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ORIGINAL ARTICLE

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

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Keywords
Cross-match, desensitization, donor-specific anti-human leukocyte antigen antibody, kidney transplantation, Luminex single antigen assay, rituximab.

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Summary

The aim of this study is to investigate the clinical impact of donor-specific anti-HLA-antibody (HLA-DSA) baseline levels, measured using the Luminex single antigen assay (LSA), in living donor kidney transplantation (LDKT). Total 129 cases of LDKT were divided into four groups according to baseline mean fluorescence intensity (MFI) HLA-DSA values: Strong ($n = 6$), >10 000; Moderate ($n = 8$), 5 000–10 000; Weak ($n = 11$), 1 000–5 000, Negative ($n = 104$), <1 000. Pretransplant desensitization (DSZ) was performed to decrease the MFI to weak or negative values before KT. Clinical outcomes in the four groups were compared. After DSZ, HLA-DSA decreased to weak or negative levels in all patients; Acute rejections developed more frequently in strong group [5/6 (83.3%)] compared with other three groups ($P < 0.05$), and especially acute antibody-mediated rejection (AAMR) developed almost exclusively in strong group [4/6 (66.7%)]. Strong HLA-DSA levels at baseline were more predictive of AAMR than either type of XM (complement-dependent lymphocytotoxicity or flow cytometry) in ROC analysis. Allograft function in this group showed significant deterioration during follow-up compared with the other groups. In conclusion, strong HLA-DSA levels at baseline are associated with worse allograft outcome even after successful desensitization; therefore, strict monitoring and strong maintenance immunosuppression may be required in such patients.

Introduction

Pre-existing sensitization to the human leukocyte antigen (HLA) is an important barrier in cases of kidney transplantation (KT), as it predisposes the patient to hyperacute or acute antibody-mediated rejection (AAMR) [1,2]. Hence, accurate determination of a patient's sensitization status is an essential pretransplantation step. Panel-reactive antibody (PRA) screening and conventional cross-match (XM) testing using complement-dependent cytotoxicity (CDC)-XM or flow cytometry-XM (FCXM) have been the most frequently employed techniques to detect patients' sensitization status [3]. The desensitization (DSZ) protocols at most transplant centers are based on XM results; conversion to a negative XM is regarded as a successful DSZ [4–9].

Recently, detection of donor-specific anti-HLA antibodies (HLA-DSA) using the Luminex single antigen (LSA)

assay technique has been introduced, and the method is reportedly superior in sensitivity to conventional XM testing [10–13]. Indeed, LSA is able to detect the presence of HLA-DSA in many patients with negative XM and in patients with conversion to a negative XM after DSZ [14–16]. However, the relevance of HLA-DSA levels, as defined by LSA, is still undetermined for two reasons. First, the high sensitivity of this method might detect clinically irrelevant DSAs [17,18]. Second, most previous studies consisted of retrospective analyses of banked samples. The clinical impact of this assay should be determined prospectively [13,14,16,17,19–23].

For the present study, we prospectively monitored HLA-DSA levels during the DSZ process. We applied a stratified DSZ protocol according to the baseline HLA-DSA values and continued DSZ until HLA-DSA values decreased to the target level. We investigated the prognostic value for clinical outcomes of HLA-DSA strength, compared with the results of conventional XM testing.

Subjects and methods

Patients and HLA-DSA typing

Between January 2010 and October 2011, 154 living donor kidney transplantations were performed at Seoul St. Mary's Hospital. Twenty-five patients with ABO-incompatible transplantation were excluded, and 129 patients were eventually included in the study. This study was approved by the institutional review board of Seoul St. Mary's Hospital (KC11RCMI0687).

Our center's pretransplant immunologic work-up is as follows (Fig. 1). As a baseline immunologic test, we performed PRA-Luminex screening and XM testing, using both CDC-anti-human globulin and FCXM in all patients. In patients with a PRA-Luminex screening result of ≥20%, or positive result of XM testing, we investigated the presence of HLA-DSA using LSA assay and performed desensitization therapy according to the strength of HLA-DSA presented as the value of mean fluorescence intensity (MFI) as described below.

Panel-reactive antibody-Luminex screening test was done by the Luminex method (Lifecodes LifeScreen Deluxe kits; Hologic Gen-Probe Inc., San Diego, CA, USA) and was presented as %PRA; CDC-XM and FCXM testing were performed in the standard manner [24,25]. Luminex single antigen assay for HLA-DSA was performed according to the manufacturer's instructions, using Lifecodes LifeScreen Deluxe kits (Tepnel Lifecodes Corp., Stamford, CT). Briefly, microbeads coated with purified HLA class I/class II glycoproteins were incubated with 12.5 μl of patient serum in 96-well plates for 30 min. After three washes with a vacuum manifold, the beads were incubated with 50 μl of a 1:10 dilution of R-phycoerythrin (PE)-conjugated goat anti-human IgG for 30 min. After washing, the test samples were analyzed using the Quick-Type User's Manual Research Use Only program, version 2.4, of the LABScan100 flow cytometer (Luminex Corp, Austin, TX); both positive and negative controls were included. The positive criterion was a MFI level of >1 000.

