Cancer Chemother Pharmacol (2013) 72:1325–1333
DOI 10.1007/s00280-013-2311-x

ORIGINAL ARTICLE

Evaluation of safety parameters and changes in serum concentration in liver transplant recipients treated with doxorubicin during the anhepatic period

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Received: 8 May 2013 / Accepted: 25 September 2013 / Published online: 13 October 2013

Abstract
Purpose Because of the recurrence of hepatocellular carcinoma (HCC) at the graft after liver transplantation, circulating HCC cells may be present during the anhepatic period. Intravenous doxorubicin (DOX) is used during the anhepatic period to combat these cells; however, pharmacokinetics data have been poorly analyzed. This study aims to investigate DOX administration during the anhepatic period.
Patients and methods We administered 5 mg/m^2 DOX immediately after liver removal and compared serum DOX concentrations at several intervals during the anhepatic period in patients who underwent liver transplantation because of liver cirrhosis and HCC ($n = 3$) and patients who underwent liver resection owing to HCC with portal vein tumor thrombi ($n = 5$). We also measured serum DOX concentrations and pharmacokinetic parameters in transplant patients that received 3–15 mg/m^2 DOX ($n = 3$ per dose level). We evaluated transplant patients' adverse drug reactions and survival.
Results At 10 and 30 min after DOX administration, serum DOX concentrations were elevated two- to threefold in transplant patients versus resection patients. Dose escalation in transplant patients exhibited a prolonged $T_{1/2}$ in the one-compartment model and $T_{1/2}$ β in the two-compartment model, as well as a dose-dependent elevation of the area under the curve. No obvious adverse drug reactions were noted at 3–15 mg/m^2 DOX. In transplant patients, 5-year recurrence-free survival was 68.8 %; overall survival was 100.0 %.
Conclusion During the anhepatic period, serum DOX concentrations were elevated two- to threefold, $T_{1/2}$ was prolonged dose dependently, and up to 15 mg/m^2 DOX could be safely administered.

Keywords Liver transplantation · Hepatocellular carcinoma · Doxorubicin · Pharmacokinetics · Anhepatic period

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Introduction

Viral hepatitis and cirrhotic liver are major risk factors associated with hepatocellular carcinoma (HCC) [1, 2]. Because these are chronic conditions that also affect liver function, and some cases of HCC are contraindicated for surgical resection because of poor liver function. In these cases, liver transplantation is becoming an alternative strategy to combat this tumor, even in patients with Child-Pugh C liver function [3, 4]. Although Milan and other criteria [4–6] have proposed indications for liver transplantation due to HCC with cirrhotic liver, the prognosis in patients with HCC exceeding these criteria is quite poor [5–8]. Accordingly, several authors have tried neo-adjuvant therapy for down-staging, as well as intra-operative and post-operative adjuvant chemotherapy [4, 9, 10]. Because of HCC recurrences at the liver graft after transplantation, some authors have suggested that circulating HCC cells may be present during the anhepatic period [11–14]. Adjuvant chemotherapies have been tried against these small clusters of HCC cells [15].

Doxorubicin (DOX) is one of the major drugs employed against HCC in several situations, both for unresectable HCC and in an adjuvant setting. For example, several clinicians have performed adjuvant chemotherapy with DOX

after the resection of HCC with portal vein tumor thrombus (PVTT) [16, 17]. In liver transplantation, several clinicians have tried chemotherapy during the anhepatic period [18, 19] or adjuvant chemotherapy with DOX [9, 20] in patients with HCC exceeding Milan criteria.

However, pharmacological analysis of DOX during the anhepatic period and after reperfusion during liver transplantation is rarely investigated. This drug is mainly metabolized in the liver, and the serum concentration would reportedly remain high in patients with liver dysfunction [21–24]. In dogs, serum DOX concentration was measured during the anhepatic period and exhibited only a 50 % reduction in total body clearance [25, 26]. In the present study, we measured serum DOX concentration during the anhepatic period in the transplant recipients. We also compared these results to serum DOX concentrations in patients who underwent liver resection. Furthermore, we evaluated safety by performing a detailed investigation of the adverse events and adverse drug reactions in these series.

Patients and methods

Patients

Between 2003 and 2011, we measured serum DOX concentration in 12 patients who underwent liver transplantation because of liver cirrhosis and HCC (TSPL group). We also measured serum DOX concentration in five patients who underwent liver resection and PVTT removal owing to HCC with PVTT (RESC group). The first three patients in the TSPL group were treated with 5 mg/m^2 DOX, and we compared pharmacokinetic data from the TSPL group with data from the RESC group. Previous data [25, 26] indicated that DOX clearance would be reduced by 50 %; therefore, for safety reasons, we administered 5 mg/m^2 DOX (the common dose for systemic administration in the context of HCC is 45–75 mg/m^2 [27–29]) and compared the pharmacokinetic data of the TSPL and RESC groups. After pharmacokinetic data were confirmed in the TSPL group at 5 mg/m^2 DOX, we administered DOX at several dose levels (3, 10, and 15 mg/m^2), calculated pharmacokinetic data, and evaluated adverse events at each dose level. Patients' characteristics were prospectively collected. All patients underwent surgery at our institution. The protocol was approved by the institutional review board at our hospital, and written informed consent was obtained from each patient.

DOX administration, sample collection, and measurement DOX concentration

The time course of DOX administration and sample collection is depicted in Fig. 1a. In the TSPL group, patients underwent liver transplantation because of liver cirrhosis with HCC. At 5 min after explantation of the cirrhotic liver, 3–15 mg/m^2 of DOX were administered intravenously. Five milliliter peripheral blood samples were obtained at 0, 10, 30, 60, and 120 min after DOX administration until reperfusion. We also collected blood samples at 0, 10, 30, and 60 min post-reperfusion. The RESC group underwent liver resection with the removal of PVTT. We administered 5 mg/m^2 DOX to each RESC patient 5 min after the liver resection was completed. Blood samples were obtained at 0, 10, 30, 60, and 120 min after DOX administration.

All blood samples were stored at 4 °C, centrifuged at 3,000 rpm for 10 min, and frozen at −80 °C before the DOX concentrations were measured. Serum DOX concentrations were measured by high-pressure liquid chromatography at Kyowa Hakko Kogyo Co., Ltd., Japan. The serum concentration curves, pharmacokinetic parameters, and area under the DOX concentration curve from 0 to 120 min (AUC_{120}) were determined for each patient. Various parameters were calculated using the one- or two-compartment infusion model ($C(t) = Ae - \alpha t$ for the one-compartment model and $C(t) = Ae - \alpha t + Be - \beta t$ for the two-compartment model) and LAB Fit Curve Fitting Software 7.2.41 (Wilton and Cleide Pereira da Silva, Brazil). AUC_{120} was calculated using the trapezoidal model.

Evaluation of adverse events and adverse drug reactions

We evaluated adverse events and adverse drug reactions according to CTACE version 4.0, retrospectively, during the first 7 days after the surgery. For adverse drug reactions, we considered events that were unrelated to liver transplantation and the use of immunosuppressant medications.

Statistical analysis

Data were expressed as mean ± standard error. Differences between groups were tested using Student's t test and the chi-squared test, and differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using StatView J-5.0 software (SAS, Cary, NC).

Results

Comparison of pharmacokinetic parameters between TSPL and RESC groups at 5 mg/m^2 DOX

We summarized these patients' characteristics in Table 1. Major characteristics (e.g., age, sex, body height and weight, and ratio of hepatitis) were similar between the groups. Characteristics specific to liver function were expected to be worse in the TSPL group than in the RESC

