RESEARCH ARTICLE

Living donor kidney transplantation after desensitization in cross-match positive high sensitized patients

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Abstract

Background: We aimed to evaluate the long-term results of the patients who had positive cross-match (XM) test results and underwent living donor renal transplantation after desensitization with different combinations of intravenous immunoglobulin (IVIG), plasmapheresis (PP), and rituximab.

Material and methods: Forty-nine patients who were positive for complement-dependent cytotoxicity (CDC), flow cytometric (FC), and Luminex-XM test were included in the study. Renal transplantation was performed in 16 patients who had XM (-) test after desensitization with different combinations of IVIG (n =15), PP (n =13), and rituximab (n =10). Anti-human leukocyte antigens (HLA) antibodies (anti-HLA Abs) were detected by the Luminex single antigen bead assay. Anti-thymocyte globulin was used for induction, and tacrolimus, mycophenolic acid, and prednisolone were used for maintenance therapy. Also, we evaluated the relationship between different donor-specific anti-HLA Abs and the parameters mentioned above.

Results: Antibody-mediated rejection (AMR) and acute T cell-mediated rejection rates were 18.8 % and 6.3 %, respectively. Graft survival rates at the first, third, and fifth years post-transplantation were 93.8 %, 85.2 %, and 85.2 %, respectively, and the patients' survival rates were found to be 100 %. Serum creatinine level and glomerular filtration rate were 1.5 ± 1.2 mg/dl and 69.9 ± 30.4 ml/min, respectively. The mean follow-up time was 39 ± 24 months.

Conclusions: Our study showed that kidney transplantation could be performed by effective desensitization in XM test positive patients. It was also shown that donor-specific anti-HLA DQ Ab and non-HLA Ab determination might be useful in diagnosing patients with positive cross-test and/or diagnosis of AMR. HIPPOKRATIA 2020, 24(4): 182-190.

Keywords: Desensitization, kidney transplantation, rituximab, intravenous immunoglobulin, plasmapheresis.

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Introduction

Recurrent transplantations, pregnancy, and transfusions are considered significant risk factors for sensitization in renal transplant candidates. Due to those factors, the risk of developing antibody-mediated rejection (AMR) and associated graft loss increases. The presence of preformed (persistent) and De-novo donor-specific antibody (Dn-DSA) with high mean fluorescence intensity (MFI) values in repeated transplants leads to the formation of a more sensitized patient in terms of rejection[1-5].

Many studies have shown that anti-human leukocyte antigens (HLA) antibodies (Anti-HLA Abs) are the leading cause of humoral rejection[5-7]. It has also been demonstrated that de-novo antibodies consist a high risk of chronic rejection than preformed antibodies[8-10]. Anti-HLA Abs bind to antigens (Ags) in endothelial cells, and the resulting Ag-Ab complex activates the complement system. This setting leads to endothelial cell damage through direct and indirect pathways allowing graft rejection and graft loss if not treated properly in time. Meanwhile, AMRs are known to be the leading cause of late graft loss[11]. Although antibodies develop against HLA A, B, DR antigens, there is a limited number of studies involving small numbers of patients concerning the antibodies against DQ, DP Ags, and non-HLA Ags (major histocompatibility complex class-1 chain-related gene A, angiotensin II type 1 receptor, endothelin -1 type A receptor, anti-endothelial cell antibodies, anti-vimentin, etc.)[12,13].

There are two alternatives for renal transplantation in patients with high MFI anti-HLA Abs positive: Kidney paired donation (KPD) and desensitization[6,7,14]. Compared to desensitization, KPD is primarily preferred in centers where donor pools are large or in countries with a shared pool among the centers[15-17]. Desensitization is an essential option for patients who have no chance of KPD.

Desensitization is generally performed in patients with complement-dependent cytotoxicity (CDC) - cross-match (XM), flow cytometric-XM, or Luminex-XM negative, but with positive PRA (panel reactive antibody) and donor-specific antibody (DSA) before or rarely immediately after transplantation. Still, it can also be performed in a very limited number of XM-positive patients[18-21]. It should also be kept in mind that non-HLA Abs may also be responsible for patients who are positive for these tests but have negative DSAs. Moreover, previous studies have provided evidence that non-HLA Abs are also the reason for AMR and lead to rejection by the same mechanism as anti-HLA Abs and are treated in the same way[5,22-24]. In summary, it should be considered that XM positivity may also be due to non-HLA Abs and that XM negativity can be achieved by desensitization, and the transplantation can be performed in this manner.

Although different desensitization protocols exist, plasmapheresis (PP), intravenous immunoglobulin (IVIG), and rituximab are generally used in various combinations and doses[20,25-27]. Studies have shown that combining these three treatment modalities (PP, IVIG, Rituximab) is more effective.

