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Impact of Low-Level Donor-Specific Anti-HLA Antibody on Posttransplant Clinical Outcomes in Kidney Transplant Recipients

Haeun Lee, M.D.[1], Hanbi Lee, M.D.[1], Sang Hun Eum, M.D.[2], Eun Jeong Ko, M.D., Ph.D.[1,3], Ji-Won Min, M.D., Ph.D.[4], Eun-Jee Oh, M.D., Ph.D.[5], Chul Woo Yang, M.D., Ph.D.[1,3], and Byung Ha Chung, M.D., Ph.D.[1,3]

[1]Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea; [2]Division of Nephrology, Department of Internal Medicine, Incheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Incheon, Korea; [3]Transplant Research Center, Convergent Research Consortium for Immunologic Disease, College of Medicine, The Catholic University of Korea, Seoul, Korea; [4]Division of Nephrology, Department of Internal Medicine, Bucheon St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Bucheon, Korea; [5]Department of Laboratory Medicine, Seoul St. Mary's Hospital, College of Medicine, The Catholic University of Korea, Seoul, Korea

Background: The clinical significance of low-level donor-specific anti-HLA antibody (low-DSA) remains controversial. We investigated the impact of low-DSA on posttransplant clinical outcomes in kidney transplant (KT) recipients.

Methods: We retrospectively reviewed 1,027 KT recipients, namely, 629 living donor KT (LDKT) recipients and 398 deceased donor KT (DDKT) recipients, in Seoul St. Mary's Hospital (Seoul, Korea) between 2010 and 2018. Low-DSA was defined as a positive anti-HLA-DSA result in the Luminex single antigen assay (LABScreen single antigen HLA class I - combi and class II - group 1 kits; One Lambda, Canoga Park, CA, USA) but a negative result in a crossmatch test. We compared the incidence of biopsy-proven allograft rejection (BPAR), changes in allograft function, allograft survival, patient survival, and post-transplant infections between subgroups according to pretransplant low-DSA.

Results: The incidence of overall BPAR and T cell-mediated rejection did not differ between the subgroups. However, antibody-mediated rejection (ABMR) developed more frequently in patients with low-DSA than in those without low-DSA in the total cohort and the LDKT and DDKT subgroups. In multivariate analysis, low-DSA was identified as a risk factor for ABMR development. Its impact was more pronounced in DDKT (odds ratio [OR]: 9.60, 95% confidence interval [CI]: 1.79–51.56) than in LDKT (OR: 3.76, 95% CI: 0.99–14.26) recipients. There were no significant differences in other outcomes according to pretransplant low-DSA.

Conclusions: Pretransplant low-DSA has a significant impact on the development of ABMR, and more so in DDKT recipients than in LDKT recipients, but not on long-term outcomes.

Key Words: Donor-specific anti-HLA antibody, Donor-specific antibody, Kidney transplantation, Rejection, Graft survival, Infection

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Corresponding author:
Byung Ha Chung, M.D., Ph.D.
Division of Nephrology, Department of Internal Medicine, Seoul St. Mary's Hospital and Transplant Research Center, Convergent Research Consortium for Immunologic Disease, College of Medicine, The Catholic University of Korea, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea
Tel: +82-2-2258-6066
Fax: +82-2-536-0323
E-mail: chungbh@catholic.ac.kr

INTRODUCTION

The presence of donor-specific anti-HLA antibody (HLA-DSA) is an important barrier to successful transplantation. A positive complement-dependent cytotoxicity crossmatch (CDC-XM) is considered a very strong risk factor for the development of anti-

body-mediated rejection (ABMR) and allograft failure [1, 2]. Flow cytometry crossmatch (FCXM) is used to predict allograft loss [3]. However, the crossmatch (XM) test has limitations in representing pretransplant immunologic risk because it does not provide a quantitative value for HLA-DSA. In addition, it is affected by non-HLA antibodies and HLA-DSA [4].

The Luminex single antigen (LSA) bead assay is used in the transplantation field. This assay enables measuring HLA-DSA at a single-antigen level and provides a semi-quantitative value [4–6]. The mean fluorescence intensity (MFI) value of HLA-DSA measured by the LSA assay correlates well with XM positivity [7]. This improvement allows for more detailed risk stratification for the prediction of ABMR or allograft failure than a traditional XM test [8–10]. Therefore, current desensitization strategies are based on the MFI values of HLA-DSA and XM test results [11–13]. However, previous studies on the clinical impact of HLA-DSA on posttransplant clinical outcomes have shown contradictory results in case the LSA assay is positive and the XM test negative [14–17]; therefore, its clinical impact remains unclear.

We investigated the impact of pretransplant low-DSA on posttransplant clinical outcomes, including ABMR and allograft survival in kidney transplant (KT) recipients. In addition, we analyzed the clinical outcomes of living donor KT (LDKT) and deceased donor KT (DDKT) recipients to evaluate the clinical significance of low-DSA according to donor type.