In all patients and donors, HLA typing was performed by the DNA molecular typing method, using reverse sequence-specific oligonucleotide probes and the RELI TM SSO HLA-A,B,C,DR,DQ Typing Kit (Dynal Biotech Ltd., Bromborough, UK). If the LSA assay-detected anti-HLA antibody in the patient corresponded to the HLA type of the donor, it was classified as a HLA-DSA. The results were presented as MFI levels, and patients were classified into four groups based on their peak level at baseline: strong, >10 000; moderate, 5 000–10 000; weak, 1 000–5 000; and negative, <1 000. If 2 or more HLA-DSAs were detected in a single patient's serum, the peak MFI level was defined as that of the HLA-DSA with the strongest reactivity.

DSZ protocol and immunosuppressive regimen

DSZ protocol

According to our center's DSZ protocol, the target HLA-DSA value at the time of KT was a weak or negative level (MFI < 5 000) by LSA assay. Stratified DSZ therapy was employed, according to the baseline MFI value (Fig. 1).

In patients with strong HLA-DSA values, rituximab (RTX) at a dose of 375 mg/m^2 (MabThera^{TM}; Genentech, Inc., San Francisco, CA, USA) was administered 2–3 weeks before transplantation, and plasmapheresis/immunoglobulin (PP/IVIG) therapy was initiated 13 days prior to transplantation and administered every other day. In addition, we initiated immunosuppressant (IS) treatment 7 days prior to transplantation in these patients (Fig. 2). HLA-DSA and XM testing were performed 2 days prior to the transplant. When HLA-DSA decreased to negative or weak levels and XM testing showed negative conversion, KT was performed. If HLA-DSA results were moderate to strong or if the XM was positive, we performed additional PP/IVIG 3 times and subsequently retested the patient for HLA-DSA and XM.

In patients who had moderate HLA-DSA levels at baseline, we also administered RTX and PP/IVIG therapy until the HLA-DSA value decreased to negative or weak levels. The difference in this group was that PP/IVIG was initiated 7 days prior to the day of transplant, and IS treatment was initiated 2 days prior to KT. In patients who had weak HLA-DSA levels, RTX (dose, 375 mg/m^2) was administered 7–10 days before transplantation, and IS treatment was initiated 2 days prior to transplantation. In patients with positive XM testing in the weak group, RTX

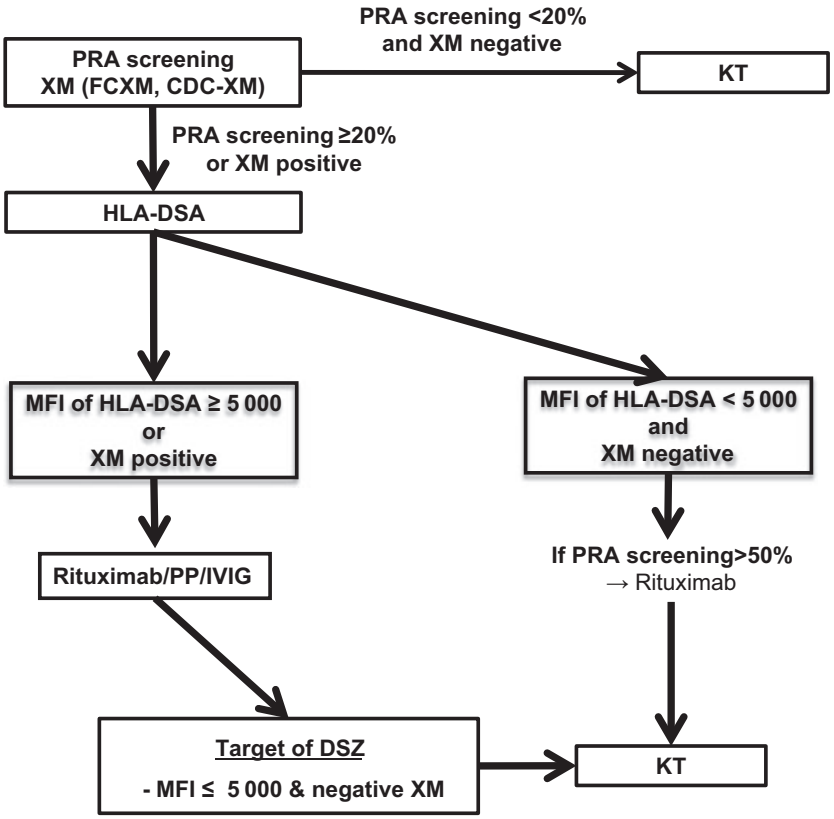

Figure 1 Immunologic work-up and desensitization strategy for highly sensitized patients to HLA antigen. As a baseline work-up, we performed PRA-Luminex screening and XM (FCXM and CDC-XM) for all patients. The desensitization strategy was as follows: (i) When the PRA-Luminex screening result was <20% and both XM tests were negative, we performed KT without desensitization therapy. (ii) When PRA-Luminex screening result was ≥20% or when either type of XM was positive, we verified the HLA-DSA by LSA assay. When the MFI of HLA-DSA was ≥5 000 or XM (CDC-XM or FCXM) result was positive, we used RTX and performed PP/IVIG until MFI value of HLA-DSA decreased to <5 000 and the XM showed a negative result. When the baseline MFI of HLA-DSA was <5 000 and XM was negative, we performed KT without pretransplant DSZ. (iii) In cases where the PRA-Luminex screening results were>50%, we used RTX at a dose of 375 mg/m^2. PRA, panel-reactive antibody; CDC-XM, complement-dependent lymphocytotoxicity cross-match; FCXM, flow cytometry cross-match; HLA, human leukocyte antigen; DSA, donor-specific antibody; MFI, median fluorescent intensity; LSA, Luminex single antigen; PP/IVIG, plasmapheresis/intravenous immunoglobulin; KT, kidney transplantation; DSZ, desensitization; KT, kidney transplantation; RTX, rituximab.

therapy was supplemented with three courses of PP/IVIG, and IS treatment was initiated 2 days prior to transplantation.