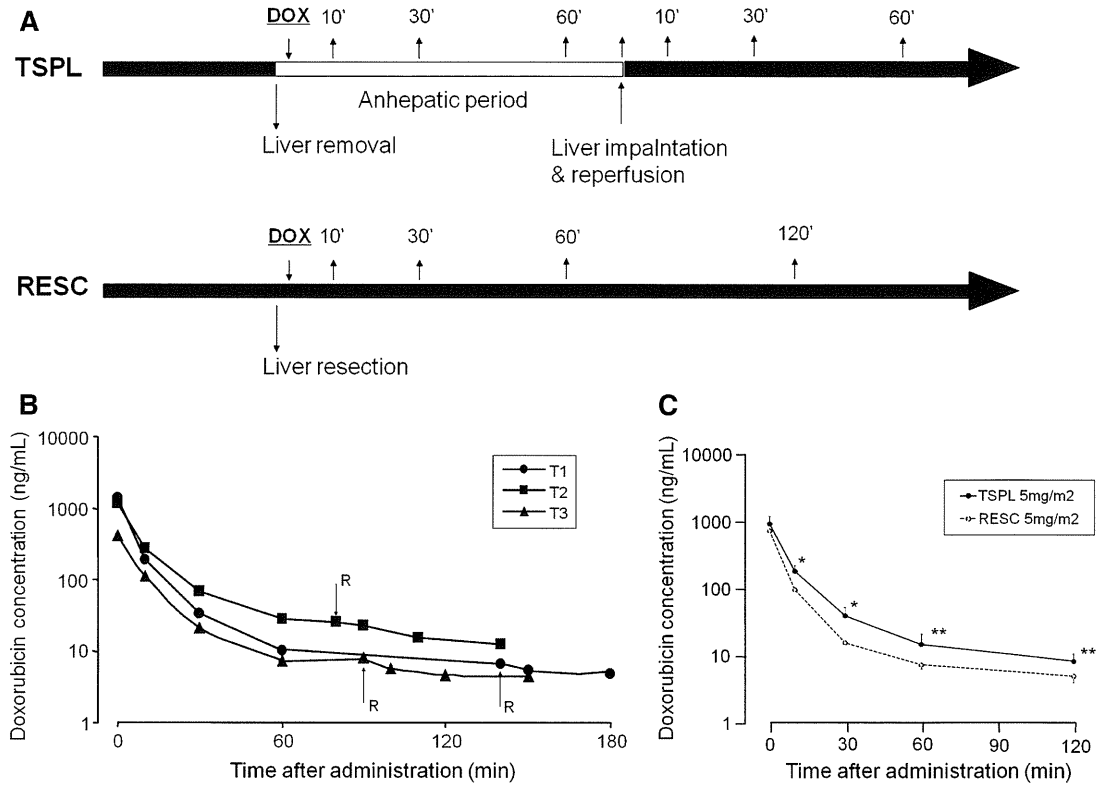

Fig. 1 Perioperative administration of doxorubicin in patients undergoing liver transplant or resection. **a** A schematic depicting doxorubicin (DOX) administration and sample collection. DOX was administered 5 min after the removal of the cirrhotic liver (TSPL) or liver resection with portal vein tumor thrombi (RESC). Peripheral blood samples were obtained at 0, 10, 30, 60, 120, and 180 min, as indicated. Blood samples were also obtained at the same intervals after reperfusion in the TSPL group. **b** Change in serum doxorubicin concentration in TSPL patients after DOX administration (5 mg/m^2). Each line indicates the serum DOX concentration in an individual TSPL patient (T1, T2, and T3). R, reperfusion. **c** The mean change in serum DOX concentration in the TSPL ($n = 3$) and RESC ($n = 5$) groups after DOX administration (5 mg/m^2). Data are expressed as mean ± standard error. $*p < 0.05$, $**p < 0.1$

group; however, only albumin and Child-Pugh classification were worse among TSPL patients. Renal function (serum creatinine level) did not differ between the groups ($p = 0.3118$).

The mean duration of the anhepatic period (from clamp of the portal vein of the recipient to reperfusion) was 101 min in the TSPL group. We compared serum DOX concentrations at 0, 10, 30, and 60 min after DOX administration; data at 120 min served as a reference. Reperfusion seemed to have almost no influence on the serum DOX concentration (Fig. 1b). The concentration from 0 min to 120 min (C0–C120) is depicted in Table 2 and Fig. 1c. At 10 and 30 min after DOX administration, serum DOX concentrations were significantly higher in TSPL patients than in RESC patients. Although the levels at 60 and 120 min were numerically higher in the TSPL group, the data only trended toward statistical significance. The other pharmacokinetic parameters are also described in Table 2. The area under curve from 0 to 120 min (AUC_{120}) was numerically higher in TSPL patients and approximately 1.5-fold higher than in RESC patients.

We employed both the one-compartment and two-compartment models to evaluate half-life ($T_{1/2}$) because the $T_{1/2}$ of alpha phase (also known as the distribution phase) was longer during the anhepatic period and the $T_{1/2}$ of beta phase (also known as the elimination phase) was unchanged in dogs [25, 26]. These findings indicated that the pharmacokinetic analysis of DOX during the anhepatic period is more important during the alpha phase, and for this reason, we decided to employ the one-compartment model. In our hands, the two-compartment model revealed that the $T_{1/2}$ of alpha phase was longer in the TSPL group than in the RESC group, although the difference did not reach statistical significance, and the $T_{1/2}$ of beta was shorter among TSPL patients than RESC patients. In contrast, the one-compartment model indicated that the $T_{1/2}$ trended longer in the TSPL group than in the RESC group.

Table 1 Characteristics of liver transplant (TSPL) and resection (RESC) patients who were treated with 5 mg/m^2 doxorubicin

Variables	TSPL	RESC	*p* value
v*n*	3	5	
Age	57 ± 5.4	56 ± 2.0	0.6939
Sex	2 (67 %)	4 (80 %)	0.6733
Male (%)			
Body high (cm)	164 ± 4.1	171 ± 2.7	0.2213
Body weight (kg)	66 ± 5.2	70 ± 2.8	0.5739
Body surface area (m^2)	1.67 ± 0.07	1.77 ± 0.04	0.3129
Hepatitis			
HBV (%)	1 (33 %)	3 (60 %)	0.4652
HCV (%)	2 (67 %)	1 (20 %)	0.1869
Preoperative liver function			
Aspartate aminotransferase (IU/L)	82 ± 30	53 ± 14	0.4460
Alanine aminotransferase (IU/L)	69 ± 31	46 ± 11	0.5300
Prothrombin time-INR	1.33 ± 0.15	1.22 ± 0.02	0.5299
Total bilirubin (mg/dL)	4.8 ± 1.31	0.9 ± 0.16	0.0918
Albumin (g/dL)	2.7 ± 0.20	3.9 ± 0.13	**0.0079**
Creatinine (mg/dL)	0.6 ± 0.10	0.8 ± 0.10	0.3118
Child-pugh score	10.7 ± 1.5	5.0 ± 0.0	0.0599
Child-pugh classification			
A	0	5 (100 %)	**0.0183**
B	1 (33 %)	0	
C	2 (67 %)	0	
MELD score	15.0 ± 2.5	8.2 ± 0.37	0.1109
Anhepatic period (min)	101 ± 22	N/A	
Cold ischemia time (min)	69 ± 18	N/A	
Warm ischemia time (min)	46 ± 12	N/A	
Operation period (min)	703 ± 41	541 ± 95	0.1767
Estimated blood loss (min)	4,307 ± 1,699	3,984 ± 2,226	0.9120
Graft or remnant liver lobe			
Left (%)	1 (33 %)	3 (60 %)	0.4652
Right (%)	2 (67 %)	2 (40 %)	
GW/SLV	0.54 ± 0.06	N/A	
Dose of doxorubicin (mg/m^2)	5	5	

Bold values indicate statistical significance at $p < 0.05$

MELD score, model for end stage liver disease score; RESC, patients who underwent liver resection and portal vein tumor thrombi removal due to hepatocellular carcinoma; TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma; and *GW/SLV,* graft weight/standard liver volume

Change in serum DOX concentration in TSPL patients at 3, 5, 10, and 15 mg/m^2 DOX

We summarized TSPL patients' characteristics in Table 3. Ninety percent of the patient population was male, and the mean body surface area was 1.78 m^2. The mean MELD score was 16.0. Because one patient was received a transplanted liver from a deceased donor, the mean cold ischemia time was 137 min and one graft liver was whole liver. However, the anhepatic period was 118 ± 11 min, and there appeared to be no large difference among the patients. We observed changes in serum DOX concentration at 3, 5, 10, and 15 mg/m^2 (Fig. 2). Pharmacokinetic parameters are summarized in Table 4. AUC_{120} increased in a dose-dependent manner, with the exception that AUC_{120} at 10 mg/m^2 was slightly lower. The $T_{1/2}$ of serum DOX concentrations was prolonged in alpha phase of the one-compartment model and in beta phase of the two-compartment model, according to dose escalation of DOX. Maximum serum concentration was 2,440 ng/mL at 15 mg/m^2 DOX administration.

Table 2 Pharmacokinetic parameters in liver transplant (TSPL) and resection (RESC) patients after administration of 5 mg/m2 doxorubicin

	TSPL	RESC	*p* value
n	3	5	
Dose of doxorubicin (mg/m^2)	5	5	
Plasma concentration (ng/mL)			
C0	975 ± 165	760 ± 171	0.2575
C10	189 ± 46	99 ± 12	**0.0233**
C30	40 ± 13.8	16 ± 2.9	**0.0315**
C60	15 ± 6.6	7.4 ± 1.1	0.0903
C120	8.3 ± 2.4	5.0 ± 1.0	0.0940
AUC_{120} (ng min/mL)	9,642 ± 2,519	6,162 ± 877	0.0808
One-compartment model			
A	974 ± 173	760 ± 171	0.2583
α	0.156 ± 0.20	0.197 ± 0.019	0.1056
$T_{1/2}$(min)	4.6 ± 0.54	3.6 ± 0.32	0.0774
Two-compartment model			
A	902 ± 267	739 ± 173	0.3040
B	73 ± 27	22 ± 5	**0.0235**
α	0.183 ± 0.020	0.218 ± 0.018	0.1301
β	0.023 ± 0.004	0.014 ± 0.002	**0.0359**
$T_{1/2}$ α(min)	3.9 ± 0.5	3.3 ± 0.3	0.1276
$T_{1/2}$ β(min)	31.7 ± 4.9	55.4 ± 10.9	0.0822

Bold values indicate statistical significance at $p < 0.05$

AUC_{120}, area under concentration curve from 0 to 120 min; RESC, patients who underwent liver resection and portal vein tumor thrombi removal due to hepatocellular carcinoma; and TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

Adverse events in TSPL patients at 5, 10, and 15 mg/m^2 DOX

We evaluated adverse events in TSPL patients using CTCAE version 4.0 during the first 7 days after liver