METHODS

Study population

Between January 2010 and December 2018, 1,284 KT procedures were performed in Seoul St. Mary's Hospital, Seoul, Korea. Patients with a positive XM test, ABO-incompatible transplantation, concurrent kidney and other solid organ or hematopoietic stem cell transplantation, and those who were pediatric (<18 years) were excluded. In total, 1,027 KT recipients (629 LDKT and 398 DDKT recipients) were included. Patients whose HLA-DSA results were positive in the LSA assay but negative in the XM test were defined as the low-DSA group. Patients without HLA-DSA in both the LSA assay and the XM test were defined as the no-DSA group. Negative XM test results were confirmed using both CDC-XM and FCXM. Among the 1,027 patients, 89 had low-DSA, including 68 LDKT recipients and 21 DDKT recipients. Finally, there were 68 low-DSA and 561 no-DSA patients in the LDKT group and 21 low-DSA and 377 no-DSA patients in the DDKT group (Fig. 1). The median follow-up period was 53.5 months (interquartile range: 30.1–82.1 months). This study was performed in accordance with the Declarations of Helsinki (2013) and Istanbul (2008) and was approved by the Institutional Review Board of Seoul St. Mary's Hospital (KC22RISI0380). The requirement for informed consent was waived because of the retrospective study design and the use of noninvasive procedures.

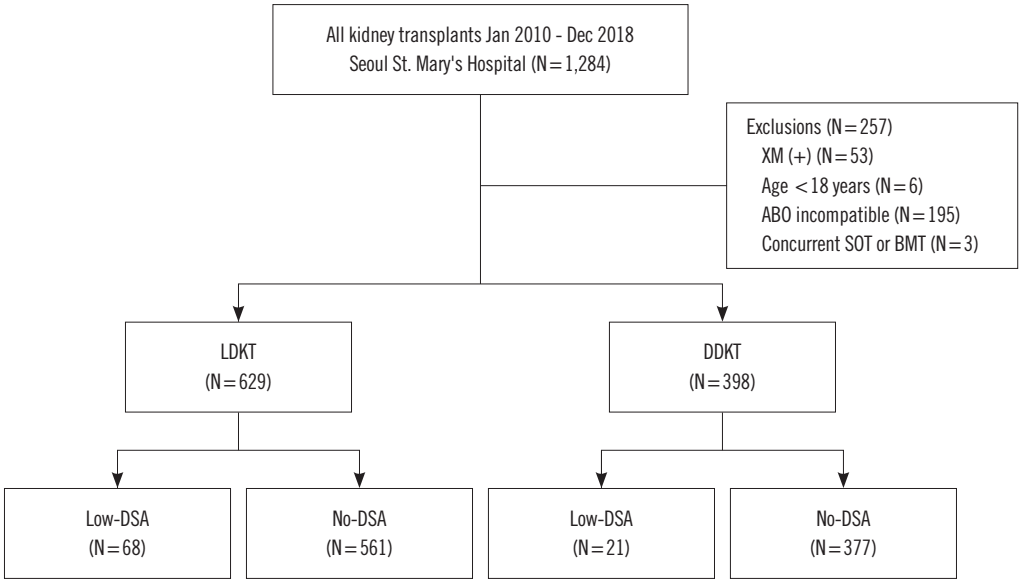

Fig. 1. Distribution of the patient population according to donor type and presence of pretransplant low-DSA.
Abbreviations: BMT, bone marrow transplantation; DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; low-DSA, low-level donor-specific anti-HLA antibody; no-DSA, negative donor-specific anti-HLA antibody; SOT, solid organ transplantation; XM, crossmatch.

Detection and definition of HLA-DSA

Our center's immunological workup protocol has been reported previously [18]. All LDKT candidates underwent panel-reactive antibody (PRA) screening and XM tests as baseline tests. XM results were reported using T-/B-CDC-XM and T-/B-FCXM tests. In patients with positive PRA screening or XM test results, we investigated the presence of HLA-DSA using the LSA assay. The criterion for PRA positivity was initially 20% but was changed to 0% during the study period. Eighty-three patients with low-DSA (93.3%) and 277 patients without low-DSA (29.5%) had PRA values >20%. Patients on the DDKT waitlist were screened for PRA as a pretransplant workup. If the result was positive, the patient was tested for HLA-DSA. When a kidney became available for transplantation, an XM test was performed. After June 2010, T-FCXM and B-FCXM were performed in addition to T-/B-CDC-XM. FCXM negativity was confirmed by both T-FCXM and B-FCXM in 380 of 398 DDKT recipients. PRA screening was conducted using the Luminex assay (LABScreen mixed class I and II; One Lambda, Canoga Park, CA, USA). The results are presented as %PRA. The CDC-XM and FCXM tests were conducted using standard procedures [19, 20]. HLA-DSA was identified by the LSA assay using LABScreen single antigen HLA class I-combi and class II-group 1 kits (One Lambda). The criterion for positivity was an MFI value >1,000. In all recipients and donors, HLA typing was performed using LIFECODES HLA-A, B, C, DRB1, and DQB1 Sequence-Specific Oligonucleotide Typing Kits (Immucor Transplant Diagnostics, Stamford, CT, USA). If the LSA assay-detected anti-HLA antibody in the recipient corresponded to the HLA type of the donor, it was classified as HLA-DSA. HLA-DSA was classified into three groups based on the peak MFI value: strong, >10,000; moderate, 5,000–10,000; and weak, 1,000–5,000.

