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Original Article

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The Effect of Preformed Anti-Human Leukocyte Antigens Antibodies on Graft and Patient Outcomes in Kidney Transplantation*

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ABSTRACT

Objective: Kidney transplantation is the most suitable kidney replacement therapy for patients with end-stage kidney disease. The relationship between the pretransplant antibodies detected by Luminex Single Antigen Bead test and the clinical outcome is still unclear. In this study, we aimed to examine the effects of pretransplant anti-human leukocyte antigen antibody status on graft functions, posttransplant complications, graft, and patient survival in kidney transplantation recipients.

Methods: Two hundred eleven patients who underwent kidney transplantation between January 2015 and July 2020 were included in the study. Detailed immunological assessment, donor and recipient characteristics, posttransplant immunological and non-immunological complications, graft loss, and death were analyzed.

Results: Thirty-three (15.6%) patients were donor-specific antibody positive, 58 (27.5%) patients were non-donor-specific antibody positive, and 120 (56.9%) patients were anti-human leukocyte antigen antibody negative. There was no significant difference between these groups in terms of acute rejection, opportunistic infections, urinary tract infections, malignancy, graft, and patient survival. The rate of desensitization therapy and antithymocyte globulin induction were higher in donor-specific antibody-positive group ($P < .001$ and $< .001$, respectively). Increased recipient and donor age and BK virus infection significantly decreased graft and patient survival in multivariate analyses.

Conclusion: Pretansplant donor-specific antibody and non-donor-specific antibody should not be a barrier for kidney transplantation. With the guidance of immunosuppression with advanced immunological risk assessment methods, kidney transplantation can be performed successfully in patients with high immunological risk.

Keywords: Acute rejection, anti-HLA antibody, donor-specific antibody, graft survival, kidney transplantation, patient survival

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INTRODUCTION

Preexisting sensitization against human leukocyte antigens (HLAs) is an important barrier in kidney transplantation (KTx), leading to prolonged waiting times for sensitized recipients, increased risk of antibody-mediated rejection (ABMR), and reduced graft and patient survival.[1-3] Currently, 38.2% of the patients awaiting KTx in the United States are sensitized against HLA antigens [panel reactive antibody (PRA) ≥1%], and 12.0% of the patients are highly sensitized (PRA >80%).[4] About 20%-50% of the patients on the wait-list for KTx are positive for class I and/or II anti-HLA antibodies.[5-7]

Evaluation of the immunological status of the KTx candidate is crucial for the proper management of immunosuppressive therapy. Complement-dependent cytotoxicity cross-match (CDC-XM) test is the first method demonstrated by Patel and Terasaki in 1969 to evaluate the immunological risk before KTx, and T-cell CDC-XM positivity is still a contraindication for KTx.[8]

With the improvements in technology, more sensitive and specific techniques such as flow cytometry cross-match (FC-XM) test and solid-phase methods including ELISA and Luminex assays came into use to detect antibodies.[9] The Luminex assay, using single-antigen bead (SAB), increased the ability to detect anti-HLA antibodies with low titer.[10] The presence of antibodies detected by the SAB technique that do not cause a positive CDC-XM is not a contraindication for KTx; however, it increases the risk of rejection and graft loss. The relationship between the antibodies detected by the SAB test and the clinical outcome is still imprecise.

In the pretransplant immunological risk assessment at our center, the CDC-XM test has been performed since 1970, panel reactive antibody screening test (PRA Class I and II) since 2002, and FC-XM and Luminex-based SAB assays since 2015. In this study, we aimed to explore the effects of comprehensive pretransplant immunological risk assessment, including donor-specific HLA antibody (DSA) and non-donor-specific HLA antibody (nDSA) on graft functions, immunological and non-immunological posttransplant complications, graft and patient survival in KTx patients.

