Transplant International

ORIGINAL ARTICLE

Outcome of the risk-stratified desensitization protocol in donor-specific antibody-positive living kidney transplant recipients: a retrospective study

Daigo Okada[1], Masayoshi Okumi[1], Yoichi Kakuta[1], Kohei Unagami[2], Junpei Iizuka[1], Toshio Takagi[1], Hideki Ishida[1] & Kazunari Tanabe[1]

1 Department of Urology, Tokyo Women's Medical University, Tokyo, Japan
2 Department of Nephrology, Tokyo Women's Medical University, Tokyo, Japan

Correspondence
Masayoshi Okumi MD, PhD, Department of Urology, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku, Tokyo 162-8666, Japan.
Tel.: 81-3-3353-8111;
Fax: 81-3-3356-0293;
e-mail: okumi@twmu.ac.jp

SUMMARY

Acceptable outcomes of donor-specific antibody (DSA)-positive living kidney transplantation (LKT) have recently been reported. However, LKT in crossmatch (XM)-positive patients remains at high-risk and requires an optimal desensitization protocol. We report our intermediate-term outcomes of XM-positive LKT vs. XM-negative LKT in patients who underwent LKT between January 2012 and June 2015 in our institution. The rate of acute antibody-mediated rejection (ABMR) within 90 days postoperation, graft function, and patient, and graft survival rates at 4 years were investigated. Patients were divided into three groups: XM−DSA− ($n = 229$), XM−DSA+ ($n = 36$), and XM + DSA+ ($n = 15$). The XM + DSA+ group patients underwent desensitization with high-dose intravenous immunoglobulin, plasmapheresis, and rituximab. The rates of ABMR within 90 days in the XM−DSA−, XM−DSA+, and XM + DSA+ groups were 1.3%, 9.4%, and 60.0%, respectively ($P < 0.001$). There were no significant differences in the graft function throughout the observational period, the 4-year patient or graft survival rates among three groups. This study showed that intermediate-term outcomes of XM-positive LKT were comparable to XM-negative LKT. However, our current desensitization protocol cannot avert ABMR within 90 days, and XM positivity is still a significant risk factor for ABMR. Further refinement of the current desensitization regimen is required.

Transplant International 2018; 31: 1008–1017

Key words
antibody-mediated rejection, desensitization, donor-specific antibody, living kidney transplantation

Received: 7 December 2017; Revision requested: 4 January 2018; Accepted: 13 April 2018; Published online: 11 May 2018

Introduction

Living kidney transplantation (LKT) is the treatment for end-stage renal disease and offers a better quality of life and survival advantage in comparison with chronic hemodialysis [1]. Donor-specific antibody (DSA) is associated with antibody-mediated rejection (ABMR) and significant immunologic barrier to graft and patient survival. In particular, highly-sensitized (e.g., complement-dependent cytotoxicity crossmatch [CDCXM] or flow cytometric crossmatch [FCXM] positive) patients have increased the risk of ABMR and early graft loss in LKT.

Pretransplant desensitization therapy, such as plasmapheresis (PP), B lymphocyte-depleting agents, and

© 2018 Steunstichting ESOT
doi:10.1111/tri.13269

intravenous immunoglobulin (IVIG), have been developed by various institutions for the purpose of preventing ABMR [2–9] and improving graft survival. Two desensitization regimens are now widely accepted for their efficacy: low-dose IVIG/plasma exchange and high-dose IVIG with or without rituximab. The goal of desensitization is to lower the DSA level to below the threshold capable of developing ABMR, and to maintain a low level in the immediate post-transplant period. Different protocols have been used to achieve this goal and are determined by recipients' baseline crossmatch (XM) DSA level [10,11]. These desensitization therapies have enabled highly-sensitized patients to receive kidney transplantation (KT) [12,13]. Nevertheless, XM-positive LKT leads to inferior graft survival compared with XM-negative LKT [14,15]. In addition, few studies have examined the intermediate- to long-term outcomes of XM-positive KT.

We hereby report our outcome data, emphasizing a risk-stratified desensitization protocol for DSA positive LKT.

Materials and methods

Study population

We performed a retrospective, observational study of 280 patients who underwent LKT from January 2012 to June 2015 in our institution. Data were extracted from the Japan Academic Consortium of Kidney Transplantation study (UMIN Clinical Trials Registry Number: UMIN000018327). The study protocol was approved by the research ethics committee (approval number: 3366-R), consistent with the Declaration of Helsinki. All patients have provided written informed consent. Prior to transplantation, we classified patients into three groups based on immunological risk stratification. This classification was then used to choose the specific transplant protocol for each group. In this study, we analyzed the rate of acute ABMR, graft function, patient, and graft survival at 4 years, and incidence of infectious events. Graft function was assessed by the estimated glomerular filtration rate calculated by the four-variable modification of renal disease equation.