The 104 patients with negative HLA-DSA testing made up the control group. As a rule, no DSZ protocol was used in these patients, but in those with a PRA-Luminex screening result of >50%, we administered RTX prior to KT, as in the weak HLA-DSA group.

PP and IVIG infusion

Plasmapheresis was performed using a COBE Spectra apheresis system (Gambro BCT Inc., Lakewood, CO, USA) [26]. At each PP session, 1 plasma volume was removed and replaced with either a 5% albumin solution or fresh frozen plasma. Intravenous immunoglobulin (100–200 mg/kg) was infused 1 h after each PP session.

Immunosuppressive regimen

The typical IS treatment regimen at our center has previously been described [27]. Briefly, tacrolimus (Tac) or cyclosporin (CsA) was administered in combination with mycophenolate mofetil and prednisolone. Basiliximab was administered as induction therapy 2 h before transplantation and on day 4 after transplantation. In patients with HLA-DSA, only Tac was administered as main IS regardless of the MFI strength.

Protocol biopsy versus indicated biopsy

We performed protocol biopsies in patients with stable allograft function at three months after transplantation. Stable graft function was defined as <20% increase in serum creatinine concentration over baseline through the 2 visits before biopsy and a stable (not increased) dose of IS drugs.

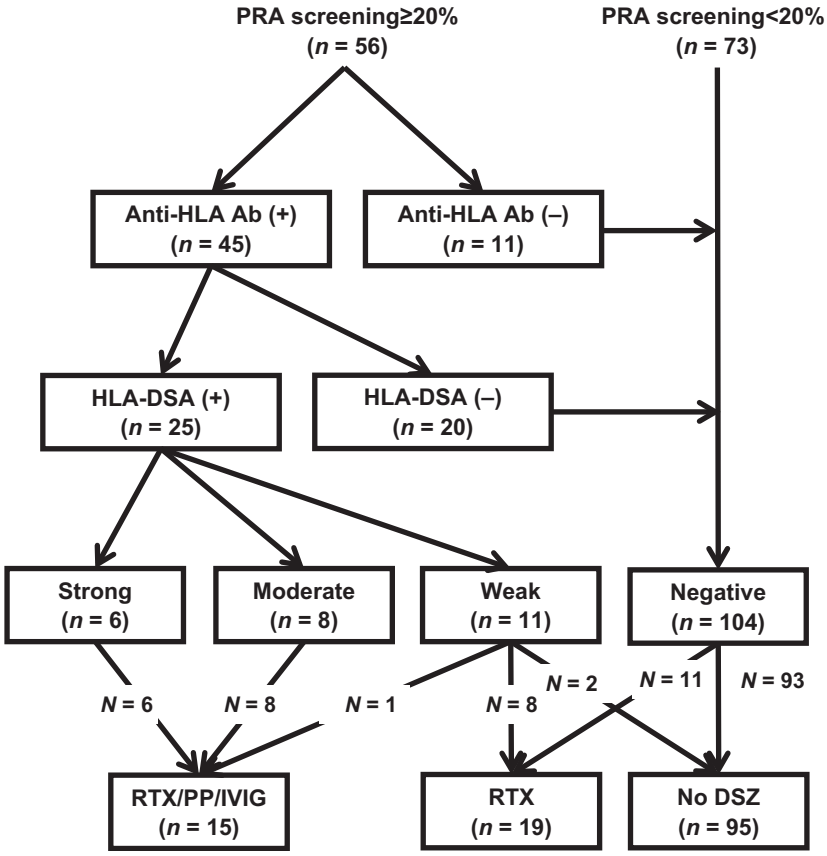

Figure 2 Distribution of the patient population according to immunologic risk and desensitization therapy. Of a total 129 patients screened, 56 patients had PRA-Luminex screening result ≥20%, 45 patients showed anti-HLA antibody by LSA assay, and 25 patients demonstrated HLA-DSA and remaining 104 patients were negative. According to the strength of HLA-DSA, the 25 patients with HLA-DSA were grouped into three groups, strong (MFI≥10 000, $n = 6$), moderate (5 000–10 000, $n = 8$), weak (1 000–5 000, $n = 11$). All patients belonging to the strong and moderate groups took the pretransplant RTX/PP/IVIG. In the weak group, one patient took RTX/PP/IVIG, while eight patients took RTX at a dose of 375 mg/m^2, and two patients did not take any DSZ therapy before kidney transplantation (KT). In the negative group, 11 patients took RTX at a dose of 375 mg/m^2 and 91 did not take any DSZ therapy before KT. PRA, panel-reactive antibody; HLA, human leukocyte antigen; DSA, donor-specific antibody; LSA, Luminex single antigen; MFI, median fluorescent intensity; RTX, rituximab; PP/IVIG, plasmapheresis/intravenous immunoglobulin; DSZ, desensitization.

Indication biopsy was performed when patients demonstrated an increase in the serum creatinine level higher than 20% of the baseline value. The biopsy procedure and the histologic diagnosis technique have been previously described [28]. Follow-up of HLA-DSA by LSA assay was carried out only in cases who needed indication biopsy.

Clinical outcome

Clinical outcomes were evaluated with the development of acute rejection (AAMR, T-cell-mediated rejection [TCMR]), allograft function, allograft survival, and patient survival between the four groups.

Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software, version 15.0 (SPSS Inc., Chicago, IL, USA). Data were calculated as the mean ± standard deviation or as numbers and percentages. For continuous variables, means were compared using Student's t-test. For categorized variables, Pearson's χ^2 test and Fisher's exact test were used. All tests were 2-tailed, and the results were considered significant at $P < 0.05$.

Results

Baseline characteristics

The mean patient age of overall patient group was 45.7 ± 11.6 years, and 77 patients (59.7%) were men. The most common indication for transplantation was primary glomerulonephritis (32.6%), followed by diabetes mellitus (22.5%) and hypertension (14.7%). Tac was administered in 85.2% (110/129) cases, and CsA was administered in 14.8% (19/129) cases. The baseline characteristics of each

group are presented in Table 1, and no significant difference was detected among four groups.

Distribution of immunologic risk according to HLA-DSA strength

The patients' immunologic characteristics are illustrated in Fig. 2. During the pre-KT work-up, a total of 56 patients showed PRA-Luminex screening value of ≥20%. Of these, 45 patients demonstrated anti-HLA antibodies by LSA assay, but only 25 had HLA-DSA. In those patients, the level of HLA-DSA was strong in six patients, moderate in eight patients, and weak in 11 patients. In the 45 patients with anti-HLA antibodies, anti-HLA-B and anti-HLA-DR were the most common. In the 25 patients with HLA-DSA, anti-HLA-DR (45.8%) was most common, followed by anti-HLA-B (41.7%) and anti-HLA-A (29.2%). The immunologic characteristics of each 25 sensitized patents are presented in Table 2.

Desensitization therapy according to the baseline HLA-DSA strength

A total of 15 patients received PP/IVIG and RTX for DSZ (six in the strong group, eight in the moderate group, one in the weak group), and 19 patients needed only RTX (eight in the weak group, 11 in the negative group). The remaining 93 patients in negative group did not receive any DSZ therapy. The number of PP/IVIG treatments required to reach weak or negative HLA-DSA status did not differ between the moderate (6.0 ± 1.0) and strong (6.6 ± 2.8) groups ($P > 0.05$). At baseline, the MFI level of HLA-DSA was significantly higher in the strong group (14472 ± 2267) than in the moderate (7200 ± 1087) and weak groups (3183 ± 1340). However, after DSZ at the time of KT, the HLA-DSA strength did not differ between the three sensitized patients groups (strong group 2535 ± 1093; moderate group, 1218 ± 1073; weak group, 2512 ± 1075; $P > 0.05$ in each comparison).

Association between HLA-DSA strength and cross-match results

A total of 13 patients had a positive result on XM testing (CDC-XM or FCXM) before transplantation; four patients demonstrated T-cell XM, five demonstrated B-cell XM, and four patients demonstrated both. The association between the XM result and the type of HLA-DSA is presented in Table 3. In 25 sensitized patients, HLA-DSA MFI value was significantly higher in FCXM-positive patients ($n = 13$, 9281 ± 1440) compared with FCXM-negative patients ($n = 12$, 4524 ± 897, $P < 0.01$ vs. FCXM positive), and it showed higher tendency in CDC-XM-positive patients ($n = 7$, 9785 ± 1503) compared with CDC-XM-negative patients ($n = 18$, 4524 ± 897, $P = 0.08$ vs. CDC-XM positive) (Fig. 3). Five of 6 patients (83.3%) in the strong group had a positive XM [CDC-XM positive in 50% (3/6) and FXCM positive in 83.3% (5/6)]. In the moderate group, 75% had a positive XM [CDC-XM positive in 37.5% (3/8) and FXCM positive in 75% (6/8)]. Only 1 of 11 (9%) weak HLA-DSA group patients (9%) had a positive result in both CDC-XM and FXCM.

Table 1. Baseline characteristics of patient population.

	Strong ($n = 6$)	Moderate ($n = 8$)	Weak ($n = 11$)	Negative ($n = 104$)
Age at KT (year)	41.4 ± 7.4	49.9 ± 9.6	46.6 ± 8.0	45.5 ± 12.2
Gender; male, *n* (%)	2 (33)	1 (13)	5 (46)	69 (66.3)
Follow-up month	21.8 ± 8.4	19.7 ± 4.6	21.3 ± 8.4	20.3 ± 6.6
Retransplantation, *n* (%)	3 (50)	2 (25)	3 (27)	11 (10.6)
LRD, n (%)	5 (83)	6 (75)	8 (73)	71 (68.3)
ABDR mismatch	3.6 ± 1.5	3.7 ± 1.4	3.5 ± 1.4	3.1 ± 1.7
Immunosuppression, *n* (%)				
Cyclosporine	0 (0)	1 (13)	0 (0)	18 (17.3)
Tacrolimus	6 (100)	7 (87)	11 (11)	86 (82.7)
Primary renal disease, *n* (%)				
Chronic GN	2 (33)	2 (25)	4 (36)	34 (32.7)
DM	2 (33)	0 (0)	2 (18)	25 (24.0)
HTN	0 (0)	2 (25)	3 (27)	14 (13.5)
ADPKD	1 (17)	0 (0)	0 (0)	4 (3.8)
SLE	0 (0)	0 (0)	0 (0)	3 (2.9)
Others	0	0	0	1 (1.0)
Unknown	1 (17)	4 (50)	2 (18)	21 (20.2)
Dialysis modality (HD/PD/Pre-emptive), *n* (%)	5/0/1 (83/0/17)	3/1/4 (38/13/50)	6/2/3 (55/27/18)	62/13/29 (60/13/28)
Dialysis vintage (month)	15.3 ± 7.0	14.7 ± 6.3	15.1 ± 22.3	19.8 ± 31.8

F/U, follow-up; LRD, Living related donor; GN, glomerulonephritis; DM, diabetes mellitus; HTN, hypertension; ADPKD, autosomal dominant polycystic kidney disease; SLE, systemic lupus erythematosus; HD, hemodialysis; PD, peritoneal dialysis.