With these treatments, all possible pathways of rejection are suppressed through many mechanisms (such as immunomodulation, anti-inflammatory effect, suppression of complement and inflammatory cytokines, suppression and apoptosis of B lymphocytes, inhibition of T lymphocyte activation and proliferation, activation of regulatory T lymphocyte, suppression of Ig production and its removal from the blood circulation), and the rejection is managed[19,24,28].

This study aimed to evaluate the long-term results of high-sensitized, XM-positive patients who underwent living donor kidney transplantation after desensitization with different combinations of IVIG, Rituximab, and PP and to emphasize the importance of anti-HLA DQ Ab or possible non-HLA Ab. Besides, we aimed to review the general characteristics of patients who did not benefit from desensitization and could not undergo transplantation due to ongoing XM positivity.

Materials and Methods:

The current study included 49 patients admitted to the hospital for living donor kidney transplantation between 2011 and 2018 and with positive XM tests. The study was performed retrospectively and approved by the Akdeniz University Clinical Research Ethics Committee (70904504/480, decision No 719, date: 10/10/2018).

XM tests were performed by CDC-XM, flow cyto-

Table 1: Comparison of the two groups (patients who underwent transplantation and patients without transplantation) in terms of age, sex, immunological tests, and the number (and percentage rate) of drugs used in the desensitization protocol.

Parameter	RTx(+) (n =16)	RTx(-) (n =33)	p value
Recipient age	35 ± 12	43 ± 11	0.027
Recipient gender (M/F)	8/8	11/22	0.208
Rtx before desensization	11 (68.8 %)	9 (27.3 %)	0.006
CDC-XM(+) BD	14 (87.5 %)	29 (87.9 %)	0.396
CDC-XM(+) AD	0	28 (82.1 %)	<0.001
FC-XM BD(+) (B/T lymphocyte)	15 (93.7 %)/14 (87.5 %)	33 (100 %)/33 (100 %)	0.059/0.031
FC-XM AD(+) (B/T lymphocyte)	4 (25 %)/2 (12.5 %)	29 (85.5 %)/29 (85.7 %)	0.001/0.001
Rituximab(+)	10 (62.5 %)	22 (68.8 %)	0.452
IVIG(+)	15 (93.7 %)	32 (97 %)	0.572
PP(+)	13 (81.2 %)	25 (75.8 %)	0.073
Number of PP	3.5 (0-14)	5 (0-10)	0.787
PRA CI/CII BD	49 ± 43 53 ± 37	68 ± 36 68 ± 35	0.087 0.175
PRA CI/CII AD	0 (0-100) 52 ± 39	80 (0-100) 76 ± 21	0.009 0.157
L-XM CI BD	8/5/3	8/18/17	0.177
CII BD (- /None/ +)	6/5/5	5/18/10	0.162
L-XM CI AD	11/4/1	4/17/12	<0.001
CII AD (- /None/ +)	9/4/3	3/17/13	0.002

Continuous variables are presented as mean ± standard deviation or median and range, as appropriate, and categorical variables as percentages, Rtx: renal transplantation, CDC: complement dependent cytotoxicity, XM: cross-match, BD: before desensitization, AD: after desensitization, FC: Flow cytometry, IVIG: intravenous immunoglobulin, PP: Plasmapheresis, L-XM: Luminex cross match, CI: Class I, CII: Class 2, PRA: Panel Reactive Antibody.

metric (FC-XM), and Luminex-XM. Desensitization was performed according to a protocol consisting of different combinations of plasmapheresis, IVIG, and rituximab. For plasmapheresis, 30-40 ml/kg fresh frozen plasma was used, IVIG was administered in a total dose of 1.5-2 g/kg and 0.4 g/kg for five days. Likewise, rituximab was administered as a single dose of 375 mg/m². The XM tests were repeated after desensitization, and renal transplantation was performed in XM negative patients.

The patients were divided into two groups: patients who had undergone transplantation (Group 1) [n =16, male/female (M/F): 8/8] and that of the patients without transplantation (Group 2) (n =33, M/F: 11/22). Mean age, sex, presence of renal transplantation before desensitization, XM results before and after desensitization, medications used in the desensitization protocol, and the data related to plasmapheresis are given in Table 1 for Groups 1 and 2.

In our clinic, HLA-A, B, and DR Ags (a total of six Ag, two major subtypes of each antigen) are examined in recipients and donors. Mismatch evaluation is based on these six Ags. If there is no DSA against these antigens in patients with AMR, DQ Ag scanning is performed.