Immunological risk stratification

Complement-dependent cytotoxicity crossmatch, FCXM, and Luminex single antigen beads assay (One Lambda Inc., Canoga, Park, CA, USA) for the detection of DSA were performed on all patients before transplantation. DSA strength was assessed by mean fluorescence intensity (MFI). Patients' MFI were checked before desensitization and at the time of KT. According to the results of CDCXM and FCXM pre-desensitization, all patients were stratified into three immunological risk groups as follows: "XM + DSA+" for positive FCXM and/or CDCXM and positive DSA; "XM−DSA+" for negative CDCXM and FCXM and positive DSA; and "XM−DSA−" for negative CDCXM and FCXM and negative DSA. Before and after KT, we determined the sensitivity status of all patients using complement-dependent cytotoxicity (CDC) and FCXM according to the manufacturer's instructions. Patients with more than 31% CDC positivity were considered a contraindication for living KTs. Patients with less than 30% CDC and/or positivity for FCXM were considered to be indicated for a desensitization protocol using high-dose IVIG treatment in this study. Briefly, FCXM was measured using an FACS Caliber (Becton Dickinson, San Jose, CA, USA). FCXM was routinely performed at the first visit, −1 day immediately before and after KT to check the status and confirm the negative conversion from positive to negative by FCXM, regardless of the DSA intensity by the Luminex single antigen beads assay. Examinations using pronase were conducted after rituximab (anti-CD20 antibody) administration to avoid the false-positive reaction of lymphocytes absorbed with rituximab. The Luminex single antigen beads assay was also conducted according to the manufacturer's instructions as previously described. We defined positive DSA as greater than 1000 MFI and positive FCXM as a shift greater than over 10 and/or ratio over 2.0 compared to the median MFI of a negative control.

Immunosuppression regimens and desensitization protocols

XM + DSA+ group: patients with XM + DSA+ received IVIG 4–6 times (total 2–4 g/kg), according to their DSA MFI level, followed by 2–4 sessions of double filtration plasmapheresis (DFPP) before transplantation. Five patients received IVIG 2 g/kg, one patient received 3 g/kg, and nine patients received 4 g/kg. Rituximab was administered intravenously at a dose of 300 mg at 1 month, and an additional 200 mg the day before transplantation. Desensitization was deemed successful when there was a negative conversion of XM. Oral tacrolimus (TAC), mycophenolate mofetil (MMF), and methylprednisolone (MP) were initiated 1 month before transplantation. Basiliximab is administered at a dose of

© 2018 Steunstichting ESOT

20 mg/day at the time of the operation and on postoperative day 4.

XM−DSA+ group: patients with XM−DSA+ received 2 DFPP before transplantation. Rituximab was administered intravenously at a dose of 200 mg the day before transplantation. Oral TAC, MMF, and MP were initiated 7 days before transplantation. Basiliximab was administered on the operative day and postoperative day 4. Desensitization was deemed successful when there was a decrease in DSA MFI.

XM−DSA− group: This group received neither pretransplant conditioning nor post-transplant therapy directed against DSA. TAC, MMF, MP, and basiliximab were administered in the same way as the XM−DSA+ group.

Diagnosis of rejection

The diagnosis of ABMR was confirmed by the detection of DSA and pathological findings, which included any of the following microvascular injuries: peritubular capillaritis (ptc > 0), glomerulitis (g > 0), thrombosis, and transplant glomerulopathy (cg > 0). The types of rejection were classified using the Banff classifications (2009 and 2013). Protocol biopsies were performed at 1, 3, and 12 months post-transplantation and, if possible, annually. A biopsy was performed whenever rejection was suspected. All biopsy specimens were evaluated using light microscopy, and the specimens obtained were evaluated for C4d using immunofluorescence staining. Two or three core biopsy samples were obtained using a spring-loaded 16-gauge biopsy gun under ultrasound guidance. A diagnosis of rejection was made in a blinded manner by the same pathologist.

Infection prophylaxis

All transplanted patients received *Pneumocystis jiroveci* pneumonia and bacterial prophylaxis with trimethoprim-sulfamethoxazole (80 mg/400 mg daily on every other day) from 2 weeks after transplantation for the rest of their lives in our center. However, routine cytomegalovirus prophylaxis (IV ganciclovir or oral valganciclovir) was not initiated, even in donor-positive, recipient-negative cytomegalovirus serostatus patients.

Statistical analysis

Statistical analyses were performed with SAS, version 9.4 TS1M5 (SAS Institute, Cary, NC, USA). One-way analysis of variance was used to compare normally distributed continuous variables, and the Kruskal–Wallis *H* test was used to evaluate skewed or discrete ordinal variables. The chi-square test was used to compare nominal scale variables. Time-to-event analyses were performed using the Kaplan–Meier method and Cox proportional hazard model. The proportional hazard assumption was confirmed by the following equation: ln [−ln (survival function)]. This sample size of 280 had more than 90%, 63%, and 37% power to detect difference of time-to-event curves on ABMR, graft survival, and patient survival with $\alpha = 0.05$. (two-tailed). A two-tailed *P*-value <0.05 was considered statistically significant by the biostatistics datacenter (STATZ Institute, Tokyo, Japan).

