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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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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

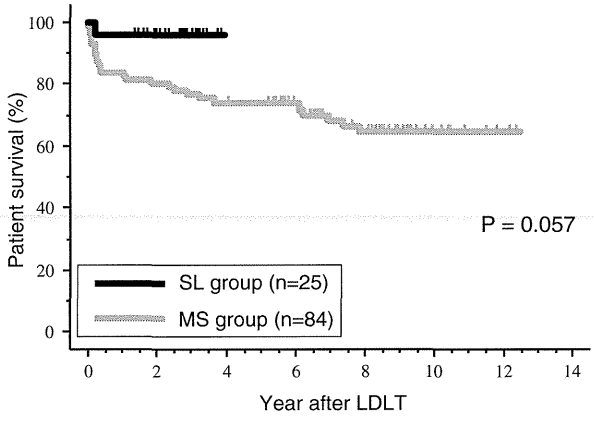

Fig. 1 Patient survival curves after LDLT. The patient survival in the surgical loupe (SL) group was better than that in the microscope (MS) group, although the difference didn't reach statistical significance (P = 0.057, log rank test). *Black line* SL group (n = 25), *Gray line* MS group (n = 84)

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.

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.

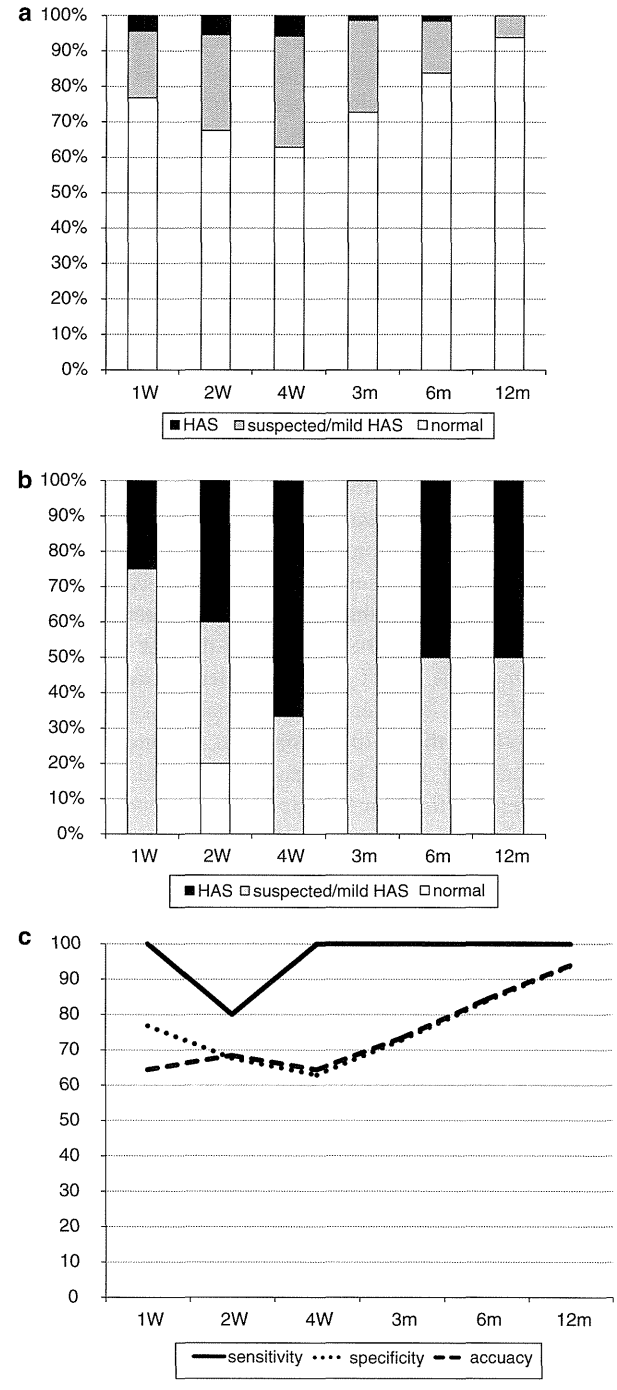

Fig. 2 HAS and MDCT diagnosis. **a** MDCT diagnosis of patients in the control group ($n = 57$), who did not fulfill the DUS-based criteria for the diagnosis of HAS. The false positive rate was relatively high until 3 months after LDLT (100 % sensitivity and 72.8 % specificity at 3 months), but it decreased 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

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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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

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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