METHODS

Study Population

This retrospective study utilized the data accessed from the medical records of 255 patients who underwent KTx between January 2015 and July 2020 at Ankara University Faculty of Medicine, Kidney Transplantation Center. After excluding 18 patients who were under 18 years of age and 16 whose follow-up was less than 6 months in our center for reasons other than death or graft loss, the study included 211 patients who underwent deceased or living donor KTx. These patients were divided into DSA-positive, nDSA-positive, and anti-HLA antibody-negative groups according to the pretransplant Luminex assays.

Recipients' age and gender, transplant type, primary kidney disease, dialysis vintage, previous organ transplantation, transfusion or pregnancy history, ABO blood group, pretransplant desensitization treatment, induction and maintenance immunosuppressive treatment, posttransplant medical complications and infections, donors' age and gender, and estimated glomerular filtration rate (eGFR) before transplantation were recorded. Immunological and non-immunological complications, eGFR, tacrolimus (TAC) trough levels, graft loss, and death were investigated in the posttransplant follow-up period. The study was approved by the Ethical Review Committee of the Ankara University (Approval No: İ7-465-20, Date: 09.07.2020).

Pretransplant Immunological Work-up

HLA typing was performed for the HLA-A, -B, -C, -DRB1, and -DQB1 loci with LIFECODES HLA-SSO Typing Kits (Immucor, Stamford, Conn, USA). HLA mismatch (MM) was evaluated according to HLA-A, -B, and -DR loci (0-6 MM). We performed PRA-Luminex screening and XM testing using CDC, CDC-anti-human globulin, and FC-XM (Beckman Navios instrument; Beckman Coulter Life Sciences, Indianapolis, IN, USA) in all patients. The sera used for HLA antibody testing and XM testing were obtained within the 1-week period preceding transplantation and treated with dithiothreitol to inactivate immunoglobulin M antibodies. The limit for PRA positivity was defined as >5%, identifying only immunoglobulin G (IgG) anti-HLA isotype-positive cases. In patients with positive PRA or positive XM test results, samples were further tested for the identification of class I (A, B, and C) and class II (DRB1, DQA1, and DQB1) antibodies using Luminex assay kits (Lifecodes; Immucor, Stamford, Conn, USA). For SAB assays, the cutoff level was a raw median fluorescence intensity (MFI) of ≥500. All KTxs performed were ABO compatible.

Immunosuppressive Protocol

In our center, KTx is not performed in patients with positive T-cell CDC-XM or positive FC-XM with median channel shift (MCS) over 250. Desensitization therapy was performed on 6 patients, 4 of whom tested positive for pretransplant HLA–DSA over 5000 MFI and 2 of whom tested positive for T-cell FC-XM (MCS value of 9 and 67). Plasmapheresis (PP) and intravenous immunoglobulin (IVIG) were initiated 13 days prior to KTx and applied every other day in all the patients. In addition, 1 patient received rituximab (RTX, 375 mg/m^2) 2 weeks before transplantation. HLA-DSA and FC-XM testing were done 2 days prior to the KTx. The transplantation was conducted assuring that the recipient is negative for HLA-DSA or positive at low titers.

In general, induction with anti-thymocyte globulin (ATG, 100 mg daily for 3-5 days) was utilized for deceased KTx recipients and living KTx recipients those considered to have higher immunological risk (>3 HLA MM, PRA >5%, presence of a DSA, history of a sensitization event). Otherwise, low-risk living-related donor recipients received no induction or basiliximab (20 mg on days 0 and 4). The initial maintenance immunosuppression regimen consisted of TAC, mycophenolate mofetil and steroids. Pulse methylprednisolone (500 mg daily for 3 days) was followed by methylprednisolone 1 mg/kg/day for 3 days, and then the corticosteroid dose was reduced by 8 mg every 3 days to 4 mg at the end of the third month. Target TAC through levels were 9-10 ng/mL for the first month, 6-8 ng/mL for 1-3 months, and 5-8 ng/mL for 6 months and beyond.