Results

Baseline demographic and transplant characteristics of the cohort are summarized in Table 1. The XM−DSA−, XM−DSA+, and XM + DSA+ groups consisted of 229 (82%), 36 (13%), and 15 (5%) patients, respectively. Unrelated donors were significantly different between the three groups. The ratio of patients with sensitizing events, previous transplant, transfusion, and pregnancy was significantly higher in the XM + DSA+ group than the XM−DSA+ and XM−DSA− groups. Both T-cell and B-cellFCXM in the XM + DSA+ group became negative at the time of transplantation. Table 2 shows characteristics in the XM + DSA+ group. Two patients were T-cell CDCXM positive, three patients were T-cell FCXM positive, and 11 patients were B-cell FCXM positive. In the XM + DSA+ group, DSA was detectable after desensitization in 10 patients, and MFIs ranged from 300 to 5897. DSA became undetectable in seven patients at the last follow-up time.

Change of estimated glomerular filtration rate (GFR) in each group is demonstrated in Fig. 1. Estimated GFR was 45.5 ± 12.3 ml/min/1.73 m^2, 42.1 ± 10.9 ml/min/1.73 m^2, and 37.7 ± 14.3 ml/min/1.73 m^2 in the XM−DSA−, XM−DSA+, and XM + DSA+ group, respectively, at 4 years post-transplant. The XM + DSA+ group had lower estimated GFR, but there was no significant difference in proteinuria incidence between the three groups at 4 years post-transplant ($P = 0.81$).

Figures 2(a and b) demonstrate the 4-year patient and graft survival rates for all patients. The 4-year patient survival rates were 98.3% (three deaths), 100% (zero deaths), and 93.3% (one death) in the XM−DSA−, XM−DSA+, and XM + DSA+ group, respectively, with no significant difference ($P = 0.18$).

© 2018 Steunstichting ESOT

Table 1. Baseline demographic and transplant characteristics.

	XM−DSA− group $n = 229$	XM−DSA+ group $n = 36$	XM + DSA+ group $n = 15$	*P*-value
Age (years)	47.3 ± 13.5	52.3 ± 12.3	48.9 ± 13.7	0.117
Gender (Male), *n* (%)	149 (65.1)	20 (55.6)	6 (40.0)	0.099
Duration of dialysis (month)	15 (4–46)	22 (3–54)	53 (15–77)	0.076
Preemptive KT, *n* (%)	40 (17.5)	7 (19.4)	1 (6.7)	0.519
BMI (kg/m^2)	21.9 ± 3.2	21.5 ± 2.9	20.8 ± 2.4	0.347
Sensitizing events, *n* (%)				
Previous transplant	13 (5.7)	2 (5.6)	7 (46.7)	<0.001
Previous transfusion	48 (21.0)	6 (16.7)	8 (53.3)	0.014
Previous pregnancy	37 (16.2)	10 (27.8)	8 (53.3)	<0.001
Cause of ESRD, *n* (%)				
Diabetic nephropathy	39 (17.0)	5 (13.9)	2 (13.3)	
Chronic glomerulonephritis	32 (14.0)	2 (5.6)	3 (20.0)	
IgA nephropathy	38 (16.6)	7 (19.4)	2 (13.3)	
PKD	18 (7.9)	3 (8.3)	3 (20.0)	
Hypoplastic kidney	6 (2.6)	–	–	
FSGS	6 (2.6)	2 (5.6)	–	
Nephrosclerosis	11 (4.8)	2 (5.6)	–	
Other/unknown	78 (34.1)	15 (41.7)	5 (33.4)	
ABO-incompatibility, *n* (%)	70 (30.6)	13 (36.1)	6 (40)	0.627
HLA-A/B/DR mismatches	3.0 ± 1.4	3.6 ± 1.6	3.4 ± 1.6	0.075
Total ischemic time (min)	71 (60–87)	72 (62–83)	66 (60–74)	0.75
Follow-up (years)	3.3 ± 0.7	3.5 ± 0.6	3.3 ± 1.0	0.306
Donor				
Gender (Men), *n* (%)	73 (31.9)	11 (30.6)	11 (73.3)	0.14
Age (years)	59.9 ± 9.5	56.9 ± 9.4	57.3 ± 8.1	0.157
Unrelated donor, *n* (%)	93 (40.6)	20 (55.6)	10 (66.7)	0.046
Graft weight (g)	175.3 ± 44.9	165.2 ± 37.6	184.9 ± 48.5	0.297

ESRD, end stage renal disease; FSGS, focal glomerular sclerosis; KT, kidney transplantation; PKD, polycystic kidney disease; XM, crossmatch.

Three patients in the XM−DSA− group died due to cardiovascular disease, while one patient in the XM + DSA+ group died of suicide. All causes of death did not relate to graft rejection. The 4-year graft survival (non-censored for death) rates were 96.9%, 97.2%, and 86.7% in the XM−DSA−, XM−DSA+, and XM + DSA+ group, respectively, with no significant difference ($P = 0.11$).