Pretransplant desensitization protocol

Our center's desensitization protocol has been reported previously [13, 18, 21]. In brief, we used a stratified protocol based on baseline MFI values. The target of desensitization was negative conversion in the XM test for XM-positive patients and reduction of the HLA-DSA MFI value to <5,000. In patients with strong HLA-DSA, rituximab (RTX) was administered at a dose of 375 mg/m^2 (MabThera; Genentech, San Francisco, CA, USA) 2–3 weeks before transplantation. Plasmapheresis/immunoglobulin (PP/IVIG) therapy was initiated 13 days before transplantation and administered every other day. Immunosuppressant treatment was initiated seven days before transplantation in these patients. In patients with moderate HLA-DSA, RTX was administered, as mentioned above. PP/IVIG therapy was administered every other day for seven days before transplantation. Immunosuppressants were initiated two days before transplantation. In patients with weak HLA-DSA, only RTX at a dose of 375 mg/m^2 was administered 7–10 days before transplantation when the PRA values were >50%.

Definition of clinical outcomes

The primary outcome was the incidence of biopsy-proven allograft rejection (BPAR). Patients with clinically diagnosed rejections without biopsies were excluded. Secondary outcomes were changes in allograft function measured as estimated glomerular filtration rate (eGFR), death-censored allograft survival rate, patient survival rate, and posttransplant infections. Allograft rejection was diagnosed using the latest version of the Banff classification at the time of biopsy, which is updated every 2 years. BPAR was classified as T cell-mediated rejection (TCMR) and ABMR according to histological scores [22]. Mixed rejection was not classified separately. Both ABMR and TCMR were added to the rejection events. Borderline change was not considered to be BPAR. Serum creatinine levels were measured at 3, 6, 12, 24, and 36 months after transplantation. The eGFR for each concordant time was assessed using the Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) equation [23]. Graft failure was defined as a return to dialysis dependence or retransplantation. In the analysis of death-censored graft failure, patients who died with a functioning transplant were censored at the time of death. An infection episode was defined as the occurrence of an infectious event requiring hospitalization. Cytomegalovirus (CMV) infection was defined as CMV DNAemia and/or any organ involvement by CMV [24]. BK virus (BKV) infection was defined as BKV DNAemia with DNA titer $\geq 10^4$ copies/mL and/or biopsy-proven BK polyomavirus-associated nephropathy (BKPyAN) [25]. Infection-free transplant survival was defined as the time from transplantation to the first infection episode.

Statistical analysis

Continuous variables are presented as means±SDs or medians with interquartile ranges. Categorical variables are presented as frequencies and percentages. Continuous variables were analyzed using the Student's *t*-test or Mann–Whitney *U* test. The chi-square test or Fisher's exact test was used to compare categorical variables. Point biserial correlation was used to confirm the correlation between XM positivity and HLA-DSA MFI values. Survival curves were generated using the Kaplan–Meier method. Groups were compared using the log-rank test. Predictors of

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ABMR were explored using multivariate logistic regression analysis. Clinical parameters showing significant differences ($P<0.05$) in univariate analysis or known to cause ABMR were fitted into the multivariate model. We selected patient sex, low-DSA, donor type, retransplantation, anti-thymocyte globulin (ATG) induction, and desensitization as predictors. PRA values (%) or high PRA values (>50%) were excluded from the model because of high variance inflation factors, suggesting multicol-

Table 1. Baseline characteristics of LDKT and DDKT recipients stratified according to the presence of low-DSA

Characteristics	LDKT recipients (N=629)		P	DDKT recipients (N=398)		P
	Low-DSA (N=68)	No-DSA (N=561)		Low-DSA (N=21)	No-DSA (N=377)	
Patients						
Age (yr)	47.0±10.8	45.6±12.0	0.317	53.7±8.8	49.9±9.6	0.080
Female sex, N (%)	47 (69.1)	207 (36.9)	<0.001	14 (66.7)	147 (39.0)	0.012
Etiology of kidney disease, N (%)			0.673			0.866
Diabetes	14 (20.6)	128 (22.8)		5 (23.8)	78 (20.7)	
Hypertension	5 (7.4)	52 (9.3)		3 (14.3)	80 (21.2)	
Glomerulonephritis	30 (44.1)	205 (36.5)		8 (38.1)	124 (32.9)	
Others	19 (27.9)	176 (31.4)		5 (23.8)	95 (25.2)	
HLA-A/B/DR mismatches	3.3±1.3	3.0±1.7	0.260	4.0±1.0	3.6±1.5	0.264
PRA I (%)	66.8±32.9	50.6±34.9	0.007	73.9±28.5	52.9±32.7	0.028
PRA II (%)	76.0±26.5	55.4±31.3	0.001	80.4±31.4	51.3±29.5	0.002
PRA >50%, N (%)	51 (75.0)	88 (15.7)	<0.001	16 (76.2)	61 (16.2)	<0.001
HLA class I	35 (51.5)		n.a.	10 (47.6)		n.a.
HLA class II	28 (41.2)		n.a.	8 (38.1)		n.a.
HLA class I+II	5 (7.4)		n.a.	3 (14.3)		n.a.
HLA-DSA peak MFI	3,676 (1,903–6,213)		n.a.	4,385 (2,608–7,952)		n.a.
Pretransplant dialysis, N (%)	47 (69.1)	372 (66.3)	0.643	21 (100.0)	377 (100.0)	n.a.
Time on dialysis, months	36.5±52.0	20.0±38.7	0.040	109.1±58.4	88.8±54.7	0.100
Follow-up period, months	47.7±31.8	55.6±31.6	0.051	50.0±27.4	56.7±28.7	0.295
Donors						
Age (yr)	41.4±12.5	43.3±12.2	0.211	53.7±8.8	49.9±9.6	0.080
Female sex, N (%)	32 (47.1)	320 (57.0)	0.117	5 (28.6)	128 (34.0)	0.612
Transplants						
Retransplant, N (%)	15 (22.1)	45 (8.0)	<0.001	9 (42.9)	28 (7.4)	<0.001
Induction therapy, N (%)			<0.001			0.001
Basiliximab	30 (44.1)	539 (96.1)		6 (28.6)	239 (63.4)	
ATG	38 (55.9)	22 (3.9)		15 (71.4)	138 (36.6)	
Initial immunosuppression, N (%)			0.236			1.000
Tacrolimus	67 (98.5)	532 (94.8)		21 (100.0)	373 (98.9)	
Cyclosporine	1 (1.5)	29 (5.2)		0 (0.0)	4 (1.1)	
Desensitization, N (%)			<0.001			<0.001
No	16 (23.5)	486 (86.6)		7 (33.3)	340 (90.2)	
Yes	52 (76.4)	75 (13.4)		14 (66.7)	37 (9.8)	