MAIN POINTS

- Graft and patient outcomes are comparable between pretransplant anti-human leukocyte antigen antibody-positive and -negative groups.
- Long graft survival can be achieved by the induction and desensitization regimens adjusted according to the level of immunological risk.
- Increased recipient and donor age and BK virus infection decrease both graft and patient survival.

Preventive Measures for Opportunistic Infections
All recipients received fluconazole, trimethoprim/sulfamethoxazole, and valganciclovir from the start of the immunosuppressive therapy until 3-6 months after KTx. BK virus (BKV) was monitored at 3, 6, and 9 months after KTx and whenever allograft dysfunction occurred by using a real-time polymerase chain reaction method.

Diagnosis and Management of Acute Rejection
A total of 54 indication biopsies were performed after KTx in 37 recipients because of progressive loss of graft function or new-onset proteinuria. In accordance with the current Banff criteria, rejection episodes were classified as T-cell-mediated rejection (TCMR), ABMR, and borderline or mixed-type rejection.[11] There were 8 optimal graft biopsies in DSA-positive patients, 14 in nDSA-positive patients, and 21 in HLA antibody-negative patients. Posttransplant graft biopsies were reevaluated and recorded according to Banff 2019 classification in Ankara University Faculty of Medicine, Department of Medical Pathology. Delayed graft function (DGF) was defined as acute kidney injury that occurred in the first week of kidney transplantation, which necessitated dialysis intervention.

A TCMR was treated with methylprednisolone (500 mg daily for 3 days) and ATG (100 mg daily for 3-5 days) in case of unresponsiveness to steroids. Two patients received alemtuzumab (a single dose of 30 mg) for resistant TCMR. Active ABMR was treated with a combination of methylprednisolone (500 mg daily for 3 days), PP (every other day for a maximum of 5 sessions or until serum creatinine is within 20%-30% of the baseline), IVIG (100 mg/kg after each session of PP), and RTX (a single dose of 375 mg/m^2) in case of rapid declining graft function without sufficient response to therapy.

Statistical Analysis
Data analysis was performed using IBM Statistical Package for Social Sciences (SPSS) version 25 (IBM SPSS Corp.; Armonk, NY, USA). Continuous data are described as mean (SD) for normally distributed numerical variables or median (minimum–maximum) for non-normally distributed numeric variables, and categorical data are expressed as number and percentage. Student's *t*-test or One-Way ANOVA was used for normally distributed numerical variables, Mann Whitney U test or Kruskal Wallis test was used for non-normally distributed numerical variables, and Chi-square or Fisher's tests were used for categorical variables in comparisons between groups. When a difference was detected between variables in more than 2 groups, pairwise comparisons were made with Bonferroni correction.

Variables with $P < .25$ in univariate analysis were taken into account to determine the independent factors affecting graft survival. Among these variables, those whose log rank *P*-value was >.25 and whose log-minus-log curve was not parallel were excluded from the analysis. In the correlation analysis, when the correlation coefficient between the 2 variables was above 0.6, the log rank *P* value which was larger or had less clinical significance was excluded from the analysis. The remaining variables were included in the Cox regression analysis and hazard ratios were calculated for independent risk factors. By evaluating the residuals resulting from the calculations, the proportionality of the periodic risk and the accuracy of the model were tested.

For $P < .05$, the results were considered statistically significant.

RESULTS

Baseline and Clinical Features
The 211 patients were divided into 3 groups as DSA-positive (33 patients, 15.6%), nDSA-positive (58 patients, 27.5%), and anti-HLA antibody negative (120 patients, 56.9%) according to the pretransplant immunological evaluation. A comparison of the general characteristics of these 3 groups regarding the pretransplant period is given in Table 1.