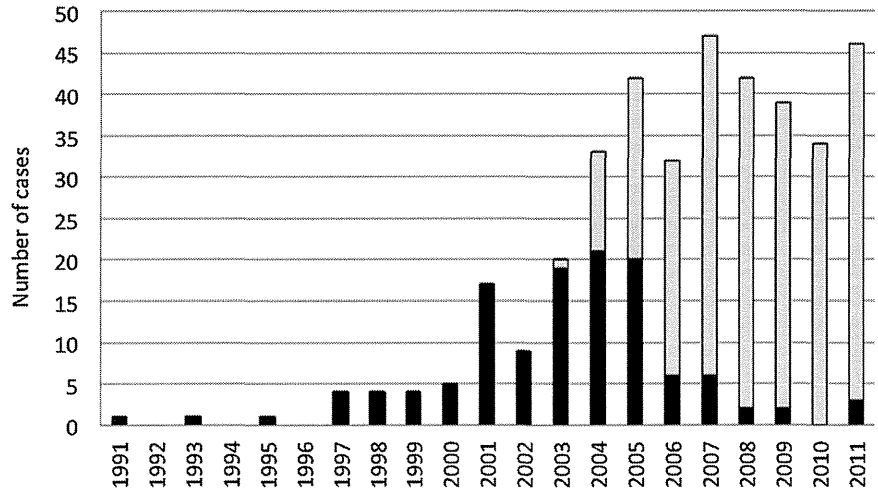

Figure 1: Annual numbers of adults undergoing ABO-I LDLT or rituximab prophylaxis at 37 institutions in Japan. ABO-blood-type-incompatible living donor liver transplantation (ABO-I LDLT) without rituximab prophylaxis (black bars); with rituximab prophylaxis (gray bars).

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 ≤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 (≥64), high IgG DSA titer at transplantation (≥16), high MELD score (≥23), absence of rituximab prophylaxis, high peak IgM and IgG DSA titers posttransplantation (both ≥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*)

Table 1: Continued

			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			
Splenectomy	No	135	1.000	–	–	–	1.000	–	–	–
	Yes	241	0.841	0.599–1.181	0.317		1.094	0.564–2.122	0.0790	
	Unknown	5	0.874	0.213–3.587	0.852		0.000	N/A	N/A	
Rituximab prophylaxis	No	119	1.000	–	–	–	1.000	–	–	–
	Yes	259	0.501	0.358–0.702	<0.001*		0.214	0.111–0.414	<0.001*	
	Unknown	3	1.554	0.380–6.358	0.540		0.000	N/A	N/A	
Prophylactic IVIG after transplantation	No	325	1.000	–	–	–	1.000	–	–	–
	Yes	56	0.859	0.523–1.409	0.547		0.392	0.117–1.313	0.129	
Anti-lymphocyte antibodies	No	345	1.000	–	–	–	1.000	–	–	–
	Yes	36	1.232	0.732–2.073	0.432		0.953	0.320–2.836	0.931	
Plasmapheresis	No	47	1.000	–	–	–	1.000	–	–	–
	Yes	320	0.723	0.454–1.152	0.172		1.132	0.422–3.038	0.806	
	Unknown	14	0.913	0.368–2.263	0.844		0.646	0.069–6.041	0.702	
Plasmapheresis (times)	0	47	1.000	–	–	0.240	1.000	–	–	0.247
	1	68	0.639	0.353–1.155	0.138		0.813	0.233–2.837	0.745	
	2	89	0.865	0.505–1.483	0.277		1.185	0.386–3.637	0.767	
	3	93	0.622	0.355–1.091	0.098		0.684	0.205–2.283	0.537	
	4	28	1.159	0.597–2.249	0.664		2.801	0.793–9.888	0.110	
	≥5	28	0.659	0.302–1.439	0.295		1.008	0.222–4.584	0.992	
	Unknown	28	0.616	0.282–1.346	0.224		1.826	0.478–6.973	0.378	
Short-term outcomes										
IgM (peak posttransplantation)	Low (<64)	251	1.000	–	–	–	1.000	–	–	–
	High (≥64)	94	1.689	1.180–2.418	0.004*		7.935	3.973–15.85	<0.001*	
	Unknown	36	1.046	0.571–1.916	0.884		0.000	N/A	N/A	
IgG (peak posttransplantation)	Low (<64)	205	1.000	–	–	–	1.000	–	–	–
	High (≥64)	126	1.484	1.043–2.110	0.028*		10.453	4.467–24.46	<0.001*	
	Unknown	50	1.142	0.671–1.945	0.624		1.805	0.450–7.244	0.405	
Acute rejection	No	296	1.000	–	–	–	1.000	–	–	–
	Yes	78	0.964	0.640–1.453	0.862		1.133	0.533–2.408	0.745	
	Unknown	7	2.023	0.746–5.487	0.166		0.000	N/A	N/A	
Chronic rejection	No	349	1.000	–	–	–	1.000	–	–	–
	Yes	5	1.905	0.703–5.158	0.205		1.827	0.199–16.74	0.594	
	Unknown	27	1.750	1.006–3.044	0.048		0.281	0.037–2.126	0.219	
Bacterial infection	No	254	1.000	–	–	–	1.000	–	–	–
	Yes	124	4.160	2.965–5.835	<0.001*		1.843	0.975–3.485	0.060	
	Unknown	3	3.650	0.890–14.97	0.072		0.000	N/A	N/A	
Fungal infection	No	342	1.000	–	–	–	1.000	–	–	–
	Yes	34	5.718	3.772–8.667	<0.001*		3.776	1.666–8.558	0.002*	
	Unknown	5	1.394	0.344–5.648	0.641		0.000	N/A	N/A	
CMV disease	No	199	1.000	–	–	–	1.000	–	–	–
	Yes	180	0.784	0.562–1.095	0.153		0.911	0.485–1.713	0.773	
	Unknown	2	1.233	0.171–8.870	0.835		0.000	N/A	N/A	
Antibody-mediated rejection	No	337	1.000	–	–	–	–	–	–	–
	Yes	44	2.493	1.654–3.759	<0.001*		–	–	–	