Figure 2(c) shows the ABMR free-survival rate within 90 days post-transplantation. Biopsy-proven acute ABMR rates within 90 days postoperation was 1.3% (3/229) in the XM−DSA− group, 19.4% (7/36) in the XM−DSA+ group, and 60% (9/15) in the XM + DSA+ group (Table 2). The ABMR incidence rate in the XM + DSA+ group was higher than the XM−DSA+ group (Hazard ratio; 3.81, 95% confidence interval; 1.41–10.30, $P = 0.008$). Similarly, without ABO-incompatible patients, the ABMR incidence rate in the XM + DSA+ group was significantly higher than that in the other two groups (XM−DSA− vs. XM−DSA+ vs. XM + DSA+: 1.3% vs. 17.4% vs. 55.6%, $P < 0.001$). Two patients in the XM + DSA+ group developed ABMR and required dialysis due to oliguria within the first week of transplantation. After IVIG therapy, graft function of both patients recovered. No patient required transient hemodialysis post-transplantation in both the XM−DSA+ and XM−DSA− groups. Four XM + DSA+ patients were diagnosed as having ABMR and received IVIG and/or PP without transient hemodialysis. All patients have stabilized serum creatinine at the last follow-up. Four, five, and two patients were diagnosed with ABMR by protocol biopsy in the XM−DSA−, XM−DSA+, and XM + DSA+ group, respectively. Interestingly, no patient developed *de novo* DSA during the study period.

Table 3 demonstrates the infectious complications within 1 year post-transplantation in all groups. There

© 2018 Steunstichting ESOT

Table 2. Detailed characteristics in XM + DSA+ group.

Patient	Cross-match	DSAs Type	Pre-desensitizaion MFI	Post-desensitization MFI	Last-FU MFI
1	T-FCXM+/B-FCXM+	B51/B52/DR51	11 186/10 808/2320	1692/2009/0	0/0/0
2	T-FCXM−/B-FCXM+	DQ6	2095	0	4173
3	T-CDCXM+/B-CDCXM+	DR4/DR53/DQ8	11 445/20 819/20 094	0/1252/1942	3238/14 847/0
4	T-FCXM+/B-FCXM+	A2	13 558	3602	2030
5	T-CDCXM+/B-CDCXM+	B35/DR12	10 062/4414	2851/288	6345/623
6	T-FCXM−/B-FCXM+	DR12	19 437	4327	0
7	T-FCXM−/B-FCXM+	unknown			
8	T-FCXM−/B-FCXM+	DR15	9016	300	0
9	T-FCXM−/B-FCXM+	B44/DR13/DQ5/DQ6	2371/4063/7169/3344	0/0/3880/5897	0/0/3829/1879
10	T-FCXM+/B-FCXM+	A24/B7/DR1	7745/8277/6821	0/0/1824	0/0/6099
11	T-FCXM−/B-FCXM+	DQ6	15 656	1622	4791
12	T-FCXM−/B-FCXM+	DR7	7972	0	0
13	T-FCXM−/B-FCXM+	DR15	1710	0	0
14	T-FCXM−/B-FCXM+	DR4	1246	0	0
15	T-FCXM−/B-FCXM+	DQ4	8685	991	0

Bx, biopsy; CDCXM, complement-dependent cytotoxicity cross-match; DSA, donor specific antibodies; FCXM, flow cross-match; FU, follow-up; MFI, mean fluorescence intensity; TG, transplant glomerulopathy; XM, cross-match.

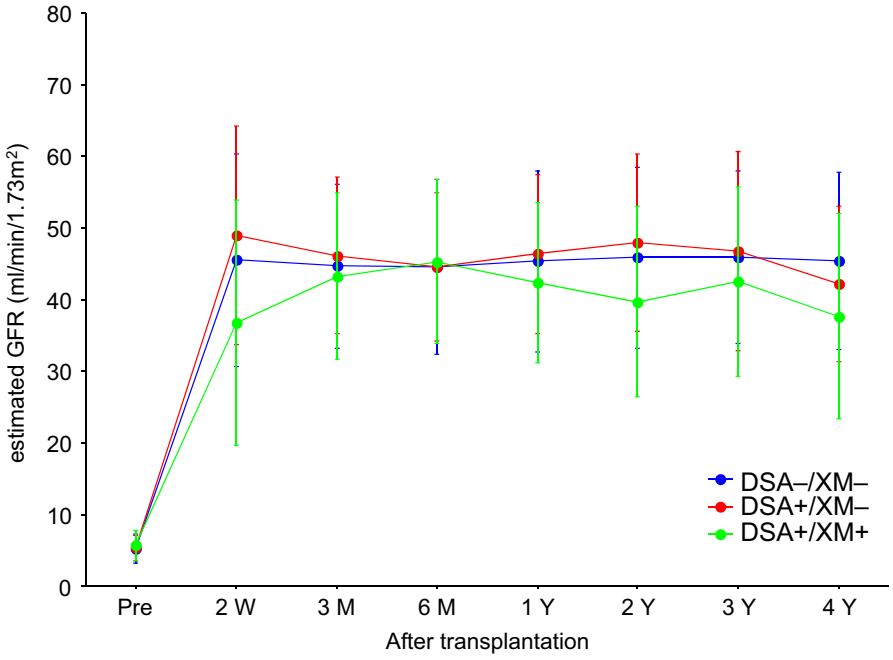


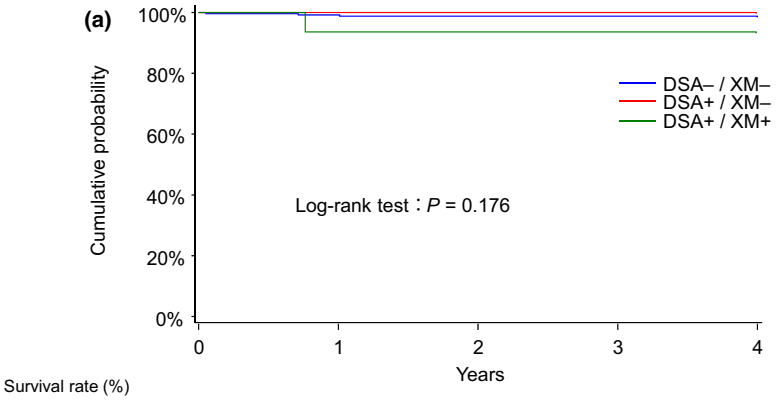
Figure 1 Changes in the estimated GFRs of each group during 4 years of follow-up.