Table 2. Immunologic characteristics of patients with HLA-DSA.

Case	Group	Highest HLA-DSA (MFI)	Cross-match	Desensitization	Rejection type	KT-acute rejection (month)	HLA-DSA at biopsy
1	Strong	B44 (15430)	T-FCXM	RTX/PP/IVIG	AAMR	2.8	DR08 (3425)
2	Strong	DR07 (17878)	Negative	RTX/PP/IVIG	AAMR	0.4	DR07 (11254)
3	Strong	B39 (12266)	T,B-CDC,FCXM	RTX/PP/IVIG	AAMR	1.4	Negative
4	Strong	DR15 (15480)	T,B-CDC,FCXM	RTX/PP/IVIG	AAMR	2.8	Negative
5	Strong	DR08 (12303)	Negative	RTX/PP/IVIG	TCMR	3.1	Negative
6	Strong	DQ02 (13956)	B-CDC/FCXM	RTX/PP/IVIG	–	–	–
7	Moderate	B62 (7343)	T-CDC/FCXM	RTX/PP/IVIG	TCMR	4.2	Negative
8	Moderate	B61 (7132)	T,B-FCXM	RTX/PP/IVIG	–	–	–
9	Moderate	A24 (6897)	Negative	RTX/PP/IVIG	–	–	–
10	Moderate	B60 (7146)	T,B-FCXM	RTX/PP/IVIG	–	–	–
11	Moderate	B54 (6746)	T-CDC/FCXM	RTX/PP/IVIG	–	–	–
12	Moderate	B58 (5148)	T-FCXM	RTX/PP/IVIG	–	–	–
13	Moderate	DR13 (7830)	Negative	RTX/PP/IVIG	–	–	–
14	Moderate	DR07 (8230)	B-CDC/FCXM	RTX/PP/IVIG	–	–	–
15	Weak	A11 (1003)	Negative	RTX	–	–	–
16	Weak	B7 (3950)	Negative	RTX	–	–	–
17	Weak	A26 (2765)	Negative	RTX	–	–	–
18	Weak	DQ05 (1560)	Negative	–	–	–	–
19	Weak	DR08 (2356)	Negative	RTX	–	–	–
20	Weak	B54 (2761)	Negative	RTX	–	–	–
21	Weak	A2 (4850)	Negative	RTX	–	–	–
22	Weak	B62 (3953)	Negative	–	TCMR	0.2	Negative
23	Weak	DQ02 (1593)	Negative	–	–	–	–
24	Weak	DR15 (2354)	Negative	–	TCMR	3.4	Negative
25	Weak	DR04 (4950)	B-CDC/FCXM	RTX/PP/IVIG	–	–	–

AAMR, acute antibody-mediated rejection; TCMR, T-cell-mediated rejection; KT, kidney transplantation; FCXM, flow cytometry cross-match; CDC-XM, complement-dependent lymphocytotoxicity cross-match; HLA, human leukocyte antigen; DSA, donor-specific antibody; MFI, median fluorescent intensity; RTX, rituximab; PP, plasmapheresis; IVIG, intravenous immunoglobulin.

Table 3. Association between the HLA-DSA and cross-match test.

	HLA-DSA class			
	I(+)II(+) (n = 4)	I(−)II(+) (n = 10)	I(+)II(−) (n = 11)	I(−)II(−) (n = 104)
T-CDC				
Positive (n = 4)	2 (50%)	0	2 (18.2%)	0 (0%)
Negative (n = 125)	2 (50%)	10 (100%)	9 (81.8%)	104 (100%)
T-FXCM				
Positive (n = 8)	3 (75%)	1 (10%)	4 (36.4%)	0 (0%)
Negative (n = 121)	1 (25%)	9 (90%)	7 (63.6%)	104 (100%)
B-CDC				
Positive (n = 5)	1 (25%)	2 (20%)	2 (18.2%)	0 (0%)
Negative (n = 124)	3 (75%)	8 (80%)	9 (81.8%)	104 (100%)
B-FXCM				
Positive (n = 9)	2 (50%)	4 (40%)	3 (27.3%)	0 (0%)
Negative (n = 120)	2 (50%)	6 (60%)	8 (72.7%)	104 (100%)

HLA, human leukocyte antigen; DSA, donor-specific antibody; KT, kidney transplantation; MFI, mean fluorescent intensity; CDC, complement-dependent cytotoxicity; XM, cross-match; FCXM; flow cytometry cross-match.