CMV, cytomegalovirus; IVIG, intravenous immunoglobulin; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

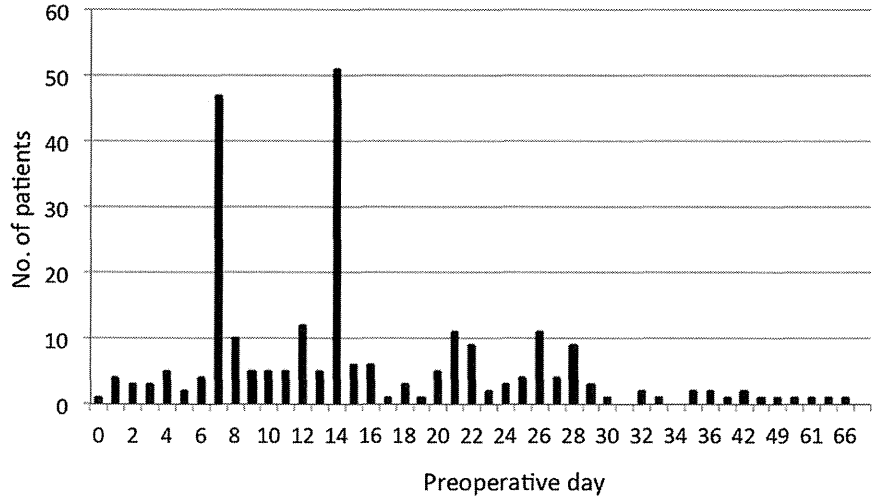

Figure 2: The timing of initial administration of rituximab ranged from preoperative days 0 to 66 and was within 6 days before transplantation in 22 cases.

Impact of rituximab on clinical outcomes

The AMR incidence was significantly lower in the rituximab group (6%) than in the nonrituximab group (23%) ($p < 0.001$; Figure 4, top); a significant difference was also observed for the subset of patients with hepatic necrosis-type AMR ($p < 0.001$; Figure 4, top). There were no significant differences between the incidences of ACR (Figure 4, top), bacterial infection or CMV disease (Figure 4, bottom) between the rituximab and nonrituximab groups. The rate of fungal infection was significantly lower in the rituximab group (4%) than in the nonrituximab group (19%) ($p < 0.001$; Figure 4, bottom).

Adverse effects of rituximab (kidney dysfunction, sepsis, neutropenia or lung edema) were observed in four patients, whose ages ranged from 56 to 62 years. Neutropenia occurred after a single dose of 300 mg/body, and the other complications manifested after the second or third dose of 500 mg/body. The patient with renal dysfunction died from a massive thrombus of the superior mesenteric artery on postoperative day 63, and the patient with sepsis died on postoperative day 202 from sepsis with an unknown focus. The other two patients are doing well.

Subgroup analysis of rituximab group

Because most ABO-I LDLT patients are currently administered rituximab, we analyzed the effects of additional desensitization therapies and the manner of rituximab administration to elucidate a better regimen. In a subgroup analysis of the rituximab group, local infusion, splenectomy, anti-lymphocyte antibodies and IVIG had no significant impact on overall survival or AMR incidence (Table 4).