Anti-HLA Ab tests were performed before and after desensitization with Luminex single antigen (LSA) bead assay in pretransplant and in post-transplant patients. However, as the LSA assay was introduced in our center in 2016, it could not be used in patients who had had transplantation before this date (n =7, Table 2). Also, since some of our transplant patients continued to be followed up in other centers for social reasons, serial LSA follow-up could not be performed in the post-transplant period (n =2). However, it was observed that the first LSA of these patients after transplantation was negative. In our clinic, DSA MFI <1,000 is accepted as negative cut-off, 1,000-2,000: low risk, 2,000-8,000: moderate risk, and >8,000: high risk for hyperacute rejection. However, since one patient (patient 3) developed antibody-mediated rejection due to DSAs below this value, these MFI levels are also given in Table 2. Demographic characteristics, induction and maintenance immunosuppressive treatment protocols, renal replacement treatment modalities, mismatch counts, pregnancy, and transfusion history of the patients who underwent renal transplantation are given in Table 3.

In induction therapy, anti-thymocyte globulin (ATG) was commenced with a dose of 1.5-2 mg/kg. This induction therapy continued until the serum creatinine level was <1.5 mg/dl, and tacrolimus level was 10-12 ng/ml in all patients. In the maintenance of immunosuppressive therapy, the initial dose of tacrolimus was determined as 0.15-0.2 mg/kg in all patients, and dose adjustments were performed targeting the serum levels as 10-12 ng/ml up to the first month, 8-10 ng/ml between 1st-6th months, 5-8 ng/ml between 6-12 months, and 4-6 ng/ml after one year. Additionally, other immunosuppressive medications and doses were administered as below: mycophenolic acid

Table 2: Luminex single antigene bead assay (LSA) results in the 16 patients who underwent living donor kidney transplantation (preformed and De-novo donor spesific anti-HLA Ab).

Patient No	Preformed Anti-HLA Ab (LSA)		Anti-HLA Ab after transplantation (LSA)		
	1.	2.	1.	2.	3.
1.	DQB1*02:01 (MFI:1568)	DQB1*05:03 (MFI:11562)	DQB1*05:03 (MFI:14660)	DQB1*05:03 (MFI:12363)	DQB1*05:03 (MFI:13058)
2.	C01:02(MFI:1676)	C01:02 (MFI:1518)	C01:02(MFI:709)	(--) / (--)	
3.	A32-B18(MFI<1000) DRB1*4(MFI<1000) DRB1*16(MFI<1000)		DRB1*4(MFI<1000) DRB1*16(MFI<1000)		
4.	DQ05:01(MFI:5860)		DQ05:01(MFI:6701)	DQ05:01 (MFI:7037)	
5.	Unknown		(--) / (--)		
6.	(--) / (--)		(--) / (--)		
7.	DQ05:03(MFI:1658)		(--) / (--)	(--) / (--)	
8.	(--) / (--)		(--) / (--)	(--) / (--)	(--) / (--)
9.	(--) / (--)		(--) / (--)		
10.	Unknown		DRB1*11:03(MFI<1000) DRB1*14:01(MFI<1000)	(--) / (--)	(--) / (--)
11.	B44:03(MFI:367) DRB1*04:01 (MFI:6595)		B44:03(MFI<1000) DRB1*04:01(MFI<1000)		
12.	Unknown		(--) / (--)		
13.	Unknown		Early graft loss (AMR+)		
14.	Unknown		(--) / (--)		
15.	Unknown		(--) / (--)		
16.	Unknown		B15:12(MFI:4774)		

Ab: Antibody.

Table 3: Demographic, immunological, immunosupressive protocols and medical history of the 16 patients who underwent living donor kidney transplantation after desensitization.

Patient No	Age (R/D)	Gender (R/D)	Number of Rtx	Induction therapy	IS protocol	Etiology of ESRD	RRT Type	RRT Time	MM number	History of pregnancy	History of transfusion
1.	19/25	M/M	2.	ATG	TAC+MPA	DDS	HD	12	5/6	(-)	(+)
2.	26/53	F/M	1.	ATG	TAC+MPA	Unknown	HD	12	2/6	(-)	(+)
3.	56/37	F/F	1.	ATG	TAC+MPA	HT	HD	12	3/6	(-)	(+)
4.	38/37	F/M	2.	ATG	CSA+MPA	Unknown	HD	12	1/6	2	(+)
5.	48/42	M/F	2.	ATG	TAC+MPA	Unknown	HD	72	6/6	(-)	(+)
6.	46/42	M/M	2.	ATG	TAC+MPA	Unknown	HD	60	5/6	(-)	(+)
7.	41/33	M/F	2.	ATG	TAC+MPA	Unknown	HD	16	2/6	(-)	(+)
8.	28/55	M/F	2.	ATG	TAC+MPA	DM	PD	48	3/6	(-)	(+)
9.	24/48	M/M	2.	ATG	TAC+MPA	Alport	PD	12	4/6	(-)	(+)
10.	38/29	F/F	1.	ATG	TAC+MPA	Unknown	HD	36	0/6	3	(-)
11.	61/27	F/M	1.	ATG	TAC+MPA	Unknown	HD	70	3/6	3	(+)
12.	27/21	M/M	2.	ATG	TAC+MPA	HT	HD	72	0/6	(-)	(+)
13.	35/35	F/F	2.	ATG	TAC+MPA	KGN	HD	84	3/6	1	(+)
14.	30/55	F/M	1.	ATG	TAC+MPA	Hypoplasic	HD	12	1/6	(-)	(-)
15.	27/32	F/M	2.	ATG	TAC+MPA	VUR	PD	36	4/6	2	(+)
16.	25/32	M/M	2.	ATG	TAC+MPA	Unknown	HD	60	5/6	(-)	(+)