Prevalence of acute rejection according to pretransplant HLA-DSA strength

A total of 33 patients received indicated allograft biopsies due to serum creatinine elevations; of these, 25 patients were diagnosed with allograft rejection. Of these, five cases involved AAMR and 20 cases involved TCMR. When we analyze the acute rejection based on HLA-DSA strength, the total number of acute rejection and AAMR was significantly higher in HLA-DSA strong group than other groups,

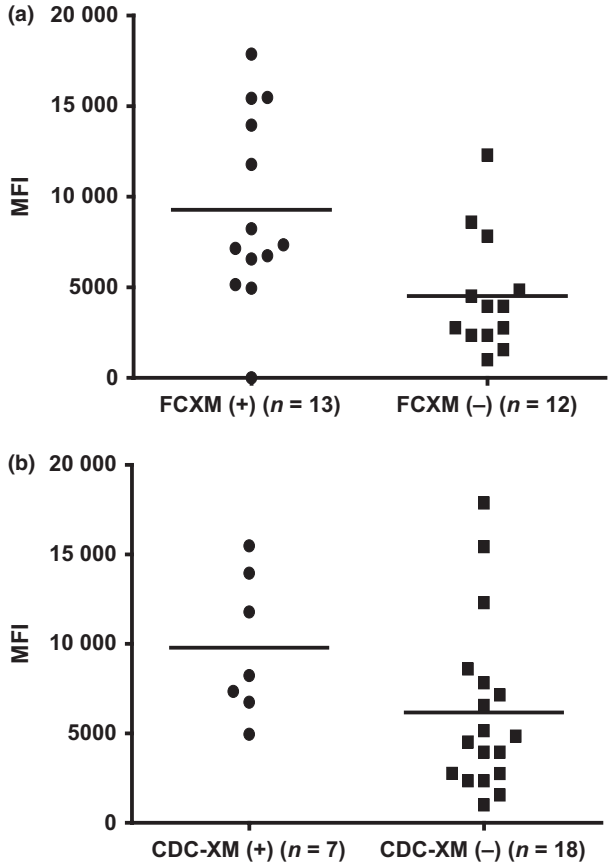

Figure 3 Correlation of HLA-DSA MFI value with CDC-XM or FCXM. HLA-DSA MFI value was significantly higher in FCXM (+) patients (n = 13, 9281 ± 1440) compared with FCXM (–) patients [n = 12, 4524 ± 897, $P < 0.01$ vs. FCXM (+)] and similarly it was higher in CDC-XM (+) patients (n = 7, 9785 ± 1503) compared with CDC-XM (–) patients [n = 18, 4524 ± 897, $P = 0.08$ vs. CDC-XM (+)]. CDC-XM, complement-dependent lymphocytotoxicity cross-match; FCXM; flow cytometry cross-match; MFI, median fluorescent intensity; HLA, human leukocyte antigen; DSA, donor-specific antibody.

suggesting the association between HLA-DSA strength and the development of AAMR (Fig. 4a, b). Indeed, most of AAMR developed in the HLA-DSA strong group (66.7%, 4/6), and there was no case of AAMR in the moderate or weak HLA-DSA groups. Only one case of AAMR developed in 104 negative groups (Fig. 4b). Subanalysis in patients with negative XM showed increase in AAMR in strong group [50% (1/2)] as well (Fig. 4c). As expected, TCMR did not show a significant association with either the presence or the strength of pretransplant HLA-DSA (Fig. 4d). Receiver operating characteristic analysis for the prediction of AAMR revealed that strong HLA-DSA status was more predictable than CDC-XM or FCXM results. The area under the curve (AUC) value for pretransplant strong HLA-DSA patients was 0.888, higher than that of pretransplant CDC-XM positivity (AUC, 0.680) or FCXM positivity (AUC, 0.764) (Fig. 5).

Allograft and patient outcomes according to HLA-DSA strength

Only one patient death, due to sudden cardiac arrest, occurred; this patient was in the HLA-DSA-negative group. No allograft loss developed in patients with any level of HLA-DSA. Allograft function did not differ between the negative, weak, or moderate HLA-DSA groups. However, the strong HLA-DSA group showed a significant deterioration in allograft function during the first post-transplant year (Fig. 6).

Development of de novo HLA-DSA at the time of indication biopsy

In the five patients with AAMR, 60% (3/5) demonstrated HLA-DSA at the time of biopsy: one strong and two weak. Of these, two patients were in the original strong HLA-DSA group and one was in the initial negative group. In the 20 patients with TCMR, only two patients who did not have pretransplant HLA-DSA (10%) showed weak de novo HLA-DSA levels. Of the eight patients whose indicated biopsies did not show evidence of allograft rejection, none demonstrated HLA-DSA at that time.

Subclinical rejection according to HLA-DSA strength on protocol biopsy

Protocol biopsy was performed in 47 patients. Three patients (6.4%) demonstrated subclinical rejection (SCR); these were all patients who had positive pretransplant HLA-DSA results. One case of SCR developed in the strong HLA-DSA group, and two developed in the moderate group. No SCR developed in the weak or negative HLA-DSA groups.

Discussion

We investigated the usefulness of HLA-DSA in the determination of DSZ and in the prediction of clinical outcomes after KT. We demonstrated that HLA-DSA values, as determined by LSA assay, enable the stratification and quantitative classification of sensitized patients at baseline and also make it feasible to accurately detect and quantify the DSZ status. In addition, the baseline HLA-DSA value shows a superior ability to predict AAMR than that by conventional XM testing.