Table 3 Characteristics of transplant (TSPL) patients

Variables	TSPL
n	12
Age	53 ± 7.1
Sex	
Male (%)	11 (92 %)
Body high (cm)	168 ± 1.7
Body weight (kg)	71 ± 3.4
Body surface area (m^2)	1.78 ± 0.05
Hepatitis	
HBV (%)	3 (25 %)
HCV (%)	7 (58 %)
Preoperative liver function	
Aspartate aminotransferase (IU/L)	58 ± 11
Alanine aminotransferase (IU/L)	47 ± 12
Prothrombin time-INR	1.68 ± 0.18
Total bilirubin (mg/dL)	5.5 ± 1.51
Albumin (g/dL)	2.9 ± 0.15
Creatinine (mg/dL)	0.8 ± 0.11
Child-pugh score	5.0 ± 0.0
Child-pugh classification	
B	4 (33 %)
C	8 (67 %)
MELD score	16.0 ± 1.43
Anhepatic period (min)	118 ± 11
Cold ischemia time (min)	137 ± 46
Warm ischemia time (min)	44 ± 4
Operation period (min)	811 ± 36
Estimated blood loss (min)	7,975 ± 1,769
Graft liver lobe	
Left (%)	2 (17 %)
Right (%)	9 (75 %)
Whole (%)	1 (8 %)
GW/SLV	0.54 ± 0.06
Dose of doxorubicin (mg/m^2)	3–15

GW/SLV, graft weight/standard liver volume; MELD score, model for end stage liver disease score; and TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

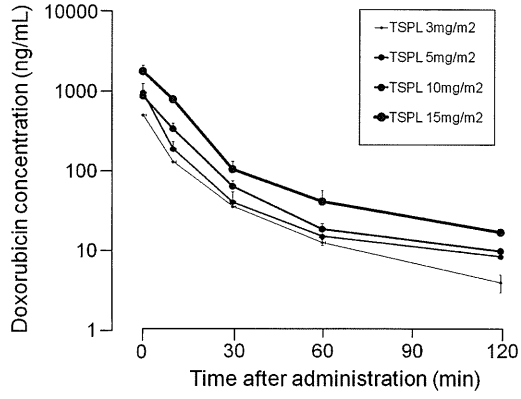

Fig. 2 The mean change in serum doxorubicin concentration in liver transplant patients (TSPL) treated perioperatively with 3, 5, 10, or 15 mg/m^2 doxorubicin. Each patient was treated with the indicated dose of doxorubicin ($n = 3$ per dose level). Data are expressed as mean ± standard error

transplantation (Table 5). Because of liver transplantation, Grade 3–4 decreased platelet count and hyperbilirubinemia was noted in almost all patients. Grade 1 diarrhea at 5 mg/m^2 was noted owing to elementary diet. Two patients at 10 mg/m^2 presented with Grade 1 abnormal echocardiogram (sinus tachycardia). Other Grade 1–2 adverse events were compatible with the regular postoperative course after liver transplantation. Regarding DOX-related adverse drug reactions, both symptoms and laboratory data were unremarkable.

Tumor factors and survival in TSPL patients

As preliminary data, we investigated the recurrence-free survival and overall survival in TSPL patients. Tumor characteristics are summarized in Table 6. Briefly, this patient population featured 58 % multiple HCCs, 50 % exceeding Milan criteria, and no portal vein tumor thrombus. One patient underwent intra-portal 5-fluorouracil infusion. With a median observation period of 4.1 years (range, 1.7–9.9 years), the 5-year recurrence-free survival was 68.8 %, and the overall survival was 100.0 %. Two patients with HCC exceeding Milan criteria experienced HCC recurrence: One patient, who suffered from over 20 HCCs (maximum diameter, 3.3 cm) with microscopic vascular invasion and was treated with 3 mg/m^2 DOX, experienced liver metastasis at 1.0 year post-transplantation. Another patient, who suffered from 4 HCCs (maximum diameter, 1.5 cm) and was treated with 5 mg/m^2 DOX, experienced lymph node metastasis at 5.0 years post-transplantation. The former patient who was treated with 3 mg/m^2 DOX died of HCC at 6.2 years post-transplantation.

Discussion

In the current study, we demonstrated the elevation of serum DOX concentration during the anhepatic period. Briefly, the concentrations at 10 and 30 min after DOX administration (C10 and C30) were elevated two- to three-fold during liver transplantation in comparison with liver resection. $T_{1/2}$ in the one-compartment model tended to be prolonged. In contrast, in the two-compartment model, $T_{1/2}$ α was prolonged, but was not significantly so, and $T_{1/2}$ β

Table 4 Pharmacokinetic data from transplant (TSPL) patients for each dose of doxorubicin

Dose of doxorubicin (mg/m^2)	AUC $_{120}$ (ng min/mL)	One-compartment model			Two-compartment model					
		A	α	$T_{1/2}$ (min)	A	B	α	β	$T_{1/2}\,\alpha$ (min)	$T_{1/2}\,\beta$ (min)
3	6,060	507	0.134	5.52	419	90	0.182	0.0285	3.8	26.6
5	9,642	974	0.156	4.57	902	73	0.183	0.0230	3.9	31.7
10	12,227	880	0.094	7.58	880	25	0.104	0.0083	6.6	83.1
15	25,882	1,804	0.084	8.87	3,038	38	0.139	0.0069	5.0	100.0

AUC_{120}, area under concentration curve from 0 to 120 min; TSPL, patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

Table 5 Adverse events during the first 7 days after liver transplantation

Dose of doxorubicin (mg/m^2)	5		10		15	
CTCAE Grade	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4	Grade 1/2	Grade 3/4
Symptom						
Diarrhea	1	0	0	0	0	0
Rash	0	0	0	0	0	0
Fever	0	0	0	0	0	0
Biliary tract infection	0	0	0	0	0	0
Other infection	0	0	0	0	0	0
Laboratory data						
Abnormal ECG	0	0	2	0	0	0
Neutropenia	1	0	2	0	1	0
Anemia	3	0	3	0	3	0
Platelet	0	3	0	3	0	3
Creatinine	0	0	2	0	1	0
Aspartate aminotransferase	3	0	3	0	3	0
Alanine aminotransferase	3	0	3	0	3	0
Alkaline phosphatase	0	0	1	0	0	0
Total bilirubin	0	3	0	3	2	1
Prothrombin time	2	0	1	0	0	0
Albumin	1	0	2	1	3	0

CTCAE Common Terminology Criteria for Adverse Events, version 4.0

tended toward being shortened. The AUC was elevated in a dose-dependent manner. No obvious adverse drug reactions were noted at the maximum dose of DOX during the anhepatic period.

Serum concentrations of drugs, including DOX, have rarely been investigated in liver dysfunction during the anhepatic period. The change in serum DOX concentration during the anhepatic period was marked by rapid decline after administration and two- to threefold elevation at C10 and C30. First, the rapid decrease after DOX administration was also noted in the normal liver [21]. This rapid decrease might be a result of distribution to the other organs and blood vessels [30] (e.g., DOX is mainly distributed to the spleen and lung in rats). At C10 and C30, serum DOX concentrations were sustained at two- to threefold; these data are similar to previous studies in dogs, which compared concentrations during the anhepatic period versus in normal whole liver [25, 26]. Using both one- and two-compartment models, we observed that DOX $T_{1/2}$ was prolonged by minutes. In contrast, $T_{1/2}$ β was shortened in the two-compartment model by approximately 10 min. These findings are compatible with previous reports of serum concentration in dogs [25].

An additional discussion point is the effect of the duration of the anhepatic period. The anhepatic period is regularly within 2 h (especially in living donor liver transplantations), and our findings demonstrate that the length of the anhepatic period appears to have limited influence on the serum DOX concentration. In comparisons of normal and cirrhotic liver, the DOX concentration in cirrhotic liver reached levels that were six- to eightfold higher than in normal liver at 48 h after DOX administration [31, 32]. In contrast, serum DOX concentration during the anhepatic period was limited two- to threefold higher

Table 6 Tumor factors in liver transplant (TSPL) patients

Variables	TSPL
n	12
HCC	
Multiple (%)	7 (58 %)
Maximum size (cm)	1.3 ± 0.3
PVTT (%)	0
Exceeding Milan criteria(%)	6 (50 %)
Preoperative treatment	
Transcatheter arterial chemo-embolization (%)	5 (42 %)
Local ablation (radiofrequency, microwave) (%)	5 (42 %)
Complete necrosis (%)	2 (17 %)
AFP (ng/mL)	527 ± 313
Histology	
Early HCC	1 (8 %)
Well differentiated HCC	2 (17 %)
Moderately differentiated HCC	4 (33 %)
Poorly differentiated HCC	3 (25 %)
Micro PVTT (%)	1 (8 %)

AFP, α-fetoprotein; *HCC,* hepatocellular carcinoma; *PVTT,* portal vein tumor thrombi; and *TSPL,* patients who underwent liver transplantation due to liver cirrhosis and hepatocellular carcinoma

in patients undergoing transplant than in those undergoing resection. In other words, the factor of liver function (anhepatic or not) appears to influence serum DOX concentration only between 30 min and 120 min after DOX administration. The elevation of the serum DOX concentration is likely limited because the anhepatic period is so short.