Recipients' mean age at transplant was 42 ± 12 years, and most of them were male (67.8%). However, in the DSA-positive group, the proportion of women was higher (51.5%, $P = .032$). The most common causes of end-stage kidney disease were diabetic kidney disease and glomerulonephritis, and in 27.0% of recipients, the cause was unidentified. Median dialysis vintage was 3 (0-300) months, which was significantly shorter in anti-HLA antibody-negative recipients ($P = .006$). Anti-HLA antibody positivity rates were increased with history of transplantation, pregnancy, and blood transfusion, but it was found to be significant only in patients with previous transplants ($P < .001$). The anti-HLA antibody-negative group had a significantly higher rate of living donors (85.0%) compared with both DSA-positive (69.7%) and nDSA-positive group (69.0%) ($P = .023$).

The median number of HLA mismatches was 3 in all groups. B- and T-cell FC-XM positivity, desensitization therapy, and ATG induction therapy were correlated with DSA positivity ($P = .001$, .004, <.001, and <.001, respectively). Almost all the recipients started standard triple-maintenance therapy.

Comparison of Acute Rejection and Posttransplant Complications
The median posttransplant follow-up period was 43 (1-81) months (Table 2). Biopsy-proven acute rejection developed in 27 recipients (12.8%), 4 of whom were DSA positive and 9 of whom were nDSA positive. Rejection episodes were classified into mixed-type rejection (n = 9, 33.3%), TCMR (n = 9, 33.3%), and ABMR (n = 4, 14.8%), furthermore, no difference was established according to anti-HLA antibody status. There was also no difference between the groups regarding the timing of acute rejection. HLA mismatch number, pretransplant anti-HLA antibody status, pretransplant DSA status and strength (MFI), and immunosuppression protocol were not related to acute rejection. Six patients had chronic active ABMR. No acute or chronic rejection was observed in 4 patients who received treatment other than

Table 1. Demographic and Clinical Data of Kidney Transplantation Recipients and Donors, According to Anti-HLA Antibody Status

Parameters	DSA Positive (n = 33, 15.6%)	Non-DSA Positive (n = 58, 27.5%)	Anti-HLA Antibody Negative (n = 120, 56.9%)	*P*
Recipient Characteristics				
Gender, n (%)				
Female	17 (51.5)*	18 (31.0)	33 (27.5)	**.032**[a]
Male	16 (48.5)*	40 (69.0)	87 (72.5)	
Age at transplant (years), mean ± SD	44.6 ± 11.8	41.6 ± 12.0	40.9 12.5	.32[b]
Dialysis modality, n (%)				
Preemptive	7 (21.2)	17 (29.3)	51 (42.5)*	**.039**[a]
HD	24 (72.7)	38 (65.5)	67 (55.8)	.15[a]
PD	9 (27.3)*	4 (6.9)	6 (5.0)	**<.001**[a]
Dialysis vintage (Months)				
Median (minimum–maximum)	24.0 (0-288)	4.5 (0-300)	1.0* (0-300)	**.006**[c]
History of organ transplantation, n (%)	13 (39.4)+	6 (10.3)+	3 (2.5)+	**<.001**[a]
History of pregnancy, n (%), n = 68	11 (64.7)	8 (44.4)	13 (39.4)	.23[a]
History of blood transfusion, n (%)	12 (36.4)	15 (25.9)	21 (17.5)	.058[a]
ESKD etiology, n (%)				
Unknown	11 (33.3)	13 (22.4)	33 (27.5)	.28[a]
Diabetes mellitus	3 (9.1)	10 (17.2)	12 (10.0)	
Hypertension	3 (9.1)	4 (6.9)	10 (8.3)	
Glomerulonephritis	4 (12.1)	10 (17.2)	35 (29.2)	
Congenital	5 (15.2)	5 (8.6)	9 (7.5)	
TIN + VUR+ ON + PN	6 (18.2)	10 (17.2)	10 (8.3)	
Other	1 (3.0)	6 (10.3)	11 (9.2)	
Donor characteristics				
Gender, n (%)				
Female	23 (69.7)	33 (56.9)	69 (57.5)	.41[a]
Male	10 (30.3)	25 (43.1)	51 (42.5)	
Age at transplant (years), mean ± SD	49.5 ± 11.8	48.4 ± 12.9	48.6 ± 12.1	.91[b]
Transplant type, n (%)				
Living	23 (69.7)	40 (69.0)	102 (85.0)*	**.023**[a]
Deceased	10 (30.3)	18 (31.0)	18 (15.0)*	
Pretransplant eGFR (CKD-EPI, mL/min/1.73 m^2), mean ± SD	89.2 ± 30.2	96.7 ± 28.5	95.7 ± 19.0	.46
Cold ischemic period (deceased donors) (hours), n = 34				
Median (minimum–maximum)	10.0 (7-14)	12.0 (4-16)	11.5 (6-18)	.38[c]
Pretransplant immunological work-up and immunosuppressive regimen				
HLA mismatch number				
Median (minimum–maximum)	3 (0-6)	3 (0-6)	3 (0-6)	.45[c]