Patients who were administered multiple doses of rituximab, or a regular dose of 500 mg/body or 375 mg/m^2, tended toward a lower incidence of AMR, but this was not

Table 2: Prognostic factors for overall survival: multivariate analysis (n = 381)

Characteristics	Category	N	5-Year survival (%)	Hazard ratio	95% CI	p-Value
Era	Up to 2000	20	40.0	1.000	–	–
	2001–2004	79	50.6	0.766	0.378–1.551	0.459
	2005 onwards	282	67.5	0.742	0.346–1.591	0.443
Preoperative status	At home	143	65.8	1.000	–	–
	In-hospital	178	63.6	1.087	0.735–1.606	0.676
	In-ICU	40	44.3	1.355	0.765–2.398	0.297
	Unknown	20	60.0	0.883	0.395–1.974	0.762
MELD	Low (<23)	240	66.9	1.000	–	–
	High (≥23)	88	57.2	1.364	0.894–2.080	0.149
	Unknown	53	48.8	1.420	0.827–2.437	0.203
Rituximab prophylaxis	No	119	48.4	1.000	–	–
	Yes	259	69.6	0.629	0.377–1.051	0.077
	Unknown	3	33.3	1.875	0.445–7.900	0.391

MELD, Model for End-Stage Liver Disease.

Table 3: Prognostic factors for antibody-mediated rejection: multivariate analysis (n = 381)

Characteristics	Category	N	AMR (%)	Odds ratio	95% CI	p-Value
Era	Up to 2000	20	30.0	1.000	–	–
	2001–2004	79	21.5	0.656	0.170–2.534	0.541
	2005 onwards	282	7.5	0.625	0.143–2.742	0.534
Autoimmune disease	No	304	9.5	1.000	–	–
	Yes	74	20.3	2.023	0.940–4.356	0.072
	Unknown	3	0.0	0.000	N/A	N/A
Preoperative status	At home	143	8.4	1.000	–	–
	In-hospital	178	11.8	0.929	0.404–2.134	0.862
	In-ICU	40	25.0	1.430	0.473–4.320	0.526
	Unknown	20	5.0	0.322	0.030–3.443	0.349
IgG (preoperative)	Low (<64)	155	7.7	1.000	–	–
	High (≥64)	182	16.5	1.805	0.724–4.505	0.205
	Unknown	44	4.6	0.744	0.100–5.555	0.773
IgG (at operation)	Low (<16)	191	7.9	1.000	–	–
	High (≥16)	124	18.6	1.933	0.790–4.731	0.149
	Unknown	66	9.1	1.066	0.269–4.234	0.927
MELD	Low (<23)	240	7.5	1.000	–	–
	High (≥23)	88	20.5	2.026	0.878–4.675	0.098
	Unknown	53	15.1	0.936	0.278–3.154	0.915
Rituximab prophylaxis	No	119	23.5	1.000	–	–
	Yes	259	6.2	0.248	0.089–0.690	0.008*
	Unknown	3	0.0	0.000	N/A	N/A

AMR, antibody-mediated rejection; MELD, Model for End-Stage Liver Disease.
*$p < 0.05$.

statistically significant (Table 4). In contrast, patients given multiple doses had significantly greater incidences of fungal infection and CMV disease than those given a single dose, and patients given the regular dose had a greater incidence of CMV disease than those given a small dose of 300 mg/body or less (Table 5). Patients subjected to local infusion together with rituximab prophylaxis (RI and RIS) had greater incidences of CMV disease than patients without local infusion or splenectomy (R) (Table 5). Finally, there were no significant differences among rituximab regimens in terms of AMR incidence or patient survival (Table 4; Figure 5).

Early administration of rituximab had no significant impact on AMR incidence or patient survival (Table 4). Twenty-two FHF patients underwent LDLT, and six of them were given