The final discussion points regarding pharmacokinetic analysis are the AUC and peak serum DOX concentrations during the anhepatic period. As the administered dose of DOX escalated, the AUC increased to 25,000 ng min/mL (approximately 400 ng h/mL), the peak DOX level reached 2,500 ng/mL in actual measurements and 3,000 ng/mL in estimates calculated from the one- and two-compartment models. The peak serum DOX concentration reportedly contributes to cardiac toxicity in addition to cumulative dose [33–35]. From our findings during the anhepatic period, the AUC of 15 mg/m^2 (our maximum dose) was much lower than when DOX was administered as a systemic bolus; however, the peak level of 15 mg/m^2 was almost equal to a systemic bolus administration of 150 mg/m^2 DOX in previous studies [36, 37]. When comparing the adverse events between "reported 150 mg/m2 of bolus DOX administration" and "our 15 mg/m2 of DOX during anhepatic period," the reported data showed 50 % of febrile neutropenia and 16.7 % of Grade 3–4 nausea/vomiting [36], our data showed 100 % of Grade 3/4 thrombocytopenia, 33 % of hyper bilirubinemia, and no cardiac toxicities, and our data were compatible with "the regular postoperative course" after living donor liver transplantation. The adverse events differed markedly between the previous reports and our data in the present study and may be associated with different causes (adverse drug reaction in the previous report versus regular postoperative course in the present report). Therefore, these might be non-drug-related adverse events that depend on conditions other than peak DOX level, which would indicate that our series did not reveal any severe adverse drug reactions. There remains the possibility that the differences were caused by AUC. However, there is a persistent possibility of severe adverse drug reactions in future series. It will be necessary to check patients' vital signs, physical status, and examinations carefully during any phase II study, because of high peak serum DOX concentration during the anhepatic period.

Regarding the anticancer effect of DOX, we achieved approximately 70 % 5-year recurrence-free survival and 100 % 5-year overall survival in this series. However, a previous randomized trial revealed that adjuvant chemotherapy is ineffective after transplantation [19]. They administered 15 mg/m^2 of DOX intra-operatively (they did not describe whether or not this was during the anhepatic period). Our maximum dose was 15 mg/m^2 DOX during the anhepatic period; the serum DOX concentration did reach 10–100 ng/mL until 120 min. Our previous evaluation showed that the IC_{50} of DOX in several cultured hepatocellular carcinoma cell lines varied from 10 to 100 ng/mL [30]. Although it is difficult to keep serum DOX concentration similar to in vitro studies, the serum concentration appeared to exceed the IC_{50}s demonstrated in vitro.

The recommended dose for DOX during the anhepatic period should be 15 mg/m^2, with careful monitoring for adverse drug reactions. Additional studies, such as a phase II study, are needed to verify adverse drug reactions and should be paired with monitoring of changes in mAFP-expressing cells during the perioperative period to evaluate efficacy. Several researchers have mentioned the existence of HCC cells and/or a niche in the bone marrow in published work [38–40], and it is necessary to evaluate bone marrow cells during the perioperative period. The main limitation of this study is the difficulty of distinguishing between adverse drug reactions and regular postoperative course, and higher doses of DOX might be necessary.

In conclusion, up to 15 mg/m^2 DOX was safely administered during the anhepatic period. However, further investigation is necessary to estimate treatment efficacy, with careful monitoring of adverse events.

Conflict of interest The authors declare no conflicts of interest.

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World J Surg (2013) 37:2671–2677
DOI 10.1007/s00268-013-2188-1

World Journal of Surgery

Hepatic Artery Reconstruction in Living Donor Liver Transplantation: Risk Factor Analysis of Complication and a Role of MDCT Scan for Detecting Anastomotic Stricture

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Published online: 28 August 2013

Abstract

Background In partial liver transplantation, reconstruction of the hepatic artery is technically highly demanding and the incidence of arterial complications is high. We attempted to identify the risk factors for anastomotic complications after hepatic artery reconstruction and examined the role of multidetector-row computed tomography (MDCT) in the evaluation of the reconstructed hepatic artery in liver transplant recipients.

Methods A total of 109 adult-to-adult living donor liver transplantations (LDLT) were performed at our institute between 1999 and July 2011. Hepatic artery reconstruction was performed under a surgical microscope (MS group, $n = 84$), until we began to adopt surgical loupes (4.5×) for arterial reconstructions in all cases after January 2009 (SL group, $n = 25$). A dynamic MDCT study was prospectively carried out on postoperative days 7, 14, and 28, and at postoperative month 3, 6, and 12 after April 2005 ($n = 60$).

Results There were no cases of hepatic artery thrombosis and six cases (5.5 %) of interventional radiology-confirmed hepatic artery stenosis (HAS). Risk factor analysis for HAS showed that ABO-incompatible LDLT was associated with HAS. Use of surgical loupes provided superior results as compared to anastomosis under a surgical microscope, and it also provided the advantage of reduced operative time. The MDCT procedure was useful for detecting HAS; however, the false positive rate was relatively high until 3 months after the LDLT (100 % sensitivity and 72.8 % specificity at 3 months).

Conclusions Hepatic arterial anastomosis using surgical loupes tended to be time-saving and to yield similar or better results than traditional microscope-anastomosis. The use of MDCT aided the diagnosis of HAS, although the substantial false positive rate should be borne in mind in clinical practice.

Abbreviations

DUS	Doppler ultrasonography
HAS	Heoatic artery stenosis
IVR	Interventional radiology
LDLT	Living donor liver transplantation
MELD score	Model for end-stage liver disease score
MDCT	Multidetector-row CT
POD	Postoperative day
POM	Postoperative month
RI	Resistive index
SMA	Superior mesenteric artery

Introduction

Hepatic artery reconstruction is the most important surgical procedure for liver transplantation, and complications associated with this vascular reconstruction, such as hepatic artery thrombosis or stenosis, may have a significant influence on the recipients' prognosis. In partial liver transplantation, where the hepatic arterial system should be reconstructed using a branch of the hepatic artery, such as the right hepatic artery in right liver grafting and the left and middle hepatic arteries in left liver grafting, reconstruction of the hepatic artery is technically highly demanding and the incidence of arterial complications is

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high. The reported incidence of hepatic artery thrombosis is in the range of 3.1–22 %, and that of hepatic artery stenosis (HAS) is in the range of 4.8–24.6 % [1–7]. The anastomosis procedure using a surgical microscope, first introduced in the 1990s, aimed at better patency and a lesser degree of graft damage in partial liver transplantation [8, 9], and it has since become a standard technique in partial liver transplantation [10, 11]. However, anastomosis using surgical loupes is more popular in some programs because of its advantages over anastomosis using the surgical microscope, such as the time saved for adjusting the operative fields and better focusing in the abdominal cavity [12], with similar surgical outcomes [12–14]. Including comparative studies between the methods using the microscope and surgical loupes [12–14], very few studies have been conducted to investigate the risk factors for the development of hepatic arterial complications.

Doppler ultrasonography (DUS) is the current gold standard for evaluating hepatic arterial thrombosis and stenosis, both intraoperatively and postoperatively. Measurements of the resistive index of the reconstructed hepatic artery, the tardus–parvus waveform, or other useful parameters in a Doppler study have been shown to provide rather accurate diagnosis of HAS [6, 15–19]. Recently, multidetector row computed tomography (MDCT) has been demonstrated to be useful for the evaluation of small-arterial complications, obviating the need for the more invasive angiography, and to also be quite useful for the diagnosis of post-transplant complications [20, 21]. However, there is very little information so far about the usefulness of MDCT in the evaluation of the hepatic artery in liver transplant recipients [21, 22].

In the present study, we attempted to identify the risk factors for anastomotic complications after hepatic artery reconstruction, and examined the role of MDCT in evaluation of the reconstructed hepatic artery in liver transplant recipients.

Patients and methods

A total of 109 adult-to-adult living donor liver transplantations (LDLT), including one re-transplantation, were performed at our institute between 1999 and July 2011; the total of 108 transplant recipients comprised 57 male and 51 female patients, with a mean age of 49.8 ± 12.3 years. The indications for liver transplantation consisted of viral cirrhosis ($n = 67$), cholestatic liver disease ($n = 14$), fulminant liver failure ($n = 8$), and others ($n = 20$). Among the 109 liver transplantations, 7 transplants were ABO-incompatible. The liver grafts consisted of the right lobe in 61 cases, left lobe with or without the caudate in 38 cases, and the right posterior section in 10 cases.

Surgical techniques

Hepatic artery reconstruction was performed under a surgical microscope (OPMI Vario S88, Zeiss, Tokyo, Japan) (MS group, $n = 84$) until January 2009, when we began to adopt surgical loupes (4.5×, Zeiss, Tokyo, Japan) for arterial reconstructions in all cases (SL group, $n = 25$).

The procedures for anastomosis were similar between the MS and SL groups. First, the hepatic artery in both the donor and the recipient was carefully handled, with appropriate preservation of the surrounding connective tissue, so as to avoid skeletonization of the artery. Appropriate alignment of both the length and rotation was determined. End-to-end anastomosis was carried out by interrupted sutures using non-absorbable monofilament 8-0 (polypropylene suture). First, both the dorsal and ventral ends were anastomosed. While the sutures were stretched apart gently by the first assistant, three to four sutures were placed on one side and tied after confirmation of their correct placement through the arterial layers. The other side was then sutured after flipping the artery, keeping the two angle sutures stretched. If there were multiple arteries in the donor liver, all of the arteries were anastomosed, to the extent feasible.

All the surgical procedures were undertaken by two experienced hepatobiliary transplant surgeons.

Immediately after reperfusion of the liver, DUS was performed. Values of the resistive index of the hepatic artery in the liver hilum of less than 0.6 or peak arterial velocity values of less than 15 cm/s at the proximal part of the intrahepatic artery are considered as abnormal, and the anastomosis was always repeated if the intraoperative DUS study was abnormal.