(*Continued*)

Table 1. Demographic and Clinical Data of Kidney Transplantation Recipients and Donors, According to Anti-HLA Antibody Status (*Continued*)

Parameters	DSA Positive (n = 33, 15.6%)	Non-DSA Positive (n = 58, 27.5%)	Anti-HLA Antibody Negative (n = 120, 56.9%)	*P*
Anti-HLA antibody class, n (%)				.38[a]
Class I	15 (45.5)	12 (20.7)		
Class II	15 (45.5)	25 (43.1)		
Classes I and II	3 (9.1)	21 (36.2)		
Anti-HLA antibody strength (MFI)				
Class I, median (minimum–maximum)	1764 (172-12 647) (n = 17)	1042 (454-15 541) (n = 30)		.63[c]
Class II, median (minimum–maximum)	1476 (187-5625) (n = 17)	1092 (487-20 340) (n = 40)		.67[c]
CDC-XM positive, n (%)				
CDC-XM B-cell positive	2 (6.1)	1 (1.7)	1 (0.8)	.15[a]
CDC-XM T-cell positive	0 (0)	0 (0)	0 (0)	.067[a]
FC-XM positive, n (%)				
FCXM B-Cell positive	4 (12.1)	6 (10.3)	0 (0)*	**.001[a]**
FCXM T-Cell positive	2 (6.1)*	0 (0)	0 (0)	**.004[a]**
Desensitization, n (%)	6 (18.2)*	0 (0)	0 (0)	<.001[a]
Induction therapy, n (%)				
None	0 (0)	9 (15.5)	48 (40.0)	**<.001[a]**
Basiliximab	6 (18.2)	24 (41.4)	57 (47.5)	**.004[a]**
ATG	27 (81.8)	25 (43.1)	15 (12.5)	**<.001[a]**
Initial immunosuppression therapy, n (%)				.56[a]
CS + MMF + TAC	20 (60.6)	28 (48.3)	72 (60.0)	
CS + MPA + TAC	12 (36.4)	30 (51.7)	45 (37.6)	
Other	1 (3.0)	0 (0)	3 (2.4)	

ATG, anti-thymocyte globulin; CDC, complement dependent cytotoxicity; CS, corticosteroid; DSA, donor-specific antibody; ESKD, end-stage kidney disease; HD, hemodialysis; HLA, human leucocyte antigen; MFI, median fluorescence intensity; MMF, mycophenolate mofetil; MPA, mycophenolic acid; ON, obstructive nephropathy; PD, peritoneal dialysis; PN, pyelonephritis; TAC, tacrolimus; TIN, tubulointerstitial nephritis; VUR, vesicoureteral reflux.
[a]Chi-square; [b]1-way ANOVA; [c]Kruskal–Wallis H. *The group from which the statistical difference originates; [+]statistically significant difference between marked groups.

standard triple-maintenance therapy. BKV nephropathy findings were found in the graft biopsy of 2 of 40 patients with BKV infection. There was no difference between the anti-HLA-antibody groups in terms of posttransplant medical complications, such as BKV infection, urinary tract infection, opportunistic infection, COVID infection, and malignancy.