Continuous variables are presented as means±SDs or medians with interquartile ranges. Categorical variables are presented as numbers (proportions).
Abbreviations: ATG, anti-thymocyte globulin; DDKT, deceased donor kidney transplant; HLA-DSA, donor-specific anti-HLA antibody; LDKT, living donor kidney transplant; low-DSA, low-level donor-specific anti-HLA antibody; MFI, mean fluorescence intensity; PRA, panel-reactive antibody; n.a., not applicable.

linearity with low-DSA. Multivariate Cox regression analyses with backward selection were used to investigate independent predictors of infectious episodes. Cox models were built considering patient age, sex, cold ischemic time, low-DSA, desensitization, and ATG induction. A linear mixed model was used to compare the changes in allograft function over time. All missing data were censored from the last follow-up date. For all tests, a two-tailed $P<0.05$ indicated statistical significance. All data were analyzed using SPSS software version 24 (IBM Corporation, Armonk, NY, USA).

RESULTS

Baseline characteristics

Table 1 shows the clinical and immunological characteristics of patients with low-DSA in the LDKT and DDKT subgroups. In both donor-type subgroups, the low-DSA group comprised more females, retransplanted patients, and individuals with higher PRA values. There were more patients with class I HLA-DSA than with class II HLA-DSA. A small percentage of patients had both class I and II HLA-DSA. HLA-DSA strength was presented as the peak MFI value. HLA-DSA class and strength did not differ between the LDKT and DDKT subgroups (Supplemental Data Table S1). Patients with low-DSA received ATG more frequently as induction therapy and more frequently underwent desensitization therapy than patients without low-DSA. All DDKT recipients underwent dialysis prior to transplantation. Recipient or donor age did not differ according to low-DSA in LDKT or DDKT recipients. Additionally, among patients with low-DSA, desensitization was performed more frequently in LDKT recipients than in DDKT recipients (76.4% vs. 66.7%, $P=0.003$). Most DDKT recipients (14/21, 66.7%) received RTX alone as desensitization therapy. However, in the LDKT subgroup, 26 (38.2%) of 68 recipients received RTX and 26 (38.2%) of 68 received additional PP/IVIG (Supplemental Data Table S2).

Correlation between XM positivity and HLA-DSA MFI

Fifty-three patients had a positive XM result, and 40 of these 53 patients had HLA-DSA. However, HLA-DSA was not identified by the LSA assay in 13 patients. We analyzed the correlation between XM positivity and HLA-DSA MFI in 129 patients who were identified as having HLA-DSA (Fig. 2). The peak MFI value of HLA-DSA was significantly higher in the CDC-XM-positive group (7,255 [6,070–12,737], N=22) than in the CDC-XM-negative group (4,009 [2,127–7,270], N=107, $P<0.001$). The peak HLA-DSA MFI was significantly higher in FCXM-positive patients (7,218 [3,495–12,087], N=39) than in FCXM-negative patients (3,997 [2,089–6,796], N=90, $P<0.001$).

Immunological characteristics of HLA-DSA

The immunological characteristics of HLA-DSA were compared between LDKT and DDKT recipients according to the strength, class, and specificities of HLA-DSA. Fig. 3 presents the distribution of immunodominant HLA-DSA in the patients. MFI values or HLA-DSA specificities were missing for four patients. Overall, 46 (54.1%) patients had class I HLA-DSA, and 39 (45.9%) patients had class II HLA-DSA as the immunodominant antibody. MFI values were higher in class II HLA-DSA than in class I HLA-DSA (5,333 [2,243–7,898] vs. 3,407 [2,034–5,092], $P=0.063$). In both donor-type groups, HLA-DSA was more frequently of class I than of class II. However, the proportion of each HLA-DSA specificity differed according to donor type. Anti-HLA-B