Postoperative anticoagulant therapy

We routinely start standard anticoagulant therapy once the patient's postoperative condition has stabilized. Intravenous administration of heparin sodium is initiated at the dose of 100 U/h when the aPTT (abnormal partial thromboplastin time) is lower than 40 s. When the target aPTT increased to the range of 40–50 s, then the heparin sodium dose was titrated and could be increased to 600 U/h until postoperative day (POD) 28. The anticoagulant therapy was usually discontinued on POD 28; however, if any abnormality was detected on DUS or MDCT, it was continued beyond POD 28. Antiplatelet agents were started for interventional radiology (IVR)-confirmed or DUS-diagnosed HAS until the abnormality improved or resolved. At this point, warfarin was initiated, with the target prothrombin time/international normalized ratio (PT-INR) set at 1.5–2.5, for any portal venous or hepatic venous abnormalities, such as partial thrombosis.

Postoperative evaluation of the hepatic artery anastomosis

Doppler ultrasound was routinely performed twice a day in the immediate postoperative period (until POD 3), with the frequency of the study reduced to once daily until POD 28, and thereafter to once every other day, and finally to once a week during the remaining period of the patient's hospital stay. In addition, diagnostic DUS was also performed anytime in the event of elevation of the serum transaminase levels. The abnormal findings of hepatic artery anastomosis that were considered as warranting hepatic arterial angiography and IVR consisted of the combination of a refractive index (RI) value of less than 0.6 in the DUS study and elevation of the serum AST or ALT (DUS-based criteria).

Interpretation of the CT images was performed by expert radiologists on staff in the hospital. Hepatic arterial complications were classified by these experts into four categories; (1) hepatic arterial thrombosis; (2) hepatic arterial stenosis, defined as anastomotic narrowing of >50 %; (3) suspected or mild hepatic arterial stenosis, defined as anastomotic narrowing of less than 50 %; and (4) normal findings. In April 2005 MDCT was introduced in our hospital; before that date CT had been performed whenever needed for diagnosing suspected hepatic arterial complications. After April 2005, we started prospective dynamic MDCT studies in recipients of liver transplants ($n = 60$), in which dynamic MDCT was performed in the recipients on POD 7, 14, 28, and at postoperative month (POM) 3, 6, and 12.

In contrast to the absolute indication of angiography/IVR in cases fulfilling DUS-based criteria, abnormal findings such as suspected hepatic arterial stenosis on CT or MDCT alone, in the absence of DUS-based criteria, are not considered clinically significant; therefore IVR was not performed. We defined IVR-confirmed HAS cases as those in which the HAS was confirmed by angiography, and control cases as those not fulfilling the DUS-based criteria.

Risk factor analysis for HAS and evaluation of the role of MDCT

To identify the risk factors for the development of HAS, the following factors were analyzed and compared between the IVR-confirmed HAS group ($n = 6$) and the control group not fulfilling the DUS-based criteria for HAS ($n = 101$): recipient age, preoperative model for end-stage liver disease score (MELD score), donor age, donor arterial diameter, number of anastomoses, anastomosis method (microscope versus surgical loupes), time for anastomosis, graft type (right lobe, left lobe, right lateral sector), ABO incompatibility between donor and recipient, and presence/absence of acute rejection.

Furthermore, the usefulness of MDCT in the diagnosis of hepatic arterial complications was investigated in the participants of the prospective MDCT study ($n = 60$). The MDCT findings were compared between the IVR-confirmed HAS group ($n = 3$) and the control group not fulfilling the DUS-based criteria for HAS ($n = 57$).

Statistical analysis

Results are expressed as mean ± standard deviation. Statistical examination of the correlations was based on the Pearson's product-moment correlation. Clinical data of the donors were compared with Student's t test. P values less than 0.05 were considered to indicate statistical significance.

Results

The patient characteristics and summary of the hepatic anastomosis procedure are described in Table 1. The patient background characteristics were similar between the MS group ($n = 85$) and the SL group ($n = 24$). With regard to the graft type, the frequency of right lobe grafts, as compared to left lobe and other grafts, tended to be higher in the MS group than in the SL group, and the graft weight/recipient standard liver volume (GW/SLV) ratio was larger in the MS group than in the SL group ($P = 0.036$ for both). The cold ischemia time was significantly longer in the SL group, while the warm ischemia time was shorter in the SL group than that in the MS group ($P = 0.0001$ and 0.029, respectively). The patient survival curves of the SL and MS groups are shown in Fig. 1. Survival in the SL group was better than in the MS group, although the difference did not reach statistical significance ($P = 0.057$, log rank test).

A single hepatic artery anastomosis was performed in 96 patients (88.1 %), while double anastomoses were performed in 12 cases (11.0 %) and a triple anastomosis was needed in 1 case (0.9 %). The diameter of the main hepatic artery was similar between the MS and SL groups. None of the 109 patients developed the complication of hepatic artery thrombosis, but HAS was diagnosed according to DUS-based criteria in 8 patients (7.3 %); of those eight patients, all of whom went on to have IVR, the diagnosis was confirmed by IVR in 6 (5.5 %). Treatment with percutaneous transarterial balloon dilatation was successful in two cases, whereas failure due to an intimal flap occurred in one case (12.5 %). In another three cases, treatment was not indicated because of technical difficulties, such as meandering proximal artery or arterial spasm (37.5 %). The two cases with HAS diagnosed according to DUS-based criteria alone had trivial stenosis that did not warrant

Table 1 Patient characteristics

	Total ($n = 109$)	Microscope (MS group) ($n = 85$)	Surgical loupes (SL group) ($n = 24$)	*P* value
Recipient age	49.8 ± 12.3	49.1 ± 12.5	53.1 ± 11.1	0.231
Recipient gender (M/F)	58/51	48/37	10/14	0.199
Indication (viral/cholestatic/fulminant/others)		54/13/9/18	15/5/2/3	0.766
PreOP MELD score	20.7 ± 8.9	20.8 ± 9.0	20.2 ± 8.8	0.865
Donor age	38.1 ± 13.2	38.1 ± 13.1	38.1 ± 13.1	0.953
Donor gender				
Blood type (identical/compatible/incompatible)	76/26/7	57/23/5	19/3/2	0.328
Graft type (right/left/right lateral)	61/38/10	50/25/10	11/13/0	0.036
Graft weight/standard liver volume (%)	48.4 ± 10.2	49.4 ± 10.3	44.1 ± 8.9	0.036
Cold ischemic time (min)	82.1 ± 45.5	73.7 ± 39.6	112.7 ± 48.9	0.0001
Warm ischemic time	43.0 ± 12.0	44.3 ± 12.4	37.8 ± 8.9	0.029
Arterial diameter	2.00 ± 0.76	1.94 ± 0.71	2.18 ± 0.89	0.198
Number of anastomosis				
Single	96 (88.1 %)	76 (89.4 %)	20 (83.3 %)	0.534
Double	12 (11.0 %)	8 (9.4 %)	4 (16.7 %)	
Triple	1 (0.9 %)	1 (1.2 %)		
Hepatic anastomosis time per anastomosis	45.2 ± 19.5	46.4 ± 20.7	38.7 ± 13.1	0.094
Hepatic artery thrombosis	0	0	0	
Hepatic artery stenosis				
Suspected mild stenosis by CT scan image within 12 months	39 (35.8 %)	28 (32.9 %)	11 (45.8 %)	0.245
Angiography performed	8 (7.3 %)	8 (9.4 %)	0	0.118
Angiography confirmed	6 (5.5 %)	6 (7.1 %)	0	0.181

Data are expressed as mean ± standard deviation. *P* values were calculated by Student's *t* test. *MELD* model for end-stage liver disease

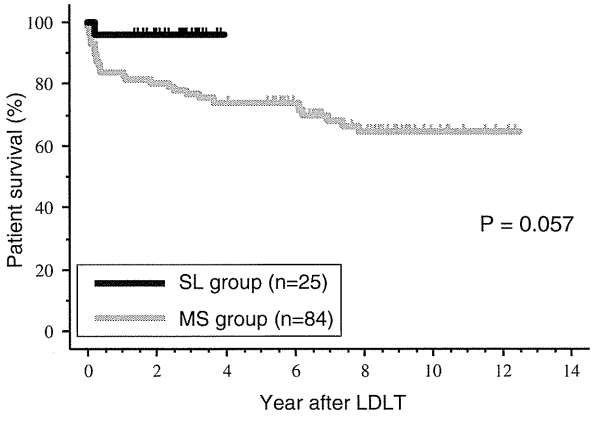

Fig. 1 Patient survival curves after LDLT. The patient survival in the surgical loupe (SL) group was better than that in the microscope (MS) group, although the difference didn't reach statistical significance ($P = 0.057$, log rank test). *Black line* SL group ($n = 25$), *Gray line* MS group ($n = 84$)

treatment. Two patients died after IVR, but in neither case was death related to the hepatic arterial complication; both died of bacterial/viral/fungal infections.

In contrast to the patients with DUS-based diagnosis of HAS ($n = 8$), including those with IVR-confirmed HAS ($n = 6$), the remaining patients (control group, $n = 101$) did not develop hepatic artery thrombosis and required no intervention for any hepatic arterial complications throughout the study period.

Risk factor analyses for HAS revealed only ABO incompatibility as being associated with a high risk of development of HAS ($P = 0.044$). None of the other factors, including arterial diameter and surgical method (microscope or surgical loupes) were found to be significant predictors of HAS (Table 2).