F: Female, M: Male, TAC: Tacrolimus, MPA: Mycophenolic acid, CSA: Cyclosporine, HD: Hemodialysis, PD: Peritoneal dialysis, ATG: Anti-thymocyte globulin, DDS: Deny Drash Syndrome, HT: Hypertension, DM: Diabetes Mellitus, CGN: Chronic glomerulonephritis, VUR: Vesicoureteral reflux, R: Recipients, D: Donor, RTx: Renal Transplantation, IS: Immunosupressive, ESRD: End Stage Renal Disease, RRT: Renal Replacement Therapy, MM: Mismatch.

(mycophenolate mofetil) 2 x 1 g/day, mycophenolate sodium 2 x 720 mg/day and prednisolone 1,000 mg on day one, 500 mg on day two, 250 mg on day three, 160 mg on day four, 80 mg on day five, 40 mg on day six, 20 mg on the 7th day, 20 mg continued until the 1st month, 15 mg/day between 2 and 3 months, 10 mg/day between 3 and 6 months, 7.5 mg/day between 6 and 12 months, and 5 mg/day after the first year.

While the protocol biopsies were performed in nine patients, this procedure could not be done in seven patients due to the graft loss in the early postoperative period (n =1) and the patients' refusals (n =6) for personal reasons. Although they were appropriately informed, there was a differentiation between the dates of our patients who underwent protocol biopsy because they agreed to have a biopsy at different times from the predetermined dates. Regarding the follow-up periods, there was a significant difference due to graft loss at two weeks (0 months) compared to patients with functional kidney at 93 months. Banff classification of Allograft Pathology was used for both protocol biopsy and the rejection diagnosis proven by biopsy[29-31].

PRA and XM (CDC, FC, Luminex) results of the patients who underwent renal transplantation before and after desensitization and after renal transplantation are shown in Table 4.

The median time between desensitization date and transplant date of renal transplantation patients was 1.5 months (min-max: 0-41 months). Also, the mean time between transplantation and protocol biopsy and the mean follow-up after transplantation was recorded at 26 ± 23 and 39 ± 24 months, respectively.

Statistical analysis

The study data were analyzed using the IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY, USA) and the Medcalc10.4.0 (MedCalc Software Ltd, Belgium) software programs. Continuous variables are presented as mean ± standard deviation or median and range, as appropriate, and categorical variables are given as percentages. Student t-test was used for normally distributed continuous variables between the two groups, while the Mann-Whitney test was used for those not normally distributed variables. Also, the Student t-test was utilized for the continuous variables and the chi-squared test for categorical variables. All hypotheses were bidirectional, and the critical alpha value was accepted as 0.05. Patient and graft survival after transplantation were calculated using Kaplan-Meier survival curves and compared using the log-rank test.

Results

The XM results of the groups before and after desensitization are given in Table 1. XM tests (CDC and/or FC) were detected positive in all patients of Group 1. In Group 1, the CDC-XM test, initially positive in 14 patients, became negative after desensitization. The FC-XM test was initially positive in 15/14 patients (B/T lymphocyte, respectively), and borderline positivity continued in 4/2 patients respectively, after desensitization. In Group 2, the CDC-XM test, initially positive in 29 patients, remained positive in 28 patients after desensitization. FC-

XM test was initially positive in all patients (B/T lymphocyte respectively 33/33), and positivity continued in 29 patients (29/29 patients B/T lymphocyte respectively) after desensitization (Table 1). Luminex-XM (LXM) test results for the desensitization period before (BD), after (AD), and after transplant (ATx) are given in Tables 1 and 4. In Group 1, it was observed that the test could not be performed in 5/4/3 patients (BD/AD/Atx, respectively). It was found that in two patients who were positive before desensitization, negative results were obtained after desensitization. In the post-transplant follow-ups, it was observed that positivity (in Class I) continued in only one patient (Table 4).

It was observed that the positivity continued in the period after desensitization in the second group. PRA titers were similar between the groups before desensitization. However, after desensitization, PRA Class I percentage was significantly higher in Group 2, while Class II levels were found similar.