was no significant difference in the incidence of each infectious complication (pneumonia, urinary tract infection, cytomegalovirus, BK virus, and adenovirus) between the three groups.

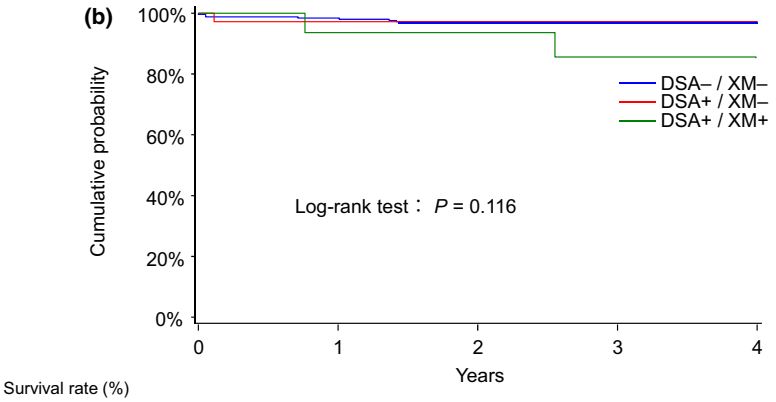
Table 4 demonstrates the relationship between DSA and the incidence of ABMR in the XM + DSA+ group. We investigated the highest and total MFI pre- and post-desensitization. There was a significant difference between ABMR+ and ABMR− in both the highest and total MFI during predesensitization (11 186 [7379] vs. 2095 [6975], $P = 0.01$; 16 947 [9285] vs. 2095 [6975], $P = 0.04$).

Histological outcomes in the latest biopsy among the three groups are shown in Table 5. Two hundred thirteen patients (93%), 35 patients (97%), and 14 patients (93%) were in the XM−DSA−, XM−DSA+, and XM + DSA+ groups. In the XM + DSA+ group, the

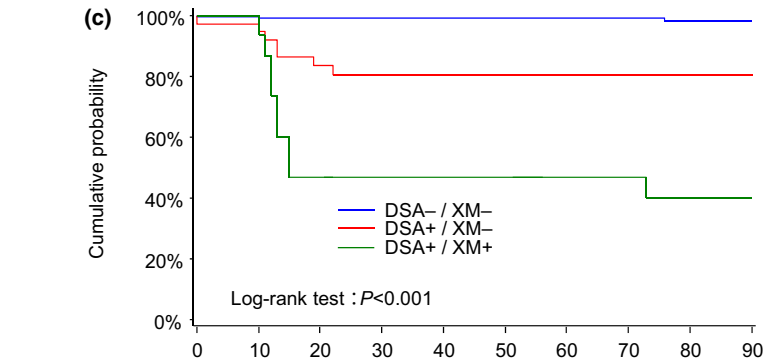
© 2018 Steunstichting ESOT



Survival rate (%)					
DSA– / XM–	100	99.1	98.7	98.7	98.7
DSA+ / XM–	100	100	100	100	100
DSA+ / XM+	100	93.3	93.3	93.3	93.3
No. at risk					
DSA– / XM–	229	227	225	152	84
DSA+ / XM–	36	36	36	27	17
DSA+ / XM+	15	14	14	9	8



Survival rate (%)					
DSA– / XM–	99.6	98.3	96.9	96.9	96.9
DSA+ / XM–	100	97.2	97.2	97.2	97.2
DSA+ / XM+	100	93.3	93.3	85.6	85.6
No. at risk					
DSA– / XM–	228	225	221	149	84
DSA+ / XM–	36	35	35	26	16
DSA+ / XM+	15	14	14	8	7



Survival rate (%)										
DSA– / XM–	99.6	99.1	99.1	99.1	99.1	99.1	99.1	99.1	98.7	98.7
DSA+ / XM–	97.2	99.4	83.3	80.6	80.6	80.6	80.6	80.6	80.6	80.6
DSA+ / XM+	100	93.3	46.7	46.7	46.7	46.7	46.7	46.7	40.0	40.0
No. at risk										
DSA– / XM–	228	227	225	225	225	225	225	225	224	224
DSA+ / XM–	35	34	30	29	29	29	29	29	29	29
DSA+ / XM+	15	14	7	7	7	7	7	7	6	6

Figure 2 (a) Kaplan–Meier estimates for patient survival. (b) Kaplan–Meier estimates for graft survival (noncensored for death). (c). Kaplan–Meier estimates for acute antibody mediated rejection free-survival within 90 days post-transplantation.

© 2018 Steunstichting ESOT

Table 3. Outcome in three groups.