At first, we made the cutoff for enough desensitization to allow transplantation based on HLA-DSA level using LSA assay rather than XM results because negative conversion

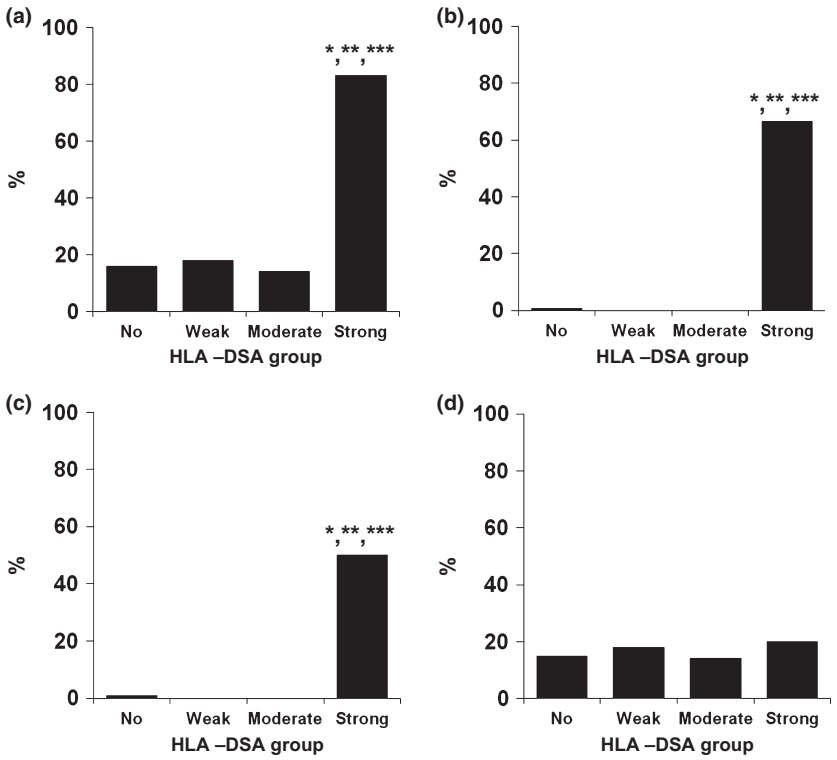

Figure 4 Development of acute rejection (a) Comparison of the incidence of total acute rejection among four groups. It was significantly higher in the strong group compared with other three groups. (b) Comparison of incidence of acute antibody-mediated rejection (AAMR) among the four groups. In strong group, most patients suffered AAMR, but it was rare in the other groups (c) Comparison of incidence of AAMR in patients with a negative cross-match and it developed in 50% of the strong group and developed rarely in the other three groups (d) Comparison of incidence of TCMR among four groups, which did not differ significantly among the four groups. CDC-XM, complement-dependent cytotoxicity cross-match; FCXM; flow cytometry cross-match; HLA, human leukocyte antigen; DSA, donor-specific antibody; AAMR. acute antibody-mediated rejection; TCMR, T-cell-mediated rejection. $*P < 0.05$ vs. negative, $**P < 0.05$ vs. weak, $***P < 0.05$ vs. moderate.

of XM often does not mean enough desensitization [14–16]. In this study, we evaluated the association between HLA-DSA strength and XM results and found that the CDC-XM-positive rate was only approximately 40% and that of FCXM was approximately 80%, even in patients with strong or moderate HLA-DSA. This finding suggests that HLA-DSA strength by LSA assay is more sensitive than XM. But, the cutoff for MFI values was not uniform in each previous report according to the HLA laboratory and the type of used LSA assay kits; hence, they should be determined in each transplant center/HLA laboratory independently [14,15]. Indeed, MFI value associated with the development of AAMR with acceptable sensitivity and specificity showed wide range from 3 000 to 10 000 in previous reports [15,19,22].

We determined our center's own cutoff according to previous reports and our center's result of the association between XM and the level of HLA-DSA. We divided patients into four groups according to the baseline HLA-DSA strength. The strong HLA-DSA group was chosen based on previous reports that showed MFI over 10 000 was commonly associated with poor allograft outcome and frequent AAMR [15,19,22]. The positivity of either type of XM is rarely detected in patients with HLA-DSA of MFI<5 000 as shown in Fig. 3. Hence, we defined it as weak group and determined the MFI 5 000 as the cutoff for enough desensitization.

Second, we performed stratified desensitization treatment according to the baseline HLA-DSA strength. In patients with strong group, we used stronger DSZ therapy, with early initiation of immune suppression and we continued DSZ, even when the XM test showed negative conversion, if the HLA-DSA did not decrease to weak or negative levels. In patients with weak HLA-DSA levels, we only administered RTX for DSZ. Finally, all sensitized patients achieved weak or negative HLA-DSA status; hence, the MFI value at the time of KT did not differ between the three previously sensitized patient groups.

Third, we investigated whether the baseline HLA-DSA level was associated with clinical outcomes after KT. The incidence of AAMR was highest in the strong HLA-DSA group, even though MFI decreased to weak or negative

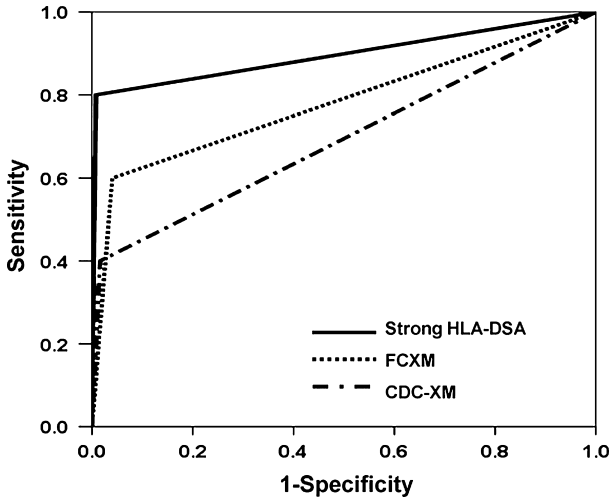

Figure 5 ROC analysis for the prediction of acute antibody-mediated rejection (AUC) was higher in the strong HLA-DSA group at baseline (0.888) compared with CDC-XM (0.680) or FCXM (0.764). AUC, area under curve; HLA, human leukocyte antigen; DSA, donor-specific antibody; CDC-XM, complement-dependent lymphocytotoxicity cross-match; FCXM; flow cytometry cross-match.