Comparison of Kidney Allograft Biopsy Re-evaluation Results

Patients with a peritubular capillaritis (ptc) score >0 were significantly less common in the nDSA-positive group, and all patients in the DSA-positive group had a higher ptc score (*P* = .004) (Table 3). However, the mesangial matrix expansion score was higher in the anti-HLA antibody-negative recipients compared with other groups (*P* < .001). Thrombotic microangiopathy was observed only in the nDSA-positive group (n = 3, *P* = .035). Other histopathological findings evaluated in Banff 2019 classification were similar between groups.

Comparison of Graft and Patient Outcomes

Among 211 patients, 25 patients (11.8%) had graft loss and 20 patients (9.5%) died. Death with a functioning graft developed in 16 patients, accounting for 80.0% of patients who died and 64.0% of patients with graft loss. Other reasons for graft loss were chronic rejection (4 patients, 16.0%), infection or sepsis (3 patients, 12.0%), acute rejection (1 patient, 4.0%), and malignancy (1 patient, 4.0%). Causes of death were septic shock (7 patients, 35.0%), unknown reason (4 patients, 20.0%), respiratory failure (3 patients, 15.0%), COVID infection (2 patients, 10.0%), ischemic cerebrovascular event (2 patients, 10.0%), subarachnoid hemorrhage (1 patient, 5.0%), and pulmonary thromboembolism (1 patient, 5.0%).

No statistically significant difference was observed in graft and patient survival between anti-HLA antibody groups. Even though we observed that median eGFR at 2 years was significantly lower in DSA-positive group compared to anti-HLA

Table 2. Kidney Transplantation Outcomes, According to Anti-HLA Antibody Status

Parameters	DSA Positive (n = 33, 15.6%)	Non-DSA Positive (n = 58, 27.5%)	Anti-HLA Antibody Negative (n = 120, 56.9%)	*P*
Follow-up (Months)				
Median (minimum–maximum)	39 (13-17)	40 (2-81)	44 (1-79)	.38[c]
Number of acute rejection, n (%)				.52[a]
0	26 (78.8)	47 (81.1)	100 (83.3)	
1	4 (12.1)	8 (13.8)	17 (14.2)	
2	3 (9.1)	2 (3.4)	2 (1.7)	
3	0 (0)	1 (1.7)	1 (0.8)	
Acute rejection type, n (%), n = 27				.75[a]
Acute TCMR	0 (0)	3 (33.3)	6 (42.9)	
Active ABMR	1 (25.0)	1 (11.1)	2 (14.3)	
TCMR + ABMR	2 (50.0)	4 (44.4)	3 (21.4)	
Borderline	1 (25.0)	1 (11.1)	3 (21.4)	
Chronic active ABMR, n (%)	3 (9.1)	1 (1.7)	2 (1.7)	.063[a]
Delayed graft function, n (%)	4 (12.1)	5 (8.6)	7 (5.8)	.45[a]
BK virus infection, n (%)	6 (18.2)	16 (27.6)	18 (15.0)	.13[a]
Urinary tract infection, n (%)	15 (45.5)	24 (41.4)	39 (32.5)	.28[a]
Opportunistic infections, n (%)	7 (21.2)	10 (17.2)	20 (16.7)	.83[a]
COVID infection, n (%)	6 (18.2)	7 (12.1)	14 (11.7)	.60[a]
Malignancy, n (%)	0 (0)	3 (5.2)	5 (4.2)	.44[a]
eGFR (CKD-EPI, mL/min/1.73 m^2)				
Discharge (n = 211) mean ± SD	69.0 ± 26.7	71.8 ± 27.2	73.1 ± 22.5	.72[b]
1 Month (n = 211) mean ± SD	67.0 ± 23.9	73.2 ± 26.7	73.7 ± 24.2	.38[b]
6 Months (n = 211) mean ± SD	67.7 ± 23.4	73.2 ± 23.4	74.5 ± 23.8	.35[b]
12 Months (n = 202) median (minimum–maximum)	70.0 (15-111)	70.0 (17-139)	75.5 (9-136)	.48[c]
24 Months (n = 174) median (minimum–maximum)	63.0[+](14-105)	67.0 (24-120)	74.0[+](32-133)	**.046**[c]
36 Months (n = 133) median (minimum–maximum)	58.5 (12-101)	61.0 (32-124)	70.5 (28-125)	.056[c]
Tacrolimus through level (ng/mL)				
1 Month (n = 211) mean ± SD	8.5 ± 2.2	9.1 ± 2.7	9.2 ± 2.7	.33[b]
6 Months (n = 211) median (minimum–maximum)	6.5 (2-13.9)	6.4 (3.1-10.2)	6.7 (0-14.5)	.44[c]
12 Months (n = 202) median (minimum–maximum)	6.7 (3.6-19.3)	6.2 (3.7-8.8)	6.4 (2.5-13.3)	.23[c]
24 Months (n = 174) median (minimum–maximum)	5.8 (3.0-20.8)	5.8 (2-11.3)	5.7 (2.6-21.7)	1.00[c]
36 Months (n = 133) median (minimum–maximum)	5.7 (4.1-10.9)	6.2 (2.9-19.4)	5.5 (2.0-11.0)	.28[c]
Graft Loss, n (%)	3 (9.1)	9 (15.5)	13 (10.8)	.58[a]
Time to graft loss (months) n = 25	n = 3	n = 9	n = 13	
Mean ± SD	45.0 ± 6.9	36.0 ± 25.2	30.3 ± 22.3	.69
Causes of graft loss, n (%)				
Death with a functioning graft chronic rejection	1 (33.3)	7 (77.8)	8 (62.5)	
Chronic rejection infection or sepsis	1 (33.3)	1 (11.1)	2 (15.3)	
Acute rejection	1 (33.3)	1 (11.1)	1 (7.7)	
Malignancy			1 (7.7)	
			1 (7.7)	
Patient loss, n (%)	2 (6.1)	7 (12.1)	11 (9.2)	.63 [a]
Time to death (months), n = 20	n = 2	n = 7	n = 11	
Mean ± SD	42.5 ± 7.5	31.1 ± 24.1	29.5 ± 22.2	.85