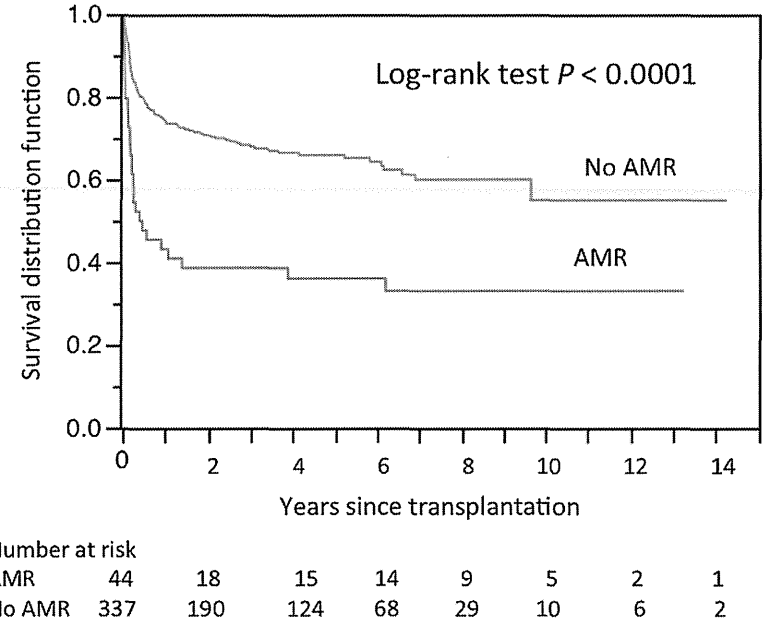

Figure 3: Comparison of overall survival between patients with and without antibody-mediated rejection. Patients with antibody-mediated rejection (AMR) had a significantly higher overall survival risk than those without AMR, $p < 0.001$.

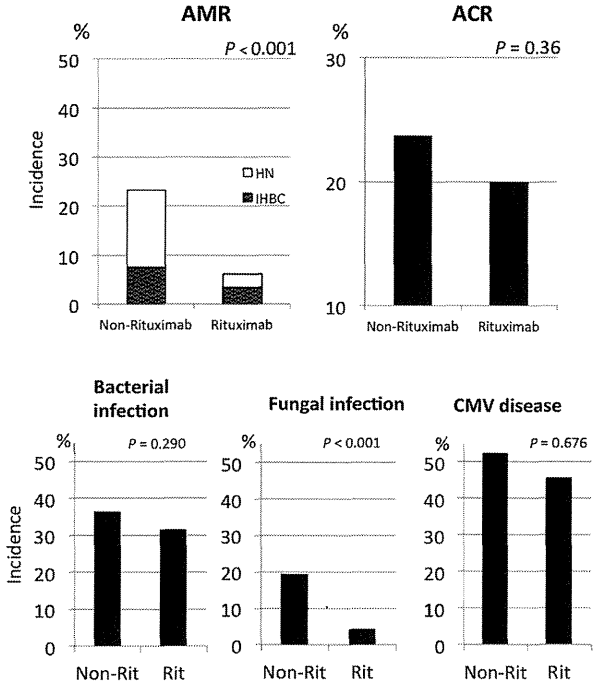

Figure 4: Comparison of incidences of complications between rituximab and nonrituximab groups. The incidences of antibody-mediated rejection (AMR) and acute cellular rejection (ACR) are shown (top); rates of intrahepatic biliary complication (IHBC) and hepatic necrosis (HN) type AMR were lower in the rituximab group than in the nonrituximab group (chi-squared test, $p < 0.0001$). The incidences of bacterial infection, fungal infection and cytomegalovirus (CMV) disease are shown (bottom); rates of bacterial infection and CMV disease were similar between the two groups (chi-squared test, $p = 0.36$), but the rate of fungal infection was significantly lower in the rituximab group (chi-squared test, $p < 0.0001$).

rituximab immediately before or during transplantation (three treated with RIS, two with RI and one with RS). All 6 patients survived transplantation without AMR, whereas AMR occurred in 7 patients and 1-year survival was 44% in the other 16 patients who were not given rituximab.

Peak IgG DSA titer before transplantation, IgG DSA titer at transplantation and peak IgG and IgM DSA titers post-transplantation showed a significant positive association with AMR incidence in the total cohort of adult ABO-I LDLT patients in the univariate analysis (Table 1). In the rituximab group, peak IgG and IgM DSA titers posttransplantation were significantly greater in patients with AMR than in those without AMR (Table 6). When the AMR incidence in the rituximab group was compared between high and low titers according to optimum cut-off values calculated from ROC curves, there were significant differences in peak IgG titers before transplantation (10% [10/104] vs. 3% [4/125] titer $\geq$128 vs. <128, $p = 0.042$), peak IgM titers post-transplantation (22% [10/45] vs. 3% [6/194], titer $\geq$64 vs. <64, $p < 0.001$) and peak IgG titers posttransplantation (19% [10/54] vs. 2% [3/171], titer $\geq$128 vs. <128, $p < 0.001$).