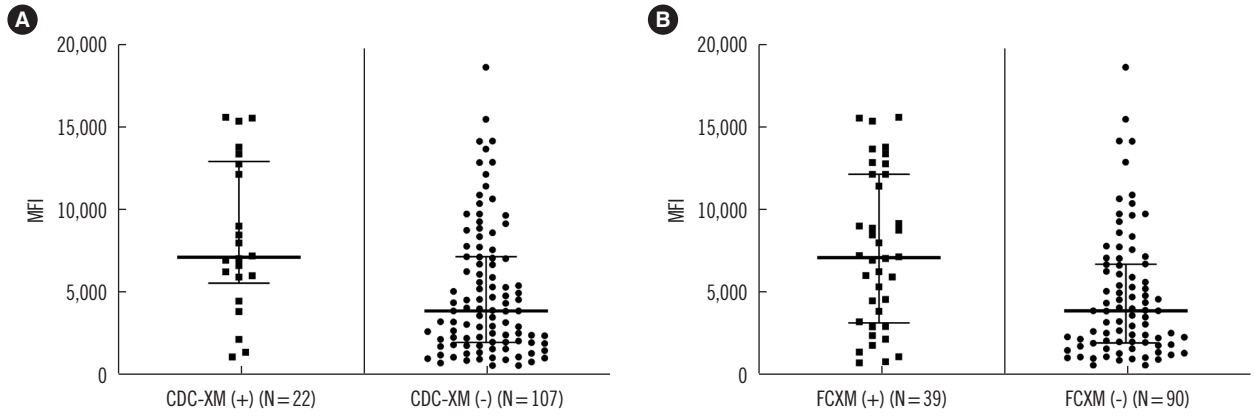

Fig. 2. Correlation between XM positivity and HLA-DSA MFI. (A) represents CDC-XM positivity and (B) represents FCXM positivity.
Abbreviations: CDC-XM, complement-dependent cytotoxicity crossmatch; FCXM, flow cytometry crossmatch; HLA-DSA, donor-specific anti-HLA antibody; MFI, mean fluorescence intensity; XM, crossmatch.

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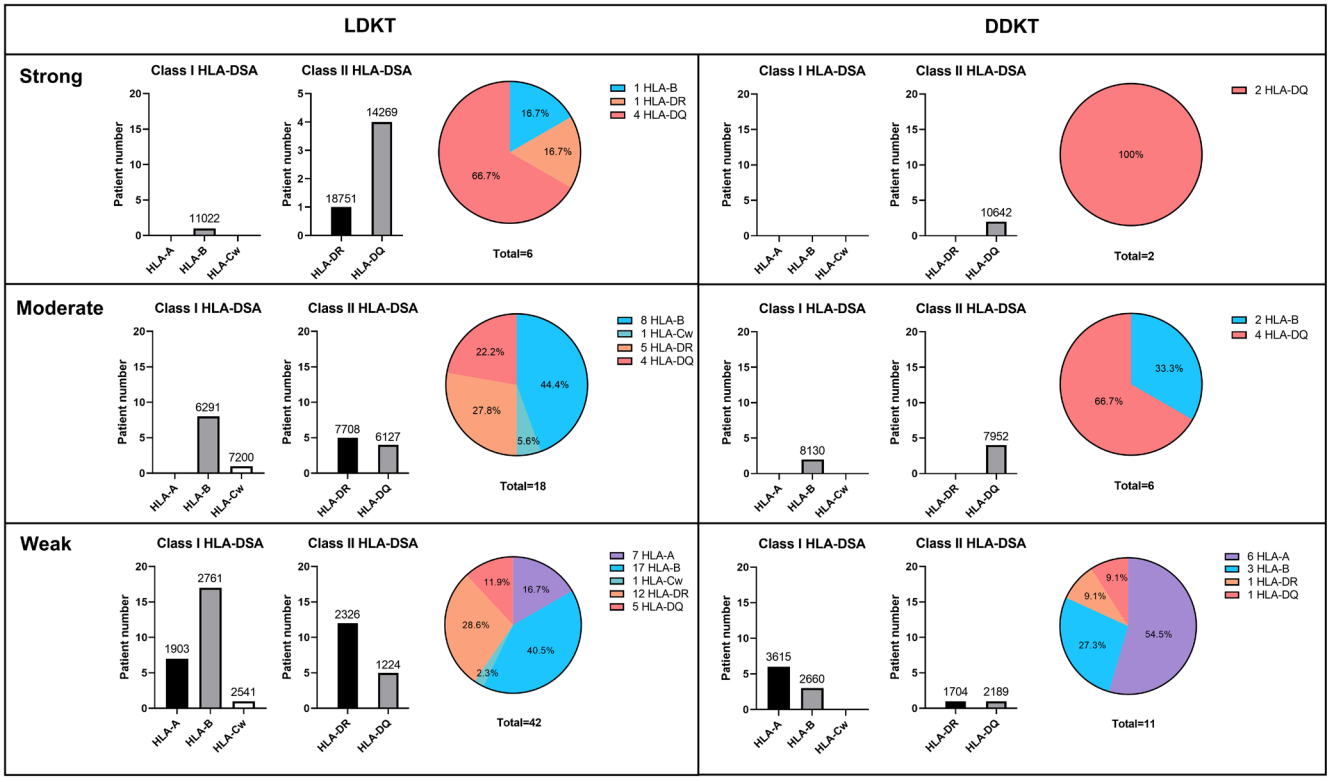