	XM−DSA− group *n* = 229	XM−DSA+ group *n* = 36	XM + DSA+ group *n* = 15	*P*-value
Biopsy (90 days)				
ABMR, *n* (%)	3 (1.3%)	7 (19.4%)	9 (60.0%)	<0.001
Infection (1 year)				
Pneumonia, *n* (%)	4 (1.7%)	–	–	0.65
UTI, *n* (%)	3 (1.3%)	2 (5.6%)	–	0.16
Cytomegalovirus, *n* (%)	51 (22.3%)	4 (11.1%)	3 (20.0%)	0.32
BK virus, *n* (%)	3 (1.3%)	–	–	0.72
Adenovirus, *n* (%)	6 (2.6%)	–	–	0.52
4-year outcome				
Patient survival	98.3%	100%	93.3%	0.26
Graft survival (non censored for death)	96.9%	97.2%	86.7%	0.11
eGFR (ml/min/1.73 m^2)	45.5 ± 12.3	42.1 ± 10.9	37.7 ± 14.3	0.29

ABMR, acute antibody mediated rejection; DSA, donor-specific antibody; UTI, urinary tract infection; XM, cross-match;.

Table 4. Relation between DSA and ABMR incidence in XM + DSA+ group.

		ABMR (+)	ABMR (−)	*P*
Highest MFI pre DS, median [IQR]	8850 [5595]	11 186 [7379]	2095 [6975]	0.01
Highest MFI post DS, median [IQR]	1723 [2566]	1942 [1980]	0 [991]	0.1
Sum MFI pre DS, median [IQR]	14 017 [10 664]	16 947 [9285]	2095 [6975]	0.04
Sum MFI post DS, median [IQR]	1723 [3425]	3194 [2079]	0 [991]	0.12
DSA negative (MFI < 1000) at post DS (*n*)	6	2	4	–
Duration of ABMR diagnosis, days, median [IQR]	13 [6]			

ABMR, acute antibody mediated rejection; DS, desensitization; DSA, donor specific antibody; IQR, interquartile range; MFI, mean fluorescence intensity; XM, crossmatch.

Table 5. Results of latest graft biopsy among three group.

	XM−DSA− *n* = 213	XM−DSA+ *n* = 35	XM + DSA+ *n* = 14	*P*-value*
Protocol, *n* (%)	150 (70)	26 (74)	9 (64)	
For cause, *n* (%)	61 (29)	8 (15)	5 (36)	
Unknown, *n* (%)	2 (1)	1 (1)	0	
Time to biopsy, months, median [IQR]	12 [13]	13.5 [20.8]	13.5 [17.8]	
i	0.2 ± 0.49	0.12 ± 0.41	0.57 ± 0.85	0.1
t	0.13 ± 0.5	0.03 ± 0.17	0.21 ± 0.8	0.5
v	0.03 ± 0.2	0	0	
MVI score	0.31 ± 0.79	0.55 ± 1.09	2.07 ± 1.86	0.004
IFTA %	16.4	14.3	33.3	0.013
TG %	2.8	5.7	33.3	0.002

DSA, donor-specific antibody; IFTA, interstitial fibrosis and tubular atrophy; IQR, interquartile range; MVI, microvascular inflammation; TG, transplant glomerulopathy; XM, crossmatch.

*Comparison between XM−DSA− vs. XM + DSA+.

microvascular inflammation score and incidences of transplant glomerulopathy and interstitial fibrosis and tubular atrophy tended to be higher than those in the other two groups.

Transplant International 2018; 31: 1008–1017
© 2018 Steunstichting ESOT

Discussion

XM-positive LKT recipients have higher risk for ABMR and early graft loss. Our study clearly showed the intermediate-term outcomes of XM-positive LKT recipients desensitized by a risk-stratified protocol. Various desensitization protocols have been introduced by many studies [14–17]. Each protocol has produced varying successful outcomes of XM-positive kidney transplantation; however, an optimal desensitization protocol has not yet to be established. The major desensitization protocol is PP and low-dose IVIG without rituximab. Haririan *et al.* [17] reported intermediate- to long-term outcome of protocol consisted of PP and low-dose IVIG. Our protocol consisted of PP, high-dose IVIG, and rituximab, and is initiated 1 month prior to transplantation. The outcome of a desensitization protocol like this is rarely reported; therefore, it was important to determine the effectiveness of our protocol. Our findings demonstrated that the intermediate-outcomes of XM-positive LKT were comparable to XM-negative LKT. In terms of patient and graft survival rates and graft function, there were no significant differences between the higher and lower immunological risk groups. However, the occurrence of ABMR was significantly higher in the XM-positive group, in spite of our strict desensitization protocol.

Bentall *et al.* [16] demonstrated the 5-year outcome in XM-positive LKT by utilizing three desensitization protocols: (i) PP with low-dose IVIG plus splenectomy at the time of transplant; (ii) PP with IVIG (2 g/kg) without splenectomy; and (iii) high-dose IVIG alone. They showed that there was significantly worse 5-year death-censored graft survival rate and incidence of ABMR in XM-positive patients (70.7% vs. 88.0%, and 37.2% vs. 2.5%, in positive and negative XM patients, respectively). Haririan *et al.* [17] showed similar intermediate- to long-term outcomes in XM-positive LKT recipients who received PP and low-dose IVIG. Previous studies have demonstrated inferior outcomes in graft survival and incidence of ABMR. In our study, death-censored graft survival rates were 100% and 93.3% at 1-year and 4-year post-transplant, respectively. In spite of the small sample size of XM-positive patients, the intermediate outcome in graft survival was comparable with the nonimmunological group.