Discussion

Worldwide, the first case report of rituximab prophylaxis in kidney transplantation was published in Japan in 2002 (9); many rituximab protocols for kidney transplantation have been reported since. Monteiro et al (10) reported the first case of ABO-I liver transplantation using rituximab in 2003, and Usuda et al (3) reported the first case of rituximab prophylaxis in ABO-I LDLT in 2005. In the Japanese registry, the first adult case of rituximab prophylaxis was reported in November 2003. In our previous multicenter study (1) of 291 patients who underwent ABO-I LDLT up to and including March 2006, 44 adult patients were administered rituximab. The current study includes 259 adult patients who underwent rituximab prophylaxis up to and including December 2011.

After 2000, the evolution of innovation in the treatment of small-for-size syndrome in adult LDLT and desensitization for DSA was achieved (11–13). The era effect on overall survival is significant. In the total cohort of 381 adult patients, after adjustment for era effects in the multivariate analysis, only rituximab prophylaxis was a significant prognostic factor for AMR, but it was not a prognostic factor for overall survival. A prospective study is required to elucidate the effect of rituximab on patient survival; however, it would be difficult to remove rituximab prophylaxis when the current results are so much improved in the most recent era and when this may be attributable to rituximab.

To find the best regimen for rituximab, the impact of additional desensitization therapies and times and doses of rituximab were addressed. Splenectomy used to be considered an essential component of a successful ABO-I desensitization regimen for renal transplantation (14); however, it has been reported that rituximab can be used in place of splenectomy with similar outcomes (15,16). The Kyoto group suggested that splenectomy should be avoided in 2007 (2,17). In LDLT, however, splenectomy is performed not only for desensitization but also for portal flow adjustment in patients with small-for-size syndrome and for future anti-viral treatment using interferon in hepatitis C patients. An assessment of the effects of preserving the spleen is required in patients without small-for-size syndrome or hepatitis C infection in future.

Plasma exchange is a standard procedure to reduce DSA titers, but the titer required to prevent AMR is not defined. If titers increase again after plasmapheresis, another plasmapheresis is often performed. When peak titer before transplantation is very low, plasmapheresis is not performed. In other words, the more times the plasmapheresis

Table 4: Prognostic factors for antibody-mediated rejection and overall postsurgical survival: univariate analysis of 259 patients given rituximab prophylaxis

			Overall survival				Antibody-mediated rejection			
			Hazard ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)
Characteristics	Category	N	Cox regression analysis				Logistic regression analysis			
Local infusion	No	40	1.000	–	–	–	1.000	–	–	–
	Yes	218	1.329	0.635–2.779	0.451	–	2.882	0.370–22.450	0.312	–
	Unknown	1	–	–	–	–	–	–	–	–
Splenectomy	No	90	1.000	–	–	–	1.000	–	–	–
	Yes	169	0.985	0.614–1.579	0.948	–	0.881	0.309–2.506	0.812	–
Anti-lymphocyte antibodies	No	244	1.000	–	–	–	1.000	–	–	–
	Yes	15	0.838	0.306–2.298	0.731	–	0.447	0.023–8.547	0.593	–
Prophylactic IVIG after transplantation	No	214	1.000	–	–	–	1.000	–	–	–
	Yes	45	0.984	0.529–1.830	0.960	–	0.664	0.146–3.031	0.598	–
Timing of rituximab administration before transplantation	≤6 days	22	1.000	–	–	–	1.000	–	–	–
	>7 days	236	1.241	0.535–2.883	0.615	–	1.425	0.179–11.330	0.738	–
	Unknown	1	–	–	–	–	–	–	–	–
Number of doses of rituximab	1	225	1.000	–	–	0.443	1.000	–	–	0.922
	2	22	1.504	0.747–3.031	0.253	–	0.947	0.161–5.560	0.730	–
	3	12	1.377	0.550–3.448	0.494	–	0.543	0.027–10.77	0.689	–
Dose of rituximab	Regular	162	1.000	–	–	–	1.000	–	–	–
	Small	66	1.282	0.745–2.207	0.370	–	2.655	0.952–7.404	0.062	–
	Unknown	31	–	–	–	–	–	–	–	–
Dose and number of doses of rituximab	Regular × 1	134	1.000	–	–	0.461	1.000	–	–	0.409
	Regular × 2	16	1.408	0.589–3.366	0.442	–	0.451	0.023–8.902	0.601	–
	Regular × 3	12	1.506	0.580–3.910	0.400	–	0.595	0.029–12.240	0.737	–
	Small × 1	60	1.264	0.694–2.310	0.444	–	2.086	0.738–5.897	0.165	–
	Small × 2	6	2.755	0.844–8.993	0.093	–	4.058	0.512–32.19	0.185	–
	Unknown	31	–	–	–	–	–	–	–	–
Regimen	RS	30	1.000	–	–	0.700	1.000	–	–	0.938
	R	10	2.053	0.490–8.597	0.325	–	0.937	0.031–28.37	0.970	–
	RI	81	1.568	0.596–4.128	0.362	–	1.693	0.266–10.790	0.577	–
	RIS	137	1.691	0.667–4.285	0.268	–	1.454	0.242–8.743	0.683	–
	Unknown	1	–	–	–	–	–	–	–	–