The rate of renal transplantation before desensitization was higher in Group 1 than Group 2 [11 (68.8 %) and 9 (27.3 %), respectively; p =0.006). Five of the eight female patients who underwent transplantation after desensitization had a history of pregnancy. Within the scope of the desensitization protocol, no difference was found between groups in terms of IVIG, Rituximab, PP administration rates, and PP counts.

Rejection episodes were observed in four (25 %) out of the 16 patients who underwent renal transplantation after desensitization (Table 5). One patient (6.3 %) had acute T Cell-Mediated Rejection (TCMR) in the first postoperative month, and complete remission was achieved with pulse steroid treatment. Chronic active antibody-mediated rejection (C4d +, cAAMR) was observed in one patient in the 26th postoperative month. In this patient, no improvement could be accomplished in graft function despite pulse steroid + PP + ATG + IVIG treatment, and subsequently, the graft was lost. Also, acute antibody-mediated rejection (AAMR) developed in two patients (12.5 %). One patient had a rejection episode (C4d-, AAMR) on the second post-transplant day, showed a rapid deterioration in graft function, and had no response to the administered treatment. Finally, the graft was lost in the first week. The other patient developed C4d (+) AAMR in the third week after transplantation, and complete remission was achieved with pulse steroid + PP + ATG + IVIG. Detailed biopsy findings of the four patients who had rejection episodes are shown in Table 5. Protocol biopsies showed interstitial inflammation and mild tubulitis in some cases but no clinically significant rejection findings (Table 6).

The graft survival rates (Kaplan-Meier/Log-rank test) were determined as 93.8 %, 85.2 %, and 85.2 % at the first, third, and fifth years of patients' follow-up, respectively. The patients' survival rates were 100 % at the first, third, and fifth years post-transplantation. In terms of graft function, the mean serum creatinine level was 1.5 ± 1.29 mg/dl, mean eGFR level was 69.9 ± 30.4 ml/min, and the proteinuria level was 150 (70-2,500) mg/day in final control (39 ± 24.2 months). In regularly following tests BK virus DNA and cytomegalovirus (CMV) DNA levels in the blood samples were negative, and BK virus nephropathy, chronic allograft dysfunction, and delayed graft function did not develop in any of the patients.

In patient one, DSA against HLA DQB1*02:01 (MFI:1,568) and HLA DQB1*05:03 (MFI: 11,562) Ags were detected but the Ab against HLA DQB1*02:01 was found to be negative in the post-transplant period. In the subsequent measurements, the presence of the Abs against the DQB1*05:03 Ag continued to be detected.

Table 4: Panel Reactive Antibody (PRA) and cross-match (XM) results in different periods [before (BD), after (AD) desensitization and after transplant (ATx)] in the Renal Transplantation Group.

Patient No	PRA (Class I-II)			CDC-XM		FC-XM B/T lymphocytes		Luminex-XM Class1(MFI)/ Class2(MFI)			
	BD	AD	ATx	BD	AD	BD	AD	BD	AD	1.ATx	2.ATx
1.	0-60	0-87	0-77	(+)	(-)	+/+	-/-	-/-	-/-	-/1261	-/-
2.	0-10	0-0	0-0	(+)	(-)	+/+	-/-	-/-	-/-	-/1523	-/-
3.	1-38	0-30	52-80→0-43	(-)	(-)	++/++	+/+	1168/-	785/-	1500-1556	-/-
4.	32-97	0-100	0-93	(+)	(+/-)	+/-	+/-	905/2857	-/3373	-/1708	-/-
5.	0-80	1-40	0-0	(+)	(-)	+/+	-/-	-/723	-/962	-/796	-/-
6.	100-56	0-97	100-100→0-42	(+)	(-)	+/+	-/-	-/1547	-/967	-/5783	
7.	6-77	0-50	0-50→0-0	(+)	(-)	+/+	-/-	-/1061	-/-	-/-	-/-
8.	98-93	92-97	88-60→88/0	(+)	(-)	+/+	-/-	-/-	-/-	-/-	
9.	0-1	0-0	0-0	(+)	(-)	+/+	-/-	-/-	-/-	-/-	-/-
10.	76-53	0-50	0-43→0-50	(-)	(-)	+/+	-/-	-/-	-/-	-/-	-/-
11.	90-100	90-94	92-63	(+)	(-)	+/+	-/-	724/945	-/-	-/-	1650/-
12.	88-16	90-26	0-68→0-0	(+)	(-)	-/-	+/-	Unknown	Unknown	-/-	-/-
13.	29-66	0-57	0-87→0-100	(+)	(-)	+/+	-/-	Unknown	Unknown	Unknown	Unknown
14.	94-96	100-100	85-80	(+++)	(-)	++/++	-/-	Unknown	Unknown	Unknown	Unknown
15.	100-0	100-0	100-0→0-0	(+)	(-)	+/+	+/+	Unknown	-/-	-/-	Unknown
16.	68-0	50-0	73-0→52-0	(+)	(-)	+/+	+/+	Unknown	Unknown	Unknown	Unknown

PRA: panel reactive antibody, CDC: complement dependent cytotoxicity, XM: cross-match, BD: before desensitization, FC: Flow cytometry, MFI: fluorescence intensity, AD: after desensitization, BD; Before desensitization AD; After desensitization ATx: After transplantation.