A comparative study of the MS and SL groups showed a tendency in the MS group toward higher frequency of use of right lobe grafts, a shorter cold ischemic time, and longer warm ischemic time, possibly due to its being a chronologically older series. In spite of the similar arterial diameter and number of anastomoses, the duration of performing each anastomosis tended to be shorter in the SL group (38.7 ± 13.1 min) than in the MS group (46.4 ± 20.7 min, $P = 0.094$). There was no patient among the study subjects

Table 2 Risk factor analyses for HAS

Clinical factors	*P* value	95 %CI
Surgical method		
Microscope versus surgical loupes	0.975	0.00 to >1000
Age, (years)	0.965	0.94 to 1.07
Gender (M/F)	0.328	0.07 to 2.39
PreOP MELD score	0.403	0.95 to 1.13
Donor age, (years)	0.085	0.82 to 1.01
Arterial diameter, (mm)	0.534	0.47 to 4.34
Anastomotic time, (min)	0.132	0.99 to 1.06
Graft weight/standard liver volume, (%)	0.472	0.00 to 213
Cold ischemia time, (min)	0.268	0.03 to 1.16
Warm ischemia time, (min)	0.416	0.97 to 1.09
Acute cellular rejection	0.983	0.00 to >1000
Graft type (left/right)	0.355	0.32 to 24.9

CI confidence interval, *MELD* model for end-stage liver disease

who developed hepatic artery thrombosis, and all of the 6 patients who developed HAS (5.5 %) confirmed by angiography belonged to the MS group.

Multidetector-row CT findings, which were categorized into three types (HAS, suspected/mild HAS, normal), were described for both IVR-confirmed HAS patients ($n = 3$) and the control group not fulfilling the DUS criteria for the diagnosis of HAS ($n = 57$) (Fig. 2). In most cases of IVR-confirmed HAS, the MDCT diagnosis was compatible with IVR-confirmed HAS, whereas a false positive MDCT diagnosis was obtained in a substantial number of cases of the control group. The false positive diagnosis rate of MDCT remained relatively high until 3 months after LDLT (100 % sensitivity and 72.8 % specificity at 3 months), but decreased thereafter until 12 months after LDLT (Fig. 2a, b).

The sensitivity, specificity, and accuracy of MDCT for the diagnosis of HAS are shown in Fig. 2c. The sensitivity was quite high throughout study period, whereas the specificity and accuracy were around 70 % until 6 months after LDLT, improving to over 90 % by 12 months after LDLT.

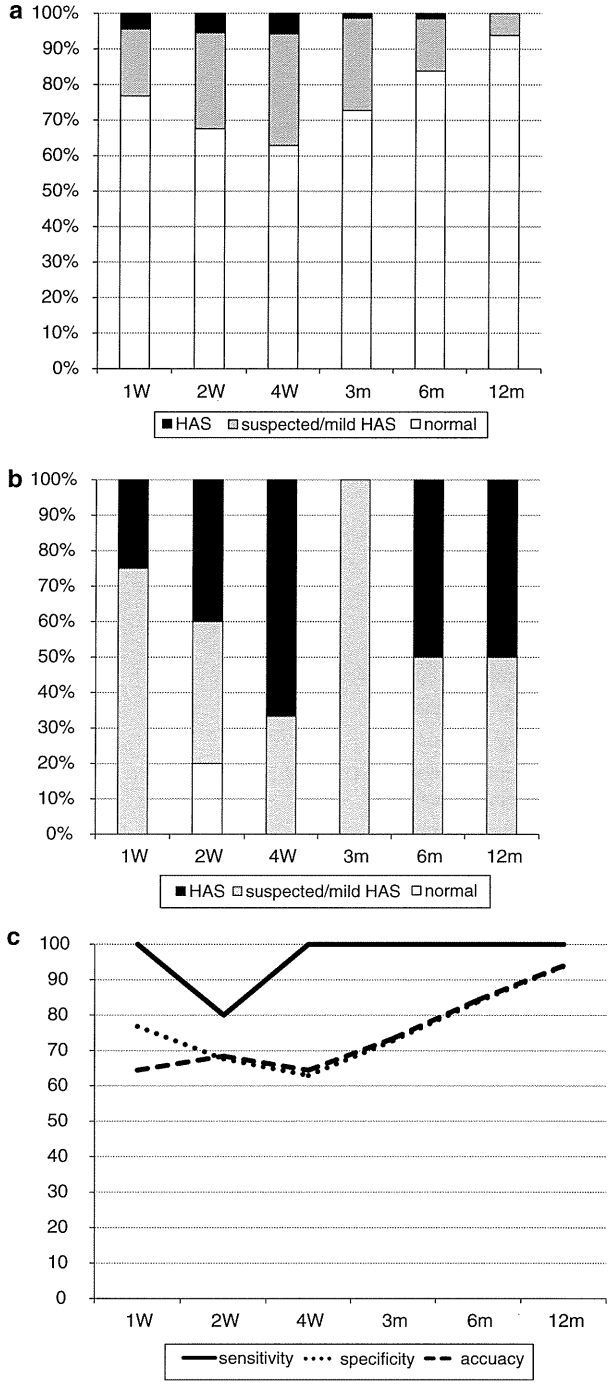

Fig. 2 HAS and MDCT diagnosis. **a** MDCT diagnosis of patients in the control group ($n = 57$), who did not fulfill the DUS-based criteria for the diagnosis of HAS. The false positive rate was relatively high until 3 months after LDLT (100 % sensitivity and 72.8 % specificity at 3 months), but it decreased thereafter up to 12 months after LDLT. **b** MDCT diagnosis in the IVR-confirmed HAS group ($n = 3$). **c** Sensitivity, specificity, and accuracy of MDCT in the diagnosis of HAS. The sensitivity was quite high throughout the study period, whereas the specificity and accuracy were around 70 % until 6 months after LDLT, improving to over 90 % by 12 months after LDLT

Discussion

In this study we investigated two different issues related to hepatic arterial anastomosis in LDLT. The first was to identify the risk factors for the development of hepatic arterial anastomotic complications, including a comparison of the surgical methods using either a microscope or surgical loupes for the arterial reconstruction. The second aim of the study was to evaluate the usefulness of MDCT in the diagnosis of hepatic arterial complications.

The risk factors for hepatic arterial complications after liver transplantation have not yet been clarified, except the anastomosis under a surgical microscope has been considered to be superior, with fewer complications, than that performed with surgical loupes in LDLT [8, 9]. Other studies have reported that continuous end-to-end suturing with a loupe yielded results equivalent to anastomosis under a microscope [23, 24]. In the present study, we found that ABO incompatibility was associated with a high risk of HAS, whereas none of the other factors examined, including the arterial diameter, history of acute cellular rejection, and the anastomosis method (microscope vs. surgical loupes) was found to be associated with the risk of development of HAS. Two (33.3 %) of the six recipients who underwent ABO-incompatible LDLT developed HAS ($P = 0.044$); therefore, this factor was considered a significant risk factor, although this interpretation should be validated with many more cases with ABO-incompatible LDLT. Both recipients survived, with an uneventful postoperative course and without antibody-mediated rejection. The reason underlying the increase in the risk of HAS in ABO-incompatible LDLT is not yet clear; however, there is a possibility of involvement of intimal injury associated with antibody-mediated immunological responses.

A comparative study between our MS and SL groups revealed that the time for hepatic arterial anastomosis was shorter in the SL group than in the MS group. The differences in the graft type, and in the warm and cold ischemic times between the two groups were considered to be mainly related to the chronological differences between the groups, and the influence of these parameters on the anastomosis time was considered to be negligible.

Similarly, the patient survival curve in the SL group was better than that in MS group, and that difference was also considered to be mainly related to the chronological differences between the groups. No case of IVR-confirmed HAS was encountered in the SL group, whereas HAS developed in six patients (7.1 %) in the MS group, although the difference did not reach statistical significance. These results show that the use of surgical loupes with a magnification power of 4.5× yielded at least similar outcomes for the anastomosis, and that the SL procedure was superior to the MS procedure in terms of the time required to perform the anastomosis. Setting up the device is much easier in the case of surgical loupes than in the case of a microscope. Surgical loupes (4.5×) can be safely substituted for a surgical microscope, but the choice should probably be left to the surgeon.

As for the second goal of our study, serial MDCT studies after LDLT showed that the sensitivity of this imaging modality for the detection of HAS was quite excellent within 12 months after LDLT, although the specificity was not optimal; up to 30 % false positive results were obtained, especially in the early post-transplant period (up to 3 months) after LDLT, whereas MDCT provided diagnosis with a rather high accuracy at 12 months after LDLT. In contrast, DUS-based criteria for HAS, namely, RI >0.6 combined with elevation of the serum AST/ALT, show 100 % sensitivity, 75 % specificity, and 93.6 % accuracy for the diagnosis of HAS during the first 12 months after LDLT. Furthermore, DUS was confirmed as being superior to MDCT for the diagnosis of HAS after LDLT.

Multidetector-row CT was also quite useful in detecting other arterial complications after LDLT [20, 21]. In contrast to a DUS study, MDCT can detect not only abnormalities in the hepatic artery but also abnormalities in other abdominal arteries, the portal vein, the hepatic vein, and the inferior vena cava. We found a superior mesenteric artery aneurysm and stenosis in two patients by MDCT, and both were successfully treated with antiplatelet agents. Blood flow to the liver graft can be evaluated easily by high-resolution MDCT. Therefore, it is worthwhile performing MDCT according to the follow-up schedule described in the present study. However, the rate of false positive diagnosis of HAS was relatively high during the first 3 months after LDLT, and this improved spontaneously over time. These data suggest that the abnormal findings on MDCT not supported by DUS-based criteria represent only a cautionary note for HAS, and that it may be sufficient to monitor the patient's course under therapy with antiplatelet agents, as long as the DUS-based criteria are not fulfilled.