IVIG, intravenous immunoglobulin; R, only rituximab; regular dose, 500 mg/body or 375 mg/m^2; RI, rituximab and infusion; RIS, rituximab and infusion and splenectomy; RS, rituximab and splenectomy; small dose, 300 mg/body or less.

Table 5: Prognostic factors for infectious complications: univariate analysis of 259 patients given rituximab prophylaxis

			Bacterial infection				Fungal infection				CMV disease			
			Odds ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)	Odds ratio	95% CI	p-Value	p-Value (global association)
Characteristics	Category	N	Logistic regression analysis				Logistic regression analysis				Logistic regression analysis			
Local infusion	No	40	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	218	1.449	0.671–3.128	0.345		0.830	0.173–3.993	0.816		2.945	1.373–6.319	0.006*	
	Unknown	1	–	–	–		–	–	–		–	–	–	
Splenectomy	No	90	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	169	0.588	0.342–1.011	0.055		0.913	0.260–3.208	0.887		1.071	0.641–1.791	0.793	
Anti-lymphocyte antibodies	No	244	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	15	2.010	0.703–5.747	0.193		1.650	0.197–13.82	0.644		1.049	0.369–2.982	0.929	
Prophylactic IVIG after transplantation	No	214	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Yes	45	1.792	0.925–3.471	0.084		1.922	0.489–7.559	0.350		1.626	0.851–3.106	0.141	
Timing of rituximab administration before transplantation	≤ 6 days	22	1.000	0.383–2.501	0.964	–	1.000	–	–	–	1.000	–	–	–
	>7 days	236	0.979	–	–		0.402	0.081–1.988	0.264		1.012	0.421–2.435	0.978	
	Unknown	1	–	–	–		–	–	–		–	–	–	
Number of doses of rituximab	1	225	1.000	–	–	0.513	1.000	–	–	0.010*	1.000	–	–	0.004*
	2	22	0.638	0.227–1.798	0.396		1.543	0.181–13.17	0.692		3.038	1.256–7.980	0.019*	
	3	12	1.549	0.475–5.050	0.468		10.288	2.278–46.47	0.002*		36.742	4.737–999.9	0.017*	
Dose of rituximab	Regular	162	1.000	–	–	–	1.000	–	–	–	1.000	–	–	–
	Small	66	1.742	0.948–3.203	0.074		0.122	0.000–0.984	0.152		0.455	0.249–0.832	0.011*	
	Unknown	31	–	–	–		–	–	–		–	–	–	
Dose and number of doses of rituximab	Regular × 1	134	1.000	–	–	0.283	1.000	–	–	0.040*	1.000	–	–	0.001*
	Regular × 2	16	0.679	0.182–2.526	0.563		2.243	0.220–12.32	0.412		14.802	3.517–137.3	0.003*	
	Regular × 3	12	2.101	0.625–7.058	0.230		8.542	1.756–37.86	0.006*		35.805	4.548–999.9	0.018*	
	Small × 1	60	1.828	0.955–3.501	0.069		0.192	0.001–1.734	0.270		0.780	0.412–1.451	0.440	
	Small × 2	6	1.471	0.258–8.390	0.664		2.108	0.015–23.08	0.657		0.110	0.000–0.964	0.167	
	Unknown	31	–	–	–		–	–	–		–	–	–	
Regimen	RS	30	1.000	–	–	0.266	1.000	–	–	0.685	1.000	–	–	0.034*
	R	10	2.611	0.574–11.71	0.221		3.105	0.232–41.87	0.366		2.609	0.574–11.71	0.221	
	RI	81	2.351	0.929–6.670	0.089		0.900	0.141–9.567	0.917		3.176	1.264–8.982	0.021*	
	RIS	137	1.566	0.642–4.318	0.357		0.980	0.195–9.654	0.983		4.053	1.688–11.07	0.004*	
	Unknown	1	–	–	–		–	–	–		–	–	–	

IVIG, intravenous immunoglobulin; R, only rituximab; regular dose, 500 mg/body or 375 mg/m^2; RI, rituximab and infusion; RIS, rituximab and infusion and splenectomy; RS, rituximab and splenectomy; small dose, 300 mg/body or less.
*$p < 0.05$.

