean age (± standard deviation [SD]) of the patients was 65 ± 12 years (range, 36–87). All 56 patients were diagnosed as having HCC or cHCC-CC by conventional radiologic imaging and FDG PET/computed tomography (CT). Thirteen patients with HCC in cirrhosis underwent LDLT, and the other 43 patients with HCC or cHCC-CC underwent LR. Among the HCC patients, 29 had a single lesion, and the other 24 had multiple lesions. Among the cHCC-CC patients, one had a single lesion and the other two had multiple lesions.

Patient follow up

After discharge, all patients were examined for recurrence by ultrasound and by tumor markers every 1–3 months. Dynamic CT was performed every 6 months. Patients with any sign of recurrence and/or inconclusive imaging studies underwent additional FDG PET/CT. All of the patients were followed up while they were alive.

FDG PET/CT

^{18}F-Fluorodeoxyglucose positron emission tomography studies were performed with Discovery ST Elite (GE Healthcare, Milwaukee, WI, USA) and Biograph mCT (Siemens AG, Erlangen, Germany) PET/CT scanners. All patients fasted for at least 4 h before FDG administration, and 185 MBq of FDG was i.v. administrated to each patient. Approximately 60 min after the FDG injection, whole-body PET images were acquired from thigh to head with 7–10 bed positions. The Discovery ST Elite scanner consists of a 16-slice multidetector CT and bismuth germanium oxide crystal. The unenhanced CT was performed first with the following parameters: 5-mm slice thickness, 120 kV, 30–250 mAs with auto mode (Smart mA). Then, PET images were obtained in 3-D mode for 3 min per bed position with a 3.27-mm slice thickness, at 70 cm field of view (FOV) in a 128 × 128 matrix. Based on the CT data, transmission maps were created and used for the attenuation correction of the PET images. The PET data were reconstructed using a 3-D ordered subset expectation maximization (3D-OSEM) algorithm (VUE Point Plus) with two iterations and 28 ordered subsets. A 6-mm post-filter of full-width at half maximum (FWHM) was applied. The Biograph mCT scanner is equipped with a 128-slice multidetector CT and lutetium crystal. The unenhanced CT was performed at 120 kV with automatic mAs adjustment (Care Dose 4D) and the slice thickness was 3 mm. The PET emission time was 2 min per bed position. The PET images were acquired with a 2-mm slice thickness, at 70 cm FOV in a 256 × 256 matrix. The concomitant CT data were used for attenuation correction. The PET data were reconstructed using a 3D-OSEM algorithm with two iterations and 21 subsets. Time of flight and point spread function techniques were also used for the image reconstruction (ultra-HD-PET). A 3-D Gaussian filter of 6-mm FWHM was applied. The PET images were qualitatively evaluated to assess whether the FDG uptake in the tumor was (PET positive status) or was not