Fig. 3. Immunological characteristics of HLA-DSA according to the strength, class, and specificities of HLA-DSA. Numbers above bar graphs are median HLA-DSA MFI values. Pie graphs represent the percentage of HLA-DSA specificity within each HLA-DSA group classified by the peak MFI value.
Abbreviations: DDKT, deceased donor kidney transplant; HLA-DSA, donor-specific anti-HLA antibody; LDKT, living donor kidney transplant.

antibody (26/66, 39.4%) in class I HLA-DSA and anti-HLA-DR antibody (18/66, 27.3%) in class II HLA-DSA were common in LDKT recipients. DDKT recipients had anti-HLA-A antibody (6/19, 31.6%) in class I HLA-DSA and anti-HLA-DQ antibody (7/19, 36.8%) in class II HLA-DSA as the most frequent HLA-DSA (P=0.030).

The strength of HLA-DSA differed according to its class and specificity. Strong HLA-DSA were mostly class II, especially anti-HLA-DQ antibody. All strong HLA-DSA in DDKT recipients was anti-HLA-DQ antibody. Class II HLA-DSA accounted for half of moderate HLA-DSA in LDKT. In DDKT, it accounted for 66.7%, all of which were anti-HLA-DQ antibody. In weak HLA-DSA, class I antibodies had a higher proportion than class II antibodies. Anti-HLA-B antibody was the most common in LDKT, and anti-HLA-A antibody was the most common in DDKT. Anti-HLA-DQ antibody accounted for a small proportion of weak HLA-DSA.

Comparison of overall BPAR and ABMR

As shown in Fig. 4, the development of overall BPAR, which was divided into TCMR and ABMR, was identified in the first year of transplantation. In the total cohort, the overall BPAR and TCMR rates did not significantly differ between the low-DSA and no-DSA groups. However, the incidence of ABMR was significantly higher in the low-DSA group than in the no-DSA group (13.5%, 12/89 vs. 2.5%, 23/938, P<0.001). In subgroup analysis, the overall BPAR rate did not significantly differ between low-DSA and no-DSA groups in LDKT or DDKT. The TCMR rate did not significantly differ according to baseline low-DSA in LDKT or DDKT. ABMR was more frequent in the low-DSA group than in the no-DSA group in both LDKT (11.8%, 8/68 vs. 2.5%, 14/561, P=0.001) and DDKT (19%, 4/21 vs. 2.4%, 9/377, P=0.003) recipients. The results of univariate and multivariate analyses correlating patient, donor, and immunological factors that can be associated with the occurrence of ABMR are shown in Table 2. Pretransplant low-DSA was the only independent predictor of ABMR development in the total cohort (OR: 5.820, 95% CI: 2.100–16.140) and in DDKT recipients (OR: 9.600, 95% CI: 1.790–51.560), whereas it had only a marginal impact in LDKT recipients (OR: 3.760, 95% CI: 0.990–14.260).

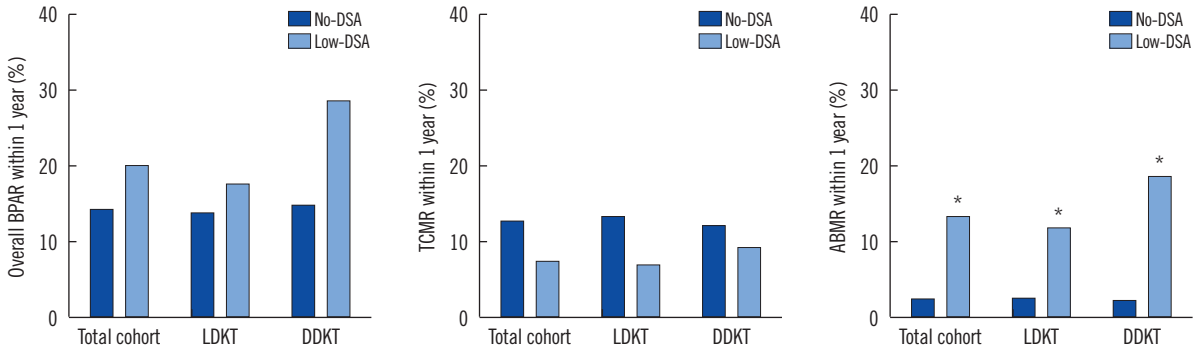

Fig. 4. Development of BPAR in the first year of transplantation according to the presence of low-DSA. The incidences of overall BPAR, TCMR, and ABMR are compared in the total cohort and the LDKT and DDKT subgroups.
Abbreviations: ABMR, antibody-mediated rejection; BPAR, biopsy-proven allograft rejection; DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; no-DSA, negative donor-specific anti-HLA antibody; low-DSA, low-level donor-specific anti-HLA antibody; TCMR, T cell-mediated rejection. *$P<0.05$.