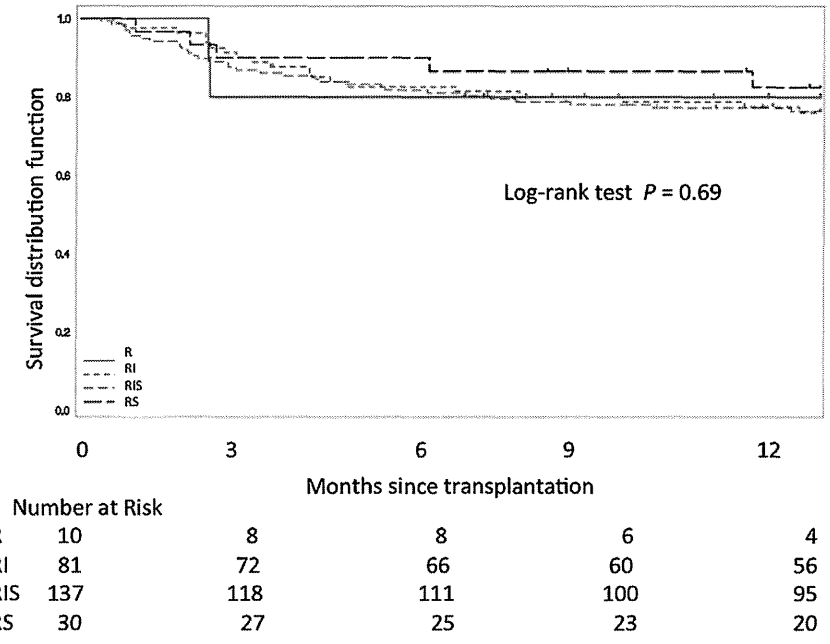

Figure 5: One-year survival of patients in the rituximab group. R, rituximab without splenectomy or local infusion (n = 10); RI, rituximab with infusion but without splenectomy (n = 81); RIS, rituximab with both infusion and splenectomy (n = 137); RS, rituximab with splenectomy but without infusion (n = 30). There were no significant differences among regimens with additional desensitization in patients with rituximab prophylaxis.

is performed, the greater the potential for an increase in DSA titer. However, we observed no significant relationship between the number of plasmapheresis procedures and clinical outcomes (Table 1).

IVIG is also a standard procedure, especially for human leukocyte antigen-related DSA in kidney transplantation, and the IVIG dose often ranged from 0.1 to 2 g/kg (18,19). In liver transplantation, Ikegami et al (4) reported a small series with desensitization by rituximab and IVIG (0.8 g/kg), and their cases were included here. We found no significant effect of IVIG on overall survival or AMR in the entire adult cohort (Table 1) and no additional effects in the rituximab group (Table 5). We analyzed the AMR incidence in each regimen with IVIG versus without IVIG (Figure 6). The AMR incidence was reduced from 26% to 9% in the local infusion and splenectomy (IS; no rituximab) regimen when IVIG was added, but this difference was not significant (p = 0.19). Among regimens with rituximab (R, RI, RIS and RS), the incidences were similar between with IVIG and without IVIG. IVIG is not approved in Japan and is not covered by insurance. IVIG costs 1.5–2.0 million yen per injection, whereas 500 mg of rituximab costs 0.3 million yen. A prospective study is required to elucidate the effects of IVIG in patients after rituximab prophylaxis.

The incidence of adverse effects of rituximab was 1.6% (4/258), and all patients recovered and underwent LDLT. Rituximab prophylaxis could be tolerated by patients with end-stage liver diseases. The incidences of bacterial

Table 6: Comparison of antibody titers between patients with and without AMR under rituximab prophylaxis

		AMR+			AMR−			
		N	Median	Mean ± SD	N	Median	Mean ± SD	p-Value
IgM	Peak before transplantation	15	64	158 ± 255	211	64	147 ± 199	0.881
	At transplantation	16	4	7 ± 8	213	4	16 ± 48	0.700
	Peak posttransplantation	16	64	593 ± 1091	223	8	49 ± 181	<0.001*
IgG	Peak before transplantation	14	128	408 ± 584	215	64	319 ± 771	0.221
	At transplantation	13	16	27 ± 35	210	8	34 ± 96	0.265
	Peak posttransplantation	13	256	1002 ± 2196	212	16	68 ± 187	<0.001*

AMR, antibody-mediated rejection. p-values are derived from Wilcoxon sum-rank test.
*p < 0.05 for AMR+ versus AMR−.