Table 5: Drug doses used for desensitization in patients with living donor kidney transplantation after desensitization and renal biopsy findings of patients who had rejection.

Patient No	Desensitisation Protocol			Acute Rejection								
	IVIG	Rituximab	PP	Type	C4d	g	i	v	t	cg	ci	ct	cv
1.	120	600	3	None									
2.	120	600	5	None									
3.	0	0	3	AAMR	++	1	2	0	1	0	0	0	0
4.	80	500	4	None									
5.	145	680	7	TCMR	(-)	0	2	0	0	0	0	0	0
6.	140	0	0	cAAMR	++	1	2	0	2	0	2	2	0
7.	140	700	0	None									
8.	130	0	4	None									
9.	120	0	0	None									
10.	140	700	4	None									
11.	120	600	7	None									
12.	55	700	3	None									
13.	90	500	3	AAMR	(-)	2	2	2	2	0	0	0	0
14.	110	600	14	None									
15.	120	0	3	None									
16.	120	0	12	None									

IVIG: intravenous immunoglobulin, PP: Plasmapheresis, AAMR: Acute antibody mediated rejection, TCMR: T Cell Mediated Rejection, cAAMR: Chronic active antibody mediated rejection, g: glomerulitis, i: interstitiel inflamation, v: intimal arteritis, t: tubulitis, cg: chronic glomerulopathy, ci: chronic interstitial inflamation, ct: chronic tubulary atrophy, cv: vasculary intimal sclerosis.

Table 6: The properties of protocol byopsi according to Banff classification of Allograft Pathology in the Renal Transplantation Group.

Patient number	g	i	v	t	cg	ci	ct	cv	C4d	Graft loss
1.	None	None	None	None	None	None	None	None	None	(-)
2.	0	1	0	1	0	1	1	0	(-)	(-)
3.	0	2	0	1	0	2	2	1	(-)	(-)
4.	0	1	0	1	0	1	1	0	(-)	(-)
5.	1	1	0	1	0	1	1	1	(-)	(-)
6.	None	None	None	None	None	None	None	None	None	(+)
7.	None	None	None	None	None	None	None	None	None	(-)
8.	1	0-1	0	1	0	0-1	0-1	0	(-)	(-)
9.	None	None	None	None	None	None	None	None	None	(-)
10.	0	1	0	1	0	1	1	1	(-)	(-)
11.	0	0-1	0	1	0	0-1	0-1	0	(-)	(-)
12.	0	1	0	1	0	1	1	0	(-)	(-)
13.	None	None	None	None	None	None	None	None	None	(+)
14.	1	1	0	1	0	1	1	0	(-)	(-)
15.	None	None	None	None	None	None	None	None	None	(-)
16.	None	None	None	None	None	None	None	None	None	(-)

g: glomerulitis, i: interstitiel inflamation, v: intimal arteritis, t: tubulitis, cg: chronic glomerulopathy, ci: chronic interstitial inflamation, ct: chronic tubulary atrophy, cv: vasculary intimal sclerosis.

The values of MFI were measured at 14,660, 12,363 MFI, and in the final assessment at 13,058 MFI. No acute rejection episode was observed in this patient,, and protocol biopsy could not be performed because the patient did not accept.

In the fourth patient, DSA against the HLA DQB1*05:01 (MFI: 5,860) was detected. While firstly, this titer was at MFI: 6,701, it was found at MFI: 7,037 at the last follow-up. There was no significant finding except interstitial inflammation and tubulitis, indicating no acute rejection development in this patient. In the 7th patient, the preformed DSA emerged against the HLA DQB1*05:03 (MFI: 1,658) Ag became negative during the post-transplant period. No acute rejection episode was observed in this patient.

In patient two, the preformed DSA against the HLA Cw*01:02 (MFI: 1,676 and subsequent MFI: 1,518) Ag continued to be detected after transplantation, at MFI: 709 and then became negative. The third patient had preformed Abs (MFI <1,000) against the HLA A32, B18, DRB1*04, and DRB1*16. Those against A and B Ags became negative after transplantation, and those against DR Ags remained positive with MFI <1,000. C4d (+) AAMR developed in this patient, and complete remission was achieved with the treatment. In the 11th patient, DSA against HLA B44:03 (MFI: 367) and HLA

DRB1*04:01 (MFI: 6,595) were detected. However, while those against B Ag remained at the same levels, a decrease (MFI: <1,000) for the antibodies against DR Ag was found after transplantation. Ultimately, no rejection was developed in this patient.