Table 2. Prediction of biopsy-proven ABMR within 1 year of transplantation

Variables	Univariate		Multivariate	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
Total cohort				
Patient age	0.99 (0.96–1.02)	0.477		
Female sex	1.11 (0.56–2.19)	0.764	0.78 (0.37–1.64)	0.513
Low-DSA	6.20 (2.97–12.94)	<0.001	5.82 (2.10–16.14)	<0.001
LDKT (vs. DDKT)	1.07 (0.53–2.16)	0.842	1.24 (0.54–2.86)	0.609
Donor age	1.01 (0.980–1.03)	0.563		
Retransplant	2.05 (0.83–5.06)	0.120	1.37 (0.50–3.71)	0.537
ATG induction (vs. basiliximab)	3.01 (1.51–5.98)	0.002	2.35 (0.95–5.82)	0.065
Desensitization	2.23 (1.07–4.64)	0.032	0.60 (0.20–1.80)	0.362
LDKT				
Patient age	1.00 (0.97–1.04)	0.919		
Female sex	1.81 (0.77–4.25)	0.174	1.26 (0.50–3.19)	0.629
Low-DSA	5.21 (2.10–12.92)	<0.001	3.76 (0.99–14.26)	0.051
Donor age	1.01 (0.98–1.05)	0.462		
Retransplant	0.95 (0.22–4.15)	0.942	0.69 (0.15–3.20)	0.636
ATG induction (vs. basiliximab)	4.88 (1.90–12.49)	0.001	3.31 (0.66–16.64)	0.146
Desensitization	2.30 (0.94–5.60)	0.068	0.53 (0.11–2.54)	0.429
DDKT				
Patient age	0.96 (0.91–1.01)	0.148		
Female sex	0.43 (0.12–1.59)	0.201	0.31 (0.07–1.31)	0.110
Low-DSA	9.62 (2.69–34.40)	<0.001	9.60 (1.79–51.56)	0.008
Donor age	1.00 (0.96–1.04)	0.926		
Retransplant	4.74 (1.38–16.23)	0.013	2.82 (0.57–13.96)	0.205
ATG induction (vs. basiliximab)	2.65 (0.85–8.25)	0.093	2.11 (0.62–7.16)	0.229
Desensitization	2.11 (0.56–7.93)	0.271	0.57 (0.08–3.84)	0.560

Abbreviations: ATG, anti-thymocyte globulin; CI, confidence interval; DDKT, deceased donor kidney transplant; LDKT, living donor kidney transplant; low-DSA, low-level donor-specific anti-HLA antibody.

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Comparison of allograft function, allograft failure, and patient survival

Allograft function did not differ between the low-DSA and no-DSA groups in the total cohort and LDKT and DDKT recipients until 36 months after KT (Supplemental Data Fig. S1). There was no significant difference in the death-censored allograft survival rate in the total cohort and LDKT and DDKT recipients according to the presence of low-DSA (Supplemental Data Fig. S2). Among patients who experienced ABMR in the first year after transplantation, one of 12 low-DSA and eight of 23 no-DSA patients eventually lost graft function. Treatment of ABMR, including steroid pulse, PP/IVIG, RTX, and bortezomib, was performed according to clinical decision. Rejection was the leading cause of allograft failure, except for one patient who lost his graft due to BKPyAN (Supplemental Data Table S3). In total, 48 patients (48/1,027, 4.7%) died, including one patient with low-DSA who died by suicide. There was no significant difference in the patient survival rate according to the presence of low-DSA in the total cohort and in LDKT recipients (Supplemental Data Fig. S3). As no patients died in the DDKT group, statistical analysis could not be performed.

Comparison of posttransplant infections

In total, 567 (55.2%) cases of infectious complications occurred during the follow-up period. The incidence of BKV infection was higher in the low-DSA group than in the no-DSA group of LDKT recipients (23.6% vs. 13.4%). The incidence of infections caused by pathogens other than BKV did not significantly differ between the low-DSA and no-DSA groups in the total cohort and LDKT and DDKT recipients (Supplemental Data Table S4). In the LDKT group, the infection-free survival rate significantly differed between the low-DSA and no-DSA groups (P=0.041). However, such a difference was not observed in the total cohort and DDKT recipients (Supplemental Data Fig. S4). The results of univariate and multivariate Cox regression analyses predicting posttransplant infections are presented in Supplemental Data Table S5. Neither low-DSA nor desensitization was identified as a predictor of posttransplant infection.

DISCUSSION

This study showed that pretransplant low-DSA is a significant risk factor for ABMR development but does not affect long-term allograft outcomes, such as changes in allograft function, allograft survival, or patient survival. Similar results were found in subgroup analysis according to donor type. The impact of low-DSA on ABMR was more significant in DDKT recipients than in LDKT recipients, likely because desensitization is more frequently applied in the latter. Desensitization did not significantly increase the risk of posttransplant infection.

First, we compared the incidence of BPAR according to the presence of pretransplant low-DSA. Low-DSA was associated with ABMR development in both LDKT and DDKT recipients. For overall BPAR or TCMR, there was no such association. These results are in line with those in previous studies [26–28]. We performed multivariate analysis to determine the impact of low-DSA on ABMR after adjusting for confounding factors, such as patient sex, donor type, retransplantation, ATG induction, and desensitization. In the final model, low-DSA was a significant predictor in DDKT recipients. In LDKT recipients, it was marginally significant. It is well known that allograft outcomes are worse in DDKT recipients than in LDKT recipients [29, 30]. This is possibly because of the poor quality of organs from brain-dead donors due to prolonged cold ischemic time, cardiovascular instability, and the use of vasopressors during organ procurement [31]. Adhesion molecules and HLA antigens are expressed at higher levels in brain-dead donor kidneys than in living donor kidneys [32]. This can lead to an increased incidence of acute ABMR. In this study, deceased donors were significantly older than living donors, which may have contributed in part to poor organ quality.