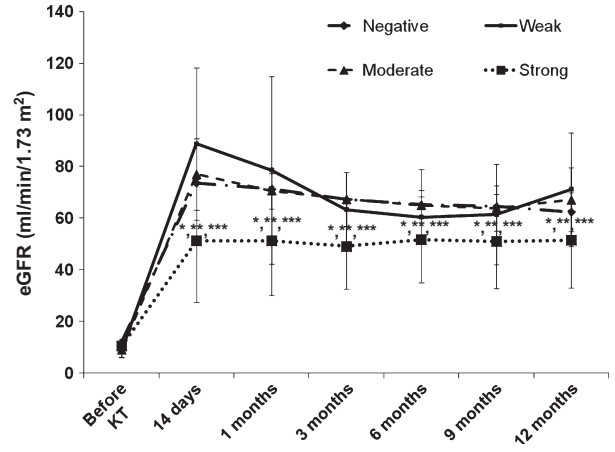

Figure 6 Comparison of alteration in allograft function among four groups. eGFR level detected by MDRD was significantly lower in the strong group compared with the three other groups during 1 year post-transplant. However, it did not differ significantly among moderate, weak, and negative groups. eGFR, estimated glomerular filtration rate; MDRD, modification of diet in renal disease; KT, kidney transplantation $*P < 0.05$ vs. negative. $**P < 0.05$ vs. weak. $***P < 0.05$ vs. moderate.

levels before KT. Interestingly, in patients with positive XM results (CDC+FXCM+ or CDC-FXCM+), AAMR only developed in patients with strong HLA-DSA levels, not in patients with weak to moderate levels. Strong HLA-DSA status is therefore a more significant predictor for the development of AAMR than CDC-XM or FCXM. Allograft function during the first post-transplant year was significantly inferior in strong-group patients as well. In addition, subclinical TCMR was found only in the moderate and strong groups and not in the weak or negative groups. All of these findings suggest that the baseline HLA-DSA is a significant predictor of poor allograft outcome, regardless of the HLA-DSA value at the time of KT.

Next, we investigated the development of de novo HLA-DSA after KT. De novo HLA-DSA was detected in 60% of patients with AAMR detected at the time of biopsy performed for increased serum creatinine. HLA-DSA at AAMR was same type with that found at baseline in two of four strong groups, suggesting that HLA-DSA associated with AAMR may represent a rebound of pre-existing antibodies. This means that patients in the strong group at baseline have a higher potential to generate de novo HLA-DSA, despite successful DSZ before KT. Considering that detection of de novo HLA-DSA serves as a biomarker for chronic renal allograft rejection, the strong group may have a higher risk for chronic antibody-mediated rejection. Determining this with certainty, however, will require long-term follow-up and further investigation [29].

In addition to its predictive value for clinical outcomes, LSA assay has other advantages over conventional methods such as cell-based XM and PRA. LSA results are not influenced by RTX treatment, which is usually included in DSZ protocols. Rituximab interferes with B-cell XM testing and provides false-positive results. Hence, cell-based XM cannot be used for the determination of DSZ after the administration of RTX in patients with B-cell XM-positive status; the decision to perform KT should be therefore based on LSA assay results. In addition, LSA assay is useful in detecting clinically significant anti-HLA antibodies. Of the 56 patients in the present study with a PRA-Luminex screening result of >20%, anti-HLA antibodies were negative by LSA assay in 11, and anti-HLA antibodies were not donor-specific in 20. Of these 31 patients, no cases of AAMR developed during follow-up. This finding is consistent with previous reports that nondonor-specific anti-HLA antibodies do not increase the risk for allograft rejection [12,14,17].

Limitation of this study is that the desensitization of the different sensitized groups is not homogeneous, and the number of cases in each group in particular the strong HLA-DSA group is small. Hence, larger patients group with longer follow-up duration may be required for clear conclusion about this issue. Second, one patient with positive B-cell XM with weak HLA-DSA levels received 3 PP/IVIG treatments. A previous retrospective study proved that patients with positive B-cell XM but negative HLA-DSA are not prone to unfavorable allograft outcomes [30]. It is possible that a positive B-cell XM result with a weak HLA-DSA level does not have a clinical impact, and such aggressive

PP/IVIG may not be necessary. Further investigation is required to clarify this issue.

In conclusion, our results suggest that the HLA-DSA level, as determined by LSA assay, presents a more stratified and quantitative value with which to determine the level of HLA sensitization. In addition, the HLA-DSA value at baseline is a more sensitive predictor for the development of AAMR than conventional XM testing. The risk for AAMR is high in patients with strong HLA-DSA levels, despite sufficient DSZ before KT; this is due to the high potential for de novo HLA-DSA formation. Hence, more strict monitoring and stronger maintenance immunosuppression may be required in patients with strong baseline HLA-DSA levels.

Authorship

BHC: participated in designing this study and writing paper. J-IK, ISM, and BSC: participated in analyzing data. CWP, Y-SK, and EJO: participated in performing study. CWY: participated in designing study.

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