Even though anti-HLA Ab screening was negative before and after the transplantation, C4d (+) cAAMR was observed in the 6th patient. This rejection was severe, unresponsive to treatment, and resulted in graft loss. Considering the XM positivity before desensitization in this patient, it was thought that this condition might be related to non-HLA Abs. Although XM tests were positive in the eighth and ninth patients, the Anti-HLA Ab tests (LSA) were negative before and after transplantation. These results indicated that XM positivity might also be related to non-HLA Ab in these patients.

In the 13th patient, the preformed Ab detection could not be performed, and graft loss developed due to C4d (-) AAMR in the early post-transplantation period. It was thought that this condition might be related to Anti-HLA Ab or non-HLA Abs. In the 10th, 12th, 14th-16th patients whose preformed Ab detection could not be performed, XM positivity was also associated with Anti-HLA Ab or non-HLA Ab.

We consider that the anti-HLA Abs, which were detected in the 10th and 16th patients after transplantation and developed as de-novo or preformed Ab positivity, might persist (Table 2). Since the detection of Anti-HLA Ab could not be performed in our clinic at that time, the levels of these antibodies could not be studied in seven patients during the desensitization period (preoperatively). Still, they were followed up in the post-transplant period.

In the thirty-three patients who could not undergo renal transplantation, anti-HLA Abs were mostly against the DR, B, and A antigens. Also, although the mean MFI values were higher in patients who could not undergo transplantation, the difference between the groups was not statistically significant [in 1st measurement the median (min-max) was 1,568 (367-5860) and 1,707 (512-65,161) for Groups 1 and 2 respectively; p =0.533, and in 2nd measurement: 9,078 (6,595-11,562) and 6,175 (934-10,977), respectively; p =0.242). This result is critical to show that the MFI value alone is not determinative and that the ability of Ab to bind complement is also important.

Discussion

In our study, among the 49 patients with positive XM tests, 16 patients were successfully renal transplanted after desensitization with IVIG, rituximab, and PP. The results proved valuable in terms of acute rejection rates, graft function, graft, and patient survival. At the same time, it was shown that XM positivity might be due to antibodies against DQ antigens being detectable by Luminex Assay and possible non-HLA Abs, and successful renal transplantation can be performed on these patients by desensitization. Desensitization is generally performed to prevent AMR development in patients with negative XM tests and anti-HLA Ab (+). Regarding XM (+) patients, the number of studies and the number of patients undergoing renal transplantation are quite limited, which is the most crucial distinguishing feature of our research.

The success of renal transplantation after desensitization strictly depends on the patients' anti-HLA Ab or non-HLA Ab positivity, which Ags these Abs are against, and MFI levels. It is known that HLA DR Ags are more immunogenic than others, and B and A Ags are less immunogenic. Additionally, it is also known that anti-HLA DQ Abs cause late rejection. The studies focusing on the relationship between the rejection and the Abs against HLA DQ Ag and non-HLA Ags are inadequate. In patients with positive XM test and negative anti-HLA Ab, the presence of non-HLA Ab must be taken into account, and they should be studied if possible.

In a study by Amrouche et al[25], it was demonstrated that 95 patients with CDC-XM (-), DSA >3,000 MFI underwent renal transplantation after desensitization. In the first year, the rates of acute AMR, cAAMR, the 5th year graft survival, and patients' survival were reported as 32.6 %, 39.5 %, 86 %, and 85 %, respectively. Although our study was performed in XM (+) patients, it was shown that acute and chronic AMR rates were lower, graft survival rates were similar, and patients' survival rates were better.

In another study[26], five patients with low levels of anti-HLA Ab (+) (MFI: 1,000-3,000) and XM (-) tests had desensitization (IVIG, rituximab, PP, ATG induction therapy) after cadaveric renal transplantation. It was shown that there was no loss of grafts or patients within a mean of 19 months, and the final control serum creatinine level was reported as 1.7 mg/dl. In our study, there were antibodies (MFI: 1,000-3,000) against anti-HLA Cw*01:02 Ab in the 2nd patient, while in the 11th patient, antibodies against HLA B44 (<1,000 MFI) and DRB1*04:01 (MFI: 6,595) were detected. The titers of the Abs decreased in the post-transplant period (MFI <1,000), and no rejection episode was observed. In contrast, acute AMR was observed in the 3rd patient who had <1,000 MFI Ab against HLA A32, B18, DRB1*04, and DRB1*16 Ags. Together with the anti-HLA Ab titer, the complement binding ability of the existent Ab is also known to be the critical determinant for AMR development.

Additionally, the study demonstrated that although the titer of DSAs could be found at MFI> 6,000, this may not activate the complement and not cause AMR. Although our study was of a small number of patients, it is in accordance with the literature and pointed out that these antibodies can bind to complement and cause rejection even if MFI is <1,000. We believe this issue should be investigated with more patients and perhaps with pathology studies performed after Ab infusion in experimental animals.