The XM + DSA+ group converted to negative XM and showed decreasing levels of DSA at transplantation following desensitization. Surprisingly, the incidence of ABMR remained considerably high in the XM + DSA+ group. Our study found that the incidence of ABMR within 3 months is 60%; importantly, all patients developed ABMR within 1 month after KT [13 days (10–16)], in spite of negative XM at transplantation. Treatment for ABMR includes steroid pulse, high-dose IVIG, and/or plasma exchange, rituximab, or bortezomib [18,19]. All patients who developed ABMR showed favorable responses to ABMR treatment. Graft survival was excellent and showed no difference with the XM−DSA− group. Our study also suggested that DSA MFI predesensitization have been associated with the incidence of ABMR, while DSA MFI post-desensitization have not been associated with ABMR. We postulate that the reason for high incidence rate of acute ABMR may be secondary to rebound DSA MFI. In our protocol, rituximab was administered at a dose of 300 mg at 1 month, and an additional 200 mg the day before transplantation. This method of administration of rituximab may not be sufficient to suppress rebound DSA MFI. To increase the transplant rates in sensitized patients, new protocols for HLA sensitization have emerged.

The effect of blocking terminal complement activation is expected for the purpose of preventing ABMR. Eculizumab is a novel desensitization agent for highly-sensitized patients and has terminal complement inhibition with the humanized anti-C5 antibody. Stegall *et al.* [20] reported the outcome of highly-sensitized patients with plasma exchange, IVIG, and eculizumab. The incidence of ABMR was only 7.7% in the eculizumab group compared to 41.2% in the control group. On the other hand, bortezomib, which inhibits 26S proteasome selectively, is widely used for plasma cell targeted therapy as a novel agent. Successful desensitization with bortezomib for high ABO titers or highly-sensitized transplantation has been reported in multiple centers [21–23]. Woodle *et al.* [24] reported consistent reduction of DSA levels in bortezomib-based desensitization without IVIG.

Previous studies suggested that highly-sensitized patients who received rituximab tended to be at an increased risk of viral and bacterial infections [25,26]. On the other hand, Kahwaji *et al.* [27] reported comparable incidence of infection in the desensitization group of ABO-incompatible transplant patients given rituximab and high-dose IVIG in comparison with the non-desensitization group. With regards to the risk of infection, we did not find significant differences in the incidence of infection between the three groups in the present study. IVIG is also considered to be the treatment of infection besides its effect of desensitization. We speculate that high- dose IVIG may have led to acceptable infection outcome post-transplantation among highly-sensitized patients.

The current study has several limitations. First, the small sample size of the high-risk group limits the

© 2018 Steunstichting ESOT

power of the study, in terms of determining the significance of outcomes between the three groups, as well as the DSA status in the high-risk group. Secondary, crossmatch assays and definitions vary between different centers; therefore, our results should be applied carefully.

In conclusion, our desensitization protocol, which consisted of high-dose IVIG, PP, and rituximab, is effective for highly-sensitized LKT recipients, and provides acceptable graft survival outcome without adverse events. Nevertheless, prevention of postoperative ABMR is still difficult in spite of strict desensitization protocol. Further refinement of our current desensitization regimen is required. In addition, the development of novel agents and more effective protocols is warranted to improve the outcomes of LKT among highly-sensitized recipients.

Authorship

MO: designed the study. DO, MO, and YK: collected and analyzed the data. DO, MO, and YK: wrote and edited the paper. DO, MO, YK, KU, JI, TT, HI, and KT: participated in performing the study and approved the final manuscript.

Funding

Astellas Pharma, Inc. (Tokyo, Japan) supported this study with a grant. The sponsor was not involved in the study design, patient enrollment, data collection, analysis, interpretation, or preparation of the manuscript.

Conflict of interest

The authors have declared no conflicts of interest.

Acknowledgements

We appreciate the support from Katsunori Shimada, PhD (STATZ Institute, Inc., Tokyo, Japan), who provided expert assistance with statistical analyses.