Percentages of class I and class II HLA-DSA were similar in LDKT and DDKT recipients. However, DDKT recipients more frequently had anti-HLA-DQ antibody as moderate to strong HLA-DSA than LDKT recipients. Class I HLA-DSA is associated with acute ABMR and early graft loss, whereas class II HLA-DSA is associated with chronic ABMR and transplant glomerulopathy [33]. The clinical significance of pretransplant anti-HLA-DQ antibody is not clear to date. Therefore, the differential impact of low-DSA on ABMR development cannot be explained by HLA-DSA characteristics alone.

Another possible reason for the greater impact of low-DSA on ABMR development in DDKT recipients may be the more frequent application of desensitization in LDKT recipients. In patients with low-DSA, desensitization therapy reduced the incidence of ABMR [34]. The discrepancy in the impact of low-DSA on clinical outcomes among studies can also be explained by differences in desensitization protocols [27, 28]. We applied desensitization treatment in patients with pretransplant low-DSA before LDKT. In DDKT recipients, there is insufficient time to conduct desensitization before transplantation, and it is crucial to minimize the total ischemic time to preserve donor kidney

quality. Desensitization was performed more frequently in LDKT recipients than in DDKT recipients in the low-DSA group (76.4% vs. 66.7%). Moreover, the intensity of desensitization was stronger in LDKT recipients than in DDKT recipients. All DDKT recipients who underwent desensitization received RTX alone. In the LDKT group, half of the desensitized patients additionally took PP/IVIG. Taken together, the more pronounced impact of pretransplant low-DSA on ABMR in DDKT recipients may have resulted from the less intensive desensitization in these recipients.

Next, we investigated long-term allograft outcomes. In contrast to the observation that low-DSA affected the development of acute ABMR, there were no significant differences in allograft function changes according to pretransplant low-DSA. Furthermore, allograft and patient survival were not associated with pretransplant low-DSA. The death-censored graft survival rate in the low-DSA group was 96.6%, which was significantly higher than that in previous studies [3, 6, 26, 35]. In the low-DSA group, only one patient who experienced ABMR in the first year after transplantation lost graft function. These favorable long-term allograft outcomes can be explained by the effect of the aggressive desensitization protocol applied in our cohort, considering that preexisting HLA-DSA is less likely to cause allograft failure than *de novo* HLA-DSA if aggressive immunosuppression is performed [36, 37].

As desensitization protocols are based on the elimination or reduction of pretransplant antibodies [38], over-immunosuppression caused by desensitization may increase the infection rate after kidney transplantation [39]. As mentioned above, LDKT recipients in the low-DSA group received more intense desensitization than the DDKT recipients. Therefore, we analyzed the association between pretransplant low-DSA and posttransplant infections. In LDKT recipients, BKV infection was more common and infection-free survival was lower in the low-DSA group than in the no-DSA group. Pretransplant low-DSA and desensitization were not identified as significant predictors of posttransplant infections using Cox regression models, but ATG induction (in the total cohort and LDKT and DDKT recipients) and female sex (in the total cohort and LDKT recipients) were. Unlike in patients with strong HLA-DSA [40], relatively weak desensitization was applied in patients with low-DSA, which did not increase the risk of posttransplant infection.

One limitation of this study was that it was a single-center study with a small number of patients who had pretransplant low-DSA; a large multicenter study is required to verify the study results. A strength of this study was that we were able to predict and precisely control the effects of immunosuppression since we used a unified protocol for desensitization, induction therapy, and immunosuppression maintenance.

In conclusion, pretransplant low-DSA has a significant impact on the development of ABMR but not on long-term allograft outcomes and posttransplant infections. The impact of low-DSA on ABMR is more significant in DDKT recipients than in LDKT recipients. One possible reason is that LDKT recipients undergo more intensive desensitization. To reduce the risk of ABMR, we recommend desensitization in both LDKT and DDKT recipients with low-DSA.

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AUTHOR CONTRIBUTIONS

Lee Haeun participated in the study design, data analysis, preparation of figures and tables, and manuscript writing. Lee Hanbi, Eum SH, Ko EJ, and Min JW collected the data. Oh EJ and Yang CW analyzed the data. Chung BH designed the study, interpreted the data, and revised the paper. All authors have read and approved the final manuscript.

CONFLICTS OF INTEREST

None declared.

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ORCID

Haeun Lee https://orcid.org/0000-0002-6661-9872
Hanbi Lee https://orcid.org/0000-0001-7326-0602
Sang Hun Eum https://orcid.org/0000-0002-5533-0459
Eun Jeong Ko https://orcid.org/0000-0002-5604-1296
Ji-Won Min https://orcid.org/0000-0001-6295-8095
Eun-Jee Oh https://orcid.org/0000-0001-5870-915X
Chul Woo Yang https://orcid.org/0000-0001-9796-636X
Byung Ha Chung https://orcid.org/0000-0003-0048-5717

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