In conclusion, our retrospective study revealed ABO-incompatible LDLT as a risk factor for HAS. Hepatic arterial anastomosis using surgical loupes tended to be time-saving and to yield similar or better results than traditional microscope anastomosis. Also, MDCT was a useful adjunct to a DUS study for the diagnosis of HAS; however, the substantially high rate of false positive diagnosis of HAS should be borne in mind in clinical practice.

Conflict of interest None declared

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American Journal of Transplantation 2014; 14: 102–114
Wiley Periodicals Inc.

doi: 10.1111/ajt.12520

Impact of Rituximab Desensitization on Blood-Type-Incompatible Adult Living Donor Liver Transplantation: A Japanese Multicenter Study

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We evaluated the effects of rituximab prophylaxis on outcomes of ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) in 381 adult patients in the Japanese registry of ABO-I LDLT. Patients underwent dual or triple immunosuppression with or without B cell desensitization therapies such as plasmapheresis, splenectomy, local infusion, intravenous immunoglobulin and rituximab. Era before 2005, intensive care unit-bound status, high Model for End-Stage Liver Disease score and absence of rituximab prophylaxis were significant risk factors for overall survival and antibody-mediated rejection (AMR) in the univariate analysis. After adjustment for era effects in the multivariate analysis, only absence of rituximab prophylaxis was a significant risk factor for AMR, and there were no significant risk factors for survival. Rituximab prophylaxis significantly decreased the incidence of AMR, especially hepatic necrosis ($p < 0.001$). In the rituximab group, other B cell desensitization therapies had no add-on effects. Multiple or large rituximab doses significantly increased the incidence of infection, and early administration had no advantage. In conclusion, outcomes in adult ABO-I LDLT have significantly improved in the latest era coincident with the introduction of rituximab.

Keywords: Antibody-mediated rejection, blood-type incompatible, desensitization, living donor liver transplantation, rituximab

Abbreviations: ABO-I, ABO-blood-type incompatible; ACR, acute cellular rejection; AIH, autoimmune hepatitis; AMR, antibody-mediated rejection; AUC, area under the curve; CMV, cytomegalovirus; DSA, donor-specific antibody; FHF, fulminant hepatic failure; ICU, intensive care unit; IHBC, intrahepatic biliary complication; IVIG, intravenous immunoglobulin; LDLT, living donor liver transplantation; MELD, Model for End-Stage Liver Disease; RBC, red blood cell; ROC, receiver operating characteristic

Received 03 June 2013, revised and accepted for publication 24 September 2013

Introduction

Advances in ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) through innovations in B cell desensitization aimed at preventing antibody-mediated rejection (AMR) have expanded the donor pool in Japan. Local infusion through the portal vein or hepatic artery to decrease inflammatory reaction at the epithelium was introduced in 2000, and rituximab prophylaxis was introduced widely in 2004 in Japan (1). Although there have been several single-center reports of rituximab prophylaxis in ABO-I LDLT, all describe small numbers of patients (2–4). There is no information about how much, how many times or when rituximab should be administered, and there have been no comparisons of patient outcomes with and without rituximab in a large cohort.

Age is an important prognostic factor for AMR and patient and graft survival (5). Demand for an effective desensitization method is especially strong in adult ABO-I LDLT. This study aimed to assess the effects of rituximab prophylaxis in ABO-I LDLT and to determine an effective and safe rituximab regimen.

Patients and Methods

Data collection

The Japan Study Group for ABO-Blood-Type-Incompatible Transplantation and a national registry for liver transplantation were established in 2001 by transplant centers performing ABO-I LDLT in Japan. The study group meets yearly to report experiences and has established a consensus for AMR diagnosis, treatment strategies and quality control of antibody titer measurements. Questionnaires are updated yearly and were sent in 2012 to registered surgeons and hepatologists in transplant centers, inquiring about patient characteristics, treatments and clinical courses. Information assayed included age, sex, disease, blood types of the recipient and donor, preoperative status, Model for End-Stage Liver Disease (MELD) score, relation of donor to recipient, peak titer of anti-donor-blood-type antibodies before transplantation and anti-donor antibody titer at the time of operation. Each center was classified as a large (≥10 ABO-I cases) or small (<10 ABO-I cases) volume center. Patients who required hospitalization in an intensive care unit (ICU) or a ward before surgery were classified as "in-ICU" or "in-hospital," respectively. Patients who required medical care other than in an ICU or ward were classified as "at home" at the time of transplantation. Treatment data included graft type, splenectomy, immunosuppression, local infusion, plasmapheresis, intravenous immunoglobulin (IVIG) and rituximab. Data concerning dose, frequency and timing of rituximab treatment and its adverse effects were collected in 2012. Clinical course data included peak titer of anti-donor-blood-type antibodies after transplantation, as well as rejection, bacterial infection, fungal infection, cytomegalovirus (CMV) disease requiring treatments and patient survival. Data on mortality and cause of death were also collected.

Measurement of anti-A/B antibody levels

Titers of anti-donor-blood-type antibodies were measured at each institution and a quality control survey was performed yearly by The Japan Study Group for ABO-Blood-Type-Incompatible Transplantation (6). The standard protocol for the test tube agglutination test is described briefly below (6,7). For both IgM and IgG assays, red blood cells (RBCs) were combined with the patient's serum sample at a ratio of 1:2 and centrifuged for 15 s. For the IgM assay, serum samples were first serially diluted with saline, and then incubated with RBCs at room temperature for 15 min. For the IgG assay using anti-human globulin, serum samples were preincubated with 0.01 M dithiothreitol at 37°C for 30 min, and then serially diluted and incubated with RBCs at 37°C for 30 min. The final dilution at which the agglutination reactivity was positive (1+), not equivocal (+/−), was determined as the antibody titer.

Definitions

Clinical AMR was diagnosed on the basis of radiological findings and clinical course, as described previously (1,5). The clinical manifestations of AMR were hepatic necrosis and intrahepatic biliary complication (IHBC). Hepatic necrosis was diagnosed when hepatic enzyme levels increased markedly in laboratory studies and liver necrosis was observed by computed tomography, usually 1 week after transplantation. IHBC was diagnosed when refractory cholangitis had developed and sclerosing change of the hepatic duct was observed by cholangiography. Diagnosis of acute cellular rejection (ACR) and chronic rejection was based on Banff criteria (8). Infectious diseases were defined as infections requiring treatment.

Statistical analysis

Survival curves were constructed with the Kaplan–Meier method (1). In univariate and multivariate analyses, Cox regression and logistic regression were used to evaluate the association between patient characteristics and overall survival and AMR, respectively. In the multivariate analyses, all potential confounders ($p < 0.05$ in the univariate analysis), including the era of operation, were included, and all patient data, including those for which values were missing, were used to minimize confounding and biases. The incidences of clinical complications were compared by using the chi-squared test.

Receiver operating characteristic (ROC) curves were plotted and areas under the curve were calculated to assess the optimum cut-off values for independent predictors of AMR. In analyses of prognostic factors for AMR and patient survival, the antibody cut-off titers that we calculated previously (1) were used. In the subgroup analysis of patients treated with rituximab, the cut-off titers for antibodies were newly calculated. SAS version 9.3 (SAS Institute, Inc., Cary, NC) was used for statistical analysis, and JMP version 10.0 (SAS Institute, Inc.) was used for the ROC curve analysis.

This study was performed in accordance with the provisions of the Declaration of Helsinki (as revised in Seoul, Korea, October 2008).

Results

Patients

By December 2011, clinical and laboratory data on 663 patients who underwent ABO-I LDLT in 37 institutions were available in the Japanese registry of ABO-I LDLT; of these patients, 381 who were aged 16 years or older were included as adults in the study. All 136 adult patients enrolled in our previous study (1) were included in the current study. The annual number of adults undergoing ABO-I LDLT was higher in 2001 and 2004 than in the previous years (Figure 1).

Demographic data on the 381 patients are listed in Table 1. Recipient age ranged from 16 to 70 years (median, 52 years). MELD scores ranged from 17 to 66 (median, 18), and donor age ranged from 18 to 66 (median, 45). Graft type was left-side liver in 146 patients, right-side liver in 231 patients and unknown in 4 patients. The original diseases were hepatocellular carcinoma in 104 patients, hepatitis C cirrhosis in 58 patients, hepatitis B cirrhosis in 22 patients, alcoholic cirrhosis in 14 patients, primary biliary cirrhosis in 57 patients, primary sclerosing cholangitis in 10 patients, cirrhosis secondary to autoimmune hepatitis (AIH) in 5 patients, cirrhosis after Kasai operation for biliary atresia in 24 patients, fulminant hepatic failure (FHF) in 22 patients (including 2 cases of FHF due to AIH), Wilson's disease in 8 patients, cirrhosis secondary to nonalcoholic steatohepatitis in 6 patients, cryptogenic cirrhosis in 5 patients, idiopathic portal hypertension in 5 patients, re-transplantation in 16 patients and other diseases in 25 patients. In an analysis of the impact of the original disease, 7 patients with AIH (5 cases of cirrhosis and 2 of FHF), 57 patients with primary biliary cirrhosis and 10 patients with primary sclerosing cholangitis were classified as having autoimmune disease.