REFERENCES

1. Evans RW, Manninen DL, Garrison Jr LP, *et al.* The quality of life of patients with end-stage renal disease. *N Engl J Med* 1985; **312**: 553.
2. Abu Jawdeh BG, Cuffy MC, Alloway RR, Shields AR, Woodle ES. Desensitization in kidney transplantation: review and future perspectives. *Clin Transplant* 2014; **28**: 494.
3. De Sousa-Amorim E, Revuelta I, Blasco M, *et al.* Desensitization before living donor kidney transplantation in highly HLA-sensitized patients: a single-center study. *Transplant Proc* 2015; **47**: 2332.
4. Jin MK, Cho JH, Kwon O, *et al.* Successful kidney transplantation after desensitization using plasmapheresis, low-dose intravenous immunoglobulin, and rituximab in highly sensitized patients: a single-center experience. *Transplant Proc* 2012; **44**: 200.
5. Vo AA, Lukovsky M, Toyoda M, *et al.* Rituximab and intravenous immune globulin for desensitization during renal transplantation. *N Engl J Med* 2008; **359**: 242.
6. Machimoto T, Guerra G, Burke G, *et al.* Effect of IVIG administration on complement activation and HLA antibody levels. *Transpl Int* 2010; **23**: 1015.
7. Bentall A, Tyan DB, Sequeira F, *et al.* Antibody-mediated rejection despite inhibition of terminal complement. *Transpl Int* 2014; **27**: 1235.
8. Huh KH, Kim MS, Kim HJ, *et al.* Renal transplantation in sensitized recipients with positive luminex and negative CDC (complement-dependent cytotoxicity) crossmatches. *Transpl Int* 2012; **25**: 1131.
9. Kahwaji J, Jordan SC, Najjar R, *et al.* Six-year outcomes in broadly HLA-sensitized living donor transplant recipients desensitized with intravenous immunoglobulin and rituximab. *Transpl Int* 2016; **29**: 1276.
10. Bartel G, Schwaiger E, Böhmig GA. Prevention and treatment of alloantibody-mediated kidney transplant rejection. *Transpl Int* 2011; **24**: 1142.
11. Chung BH, Choi BS, Oh EJ, *et al.* Clinical impact of the baseline donor-specific anti-human leukocyte antigen antibody measured by Luminex single antigen assay in living donor kidney transplant recipients after desensitization therapy. *Transpl Int* 2014; **27**: 49.
12. Gloor JM, Winters JL, Cornell LD, *et al.* Baseline donor-specific antibody levels and outcomes in positive crossmatch kidney transplantation. *Am J Transplant* 2010; **10**: 582.
13. Morath C, Beimler J, Opelz G, *et al.* Living donor kidney transplantation in crossmatch-positive patients enabled by peritransplant immunoadsorption and anti-CD20 therapy. *Transpl Int* 2012; **25**: 506.
14. Glotz D, Antoine C, Julia P, *et al.* Desensitization and subsequent kidney transplantation of patients using intravenous immunoglobulins (IVIg). *Am J Transplant* 2002; **2**: 758.
15. Stegall MD, Gloor J, Winters JL, Moore SB, Degoey S. A comparison of plasmapheresis versus high-dose IVIG desensitization in renal allograft recipients with high levels of donor specific alloantibody. *Am J Transplant* 2006; **6**: 346.
16. Bentall A, Cornell LD, Gloor JM, *et al.* Five-year outcomes in living donor kidney transplants with a positive crossmatch. *Am J Transplant* 2013; **13**: 76.
17. Haririan A, Nogueira J, Kukuruga D, *et al.* Positive cross-match living donor kidney transplantation: longer-term outcomes. *Am J Transplant* 2009; **9**: 536.
18. Djamali A, Kaufman DB, Ellis TM, Zhong W, Matas A, Samaniego M. Diagnosis and management of antibody-mediated rejection: current status and novel approaches. *Am J Transplant* 2014; **14**: 255.
19. Ishida H, Furusawa M, Shimizu T, Nozaki T, Tanabe K. Influence of preoperative anti-HLA antibodies on short- and long-term graft survival in recipients with or without rituximab treatment. *Transpl Int* 2014; **27**: 371.

© 2018 Steunstichting ESOT

20. Stegall MD, Diwan T, Raghavaiah S, *et al.* Terminal complement inhibition decreases antibody-mediated rejection in sensitized renal transplant recipients. *Am J Transplant* 2011; **11**: 2405.
21. Inui M, Miyazato T, Furusawa M, *et al.* Successful kidney transplantation after stepwise desensitization using rituximab and bortezomib in a highly HLA-sensitized and ABO incompatible high titer patient. *Transplant Direct* 2016; **2**: e92.
22. Ide K, Tanaka Y, Sasaki Y, *et al.* A phased desensitization protocol with rituximab and bortezomib for highly sensitized kidney transplant candidates. *Transplant Direct* 2015; **1**: e17.
23. Koch M, Graser C, Lehnhardt A, *et al.* Four-year allograft survival in a highly sensitized combined liver-kidney transplant patient despite unsuccessful anti-HLA antibody reduction with rituximab, splenectomy, and bortezomib. *Transpl Int* 2013; **26**: e64.
24. Woodle ES, Shields AR, Ejaz NS, *et al.* Prospective iterative trial of proteasome inhibitor-based desensitization. *Am J Transplant* 2015; **15**: 101.
25. Kamar N, Milioto O, Puissant-Lubrano B, *et al.* Incidence and predictive factors for infectious disease after rituximab therapy in kidney-transplant patients. *Am J Transplant* 2010; **10**: 89.
26. Grim SA, Pham T, Thielke J, *et al.* Infectious complications associated with the use of rituximab for ABO-incompatible and positive cross-match renal transplant recipients. *Clin Transplant* 2007; **21**: 628.
27. Kahwaji J, Sinha A, Toyoda M, *et al.* Infectious complications in kidney-transplant recipients desensitized with rituximab and intravenous immunoglobulin. *Clin J Am Soc Nephrol* 2011; **6**: 2894.

© 2018 Steunstichting ESOT