In another study involving 16 patients who underwent kidney transplantation after desensitization [IVIG,

PP, Rituximab (5 patients), MFI: 3,000-14,000], the acute AMR rate was found at 38 %. It has also been reported that one patient died following graft loss due to cAAMR, and patients were followed with functional grafts[18]. In our study, all patients were evaluated with CDC, FC, and Luminex XM tests, and their positivity was presented. Moreover, rituximab was used in 10 patients, and our AMR rate was shown to be lower than in the study mentioned above.

It is known that the IVIG, PP, and rituximab used for desensitization provide immunomodulation and anti-inflammatory effect. They also inactivate and inhibit the production of the Abs and remove them from the circulation. Additionally, De-novo Abs have a significant adverse effect on graft survival and are almost always positive in the development of cAAMR. Therefore, it is concluded that removing these Abs by desensitization will prevent the development of cAAMR significantly[19]. In our study, the development of cAAMR in only one patient (6.3 %) supports this result. However, the presence of anti-HLA Ab negativity on this patient suggested that XM positivity and the current AMR status might be related to non-HLA Abs. However, this hypothesis was not proved as these Abs could not be studied in our clinic.

Many studies demonstrated that despite different combinations utilized for desensitization, the most effective protocol is generally IVIG, PP, and Rituximab combination. A study involving seven patients treated with IVIG + plasebo and six patients treated with IVIG + rituximab showed that while severe AMR and graft loss were seen in the placebo group, such results have not been reported in the Rituximab group[2]. In another study, 61 FC-XM (+) patients were included. First group with PP + IVIG (low dose) + Rituximab (n =32), second group with high dose IVIG (n =13) and the third group treated with PP + IVIG (low dose) + Rituximab + ATG (n =16) . It was shown that the FC-XM negativity rate was lower, and the AMR rate was higher in the group treated with IVIG alone, and these results were better in the other groups[7].

In our study, two patients were desensitized with only high dose IVIG, and in one of these patients, graft loss developed due to cAAMR. The other patient underwent renal transplantation by providing XM negativity with only PP. Postoperatively after developing AAMR, complete remission was achieved with medical treatment, and this patient retained a functional graft in follow-up. Nine of our patients used IVIG + PP + Rituximab, and only one patient faced C4d (-) AAMR, which resulted in early graft loss. Therefore, the current study is precious in terms of demonstrating the results supporting the literature in the XM (+) patients. In the present study, among the 49 patients, in terms of CDC or FC-XM negativity rates, there was no difference between the groups using and not using the combination of IVIG + PP + Rituximab; and the groups using and not using Rituximab alone. This result was thought to be related to the MFI values, complement binding ability, patient and treatment protocols, and presence of anti-HLA/non-HLA Ab.

There are studies regarding the relationship between anti-HLA Abs against HLA DQ Ags and the immunobiology of rejection. These studies have shown that when together with other antibodies, the anti-HLA DQ Abs are more immunogenic, increase proinflammatory response, reduce regulatory T lymphocyte expansion, lead to late AMR development, and are associated with decreased graft survival[32-34]. In our study, in a patient who had Ab (MFI 12,000-16,000) against DQB1*02:01 Ag, no rejection episode or graft loss occurred even in the 20th month after transplantation. No rejection episode was seen in the 34th month after transplantation in a patient who had Ab (MFI: 5,000-8,000) against DQB1*05:01 Ag. Although this result is with a limited number of patients, it supports the literature data that anti-HLA DQ DSA in combination with other HLA Abs predicts AMR in the late period and reduces graft survival.

It is known that non-HLA Abs cause rejection by the mechanisms similar to anti-HLA Abs and are treated as anti-HLA Ab-associated AMRs[35,36]. In our study, even though anti-HLA Abs negativity before and after transplantation, CDC-XM, and FC-XM tests were found to be positive in three patients. It was considered that this positivity could be related to non-HLA Ab. One of these patients had C4d (+) cAAMR and related graft loss. This result showed that non-HLA Ab screening would be quite significant in similar cases, and transplantation can be performed by obtaining XM negativity with desensitization.

In summary, in our study, XM (+)(anti-HLA Ab or may be non-HLA Ab related) living donor kidney transplant candidates underwent desensitization with IVIG + rituximab + PP. It was demonstrated that the patients who tested negative for XM had successful kidney transplantations with similar rejection and graft survival rates compared to transplantations performed on patients with (-) XM in the initial testing. Furthermore, it has been shown indirectly that, especially in the absence of anti-HLA Ab, anti-HLA DQ Abs do not cause early rejection, even if they have high titers, and non-HLA Abs may be the cause of XM positivity.

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