Immunosuppression

All patients underwent double (calcineurin inhibitor and steroids; n = 36) or triple (calcineurin inhibitor, steroids and antimetabolites; n = 345) immunosuppression. The

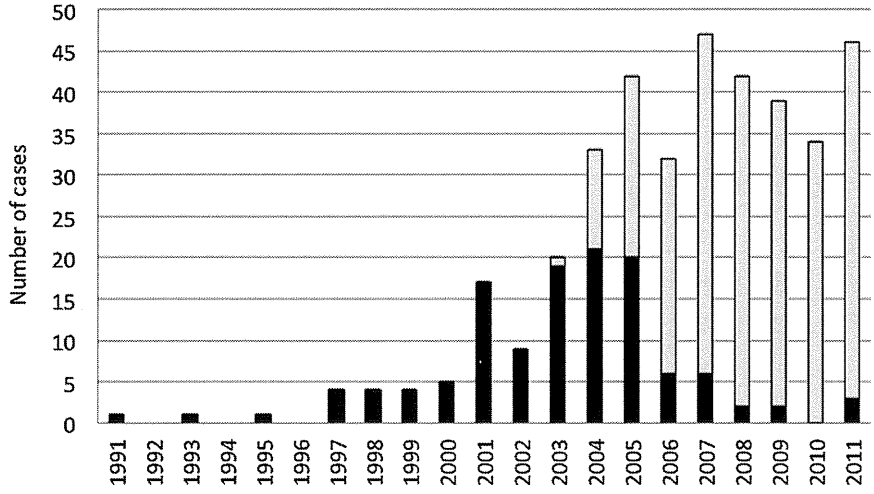

Figure 1: Annual numbers of adults undergoing ABO-I LDLT or rituximab prophylaxis at 37 institutions in Japan. ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) without rituximab prophylaxis (black bars); with rituximab prophylaxis (gray bars).

calcineurin inhibitor tacrolimus was administered in 364 cases, cyclosporine in 13 cases and an unknown drug in 4 cases. Regarding antimetabolites, cyclophosphamide was administered in 137 cases, mycophenolate mofetil in 286 cases, azathioprine in 18 cases, mizoribine in 20 cases and data were missing in 4 cases. Cyclophosphamide was switched to another antimetabolite in 105 cases. Antibody induction was performed by anti-lymphocytic antibody in 36 cases, anti-lymphocyte globulin in 15 cases, anti-IL-2 receptor antibody in 18 cases, muromonab-CD3 (OKT-3) in 2 cases and an unknown antibody in 1 case.

B cell desensitization

Plasmapheresis (n = 320), local infusion (n = 312), rituximab (n = 259), splenectomy (n = 241) and IVIG (n = 56) were performed. Local infusion, IVIG and rituximab were first used in 2000, 2003 and 2004, respectively. The number of times plasmapheresis was used before transplantation ranged from 0 to 11 (median, 2). Prophylactic IVIG was performed in seven institutions as center-specific policy, and it was performed in 6 patients before transplantation and 56 patients after transplantation. Here, we analyzed the effects of only posttransplantation IVIG. The dose ranged from 0.5 to 0.8 g/kg/injection, and the number of doses in regimens ranged from 2 to 5. There was no significant difference in titers between patients treated, or not treated, with IVIG (data not shown).

In the subgroup analysis of the rituximab group, regimens were classified into the following four groups: rituximab only without splenectomy or local infusion (R; n = 10); rituximab with splenectomy but without infusion (RS; n = 30); rituximab with infusion but without splenectomy (RI; n = 80); and rituximab with both infusion and splenectomy (RIS; n = 137).

Rituximab administration

Doses of rituximab were 500 mg/body in 113 cases, 300 mg/body in 60 cases and 375 mg/m^2 in 49 cases. The number of doses administered was 1 in 222 cases, 2 in 22 cases and 3 in 12 cases. The timing of initial administration ranged from preoperative days 0 to 66 and was $\leq$6 days before transplantation in 22 cases (Figure 2).

Analysis for prognostic factors

In univariate Cox regression analyses, prognostic factors that were significantly and favorably associated with patient survival were era (2005 onward), preoperative status (at home), low MELD score (<23), rituximab prophylaxis, low peak IgM and IgG donor-specific antibody (DSA) titers posttransplantation (<64), absence of bacterial and fungal infection and absence of AMR (Table 1). There was no significant factor among pretransplant characteristics and types of desensitization therapy in the multivariate analysis after adjustment for the era effect (Table 2).

In univariate analyses, significant risk factors for AMR were era (up to 2000 or 2001–2004), autoimmune disease, preoperative status (in-ICU), high peak IgG DSA titer before transplantation ($\geq$64), high IgG DSA titer at transplantation ($\geq$16), high MELD score ($\geq$23), absence of rituximab prophylaxis, high peak IgM and IgG DSA titers posttransplantation (both $\geq$64) and presence of fungal infection (Table 1). Among pretransplant characteristics and types of desensitization therapy, only the absence of rituximab prophylaxis was a significant indicator of risk of AMR in the multivariate analysis after adjustment for the era effect (Table 3).

AMR was a significant risk for overall survival in the univariate analysis ($p < 0.001$; Figure 3).

Table 1: Prognostic factors for overall survival and antibody-mediated rejection: univariate analysis (n = 381)

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association without unknown)	Odds ratio	95% CI	p-Value	p-Value (global association without unknown)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Characteristics before transplantation										
Sex	Male	169	1.000	–	–	–	1.000	–	–	–
	Female	212	1.062	0.762–1.479	0.723		1.455	0.759–2.789	0.259	
Center size	Less than 10 cases	49	1.000	–	–	–	1.000	–	–	–
	10 cases or more	332	1.102	0.684–1.845	0.705		1.171	0.438–3.132	0.749	
Era	Up to 2000	20	1.000	–	–	0.002*	1.000	–	–	<0.001*
	2001–2004	79	0.628	0.335–1.178	0.147		0.640	0.214–1.915	0.425	
	2005 onward	282	0.391	0.217–0.708	0.002*		0.188	0.065–0.539	0.002	
Autoimmune disease	No	304	1.000	–	–	–	1.000	–	–	–
	Yes	74	1.032	0.685–1.553	0.882		2.411	1.217–4.777	0.012*	
	Unknown	3	2.612	0.642–10.62	0.180		0.000	N/A	N/A	
Preoprative status	At home	143	1.000	–	–	0.013*	1.000	–	–	0.022*
	In-hospital	178	1.222	0.837–1.786	0.299		1.460	0.692–3.080	0.320	
	In-ICU	40	2.153	1.289–3.596	0.003*		3.639	1.438–9.208	0.006*	
	Unknown	20	1.489	0.727–3.048	0.277		0.575	0.071–4.673	0.605	
Recipient's blood type	A	91	1.000	–	–	0.860	1.000	–	–	0.116
	B	87	0.896	0.548–1.464	0.660		1.050	0.353–3.128	0.930	
	O	203	1.004	0.671–1.502	0.984		2.081	0.878–4.932	0.096	
Donor's blood type	A	183	1.000	–	–	0.654	1.000	–	–	0.654
	B	117	0.949	0.643–1.400	0.793		0.757	0.363–1.580	0.458	
	AB	81	1.166	0.772–1.762	0.465		0.726	0.311–1.693	0.459	
Antigen blood type	A	217	1.000	–	–	0.528	1.000	–	–	0.965
	B	153	0.992	0.705–1.396	0.962		1.024	0.537–1.951	0.943	
	AB	11	1.597	0.696–3.662	0.269		0.768	0.094–6.256	0.805	
Donor relative	No	188	1.000	–	–	–	1.000	–	–	–
	Yes	185	0.777	0.558–1.083	0.136		1.018	0.543–1.911	0.955	
	Unknown	8	0.350	0.049–2.523	0.298		0.000	N/A	N/A	
IgM (peak before transplantation)	Low (<256)	273	1.000	–	–	–	1.000	–	–	–
	High (≥256)	62	1.180	0.767–1.817	0.451		0.683	0.275–1.699	0.413	
	Unknown	46	0.908	0.528–1.563	0.729		0.142	0.019–1.060	0.057	
IgG (peak before transplantation)	Low (<64)	155	1.000	–	–	–	1.000	–	–	–
	High (>64)	182	1.229	0.863–1.749	0.253		2.352	1.159–4.771	0.018*	
	Unknown	44	1.112	0.627–1.973	0.717		0.568	0.122–2.637	0.470	
IgM (at transplantation)	Low (<16)	245	1.000	–	–	–	1.000	–	–	–
	High (≥16)	82	1.231	0.828–1.828	0.304		1.183	0.577–2.429	0.646	
	Unknown	54	1.007	0.613–1.653	0.979		0.130	0.017–0.976	0.047	
IgG (at transplantation)	Low (<16)	191	1.000	–	–	–	1.000	–	–	–
	High (≥16)	124	1.172	0.809–1.699	0.401		2.672	1.334–5.354	0.006*	
	Unknown	66	1.336	0.855–2.089	0.204		1.173	0.436–3.161	0.752	
MELD	Low (<23)	240	1.000	–	–	–	1.000	–	–	–
	High (≥23)	88	1.619	1.095–2.393	0.016*		3.172	1.565–6.428	0.001*	
	Unknown	53	2.039	1.325–3.138	0.001		2.193	0.898–5.352	0.085	
Desensitization therapies										
Local infusion	No	65	1.000	–	–	–	1.000	–	–	–
	Yes	312	0.904	0.582–1.405	0.655		0.929	0.410–2.105	0.861	
	Unknown	4	1.368	0.323–5.795	0.671		0.000	N/A	N/A	

(*Continued*)