ABMR, antibody-mediated rejection; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; HLA, human leucocyte antigen; TCMR, T-cell-mediated rejection.
[a]Chi-square; [b]1-way ANOVA; [c]Kruskal–Wallis H. *The group from which the statistical difference originates; [+]statistically significant difference between marked groups.

antibody-negative group (63.0 vs. 74.0, respectively, *P* = .045), the groups were similar regarding eGFR at 1 and 3 years (Table 2).

As advanced recipient age, cadaveric donor, advanced donor age, lower pretransplant donor eGFR, and lower recipient eGFR at discharge were associated with poor graft survival,

Table 3. Comparison of Histopathological Results According to Anti-HLA Antibody Status (n = 43)

	DSA Positive (n = 8)	Non-DSA Positive (n = 14)	Anti-HLA Antibody Negative (n = 21)	*P*
Number of glomeruli, median (minimum–maximum)	13.5 (10-22)	13 (6-31)	12 (9-24)	.71[c]
Interstitial inflammation (i)				
i > 0, n (%)	6 (75.0)	12 (85.7)	18 (85.7)	.76[a]
Percentage of interstitial inflammation (%)				
Median (minimum–maximum)	40 (0-70)	30 (0-70)	40 (2-90)	.91[c]
Tubulitis (t)				
t > 0, n (%)	6 (75.0)	12 (85.7)	17 (81.0)	.82[a]
Intimal arteritis (v)				
v > 0, n (%)	3 (37.5)	2 (14.3)	5 (23.8)	.46[a]
Glomerulitis (g)				
g > 0, n (%)	8 (100.0)	9 (64.3)	17 (81.0)	.14[a]
Peritubular capillaritis (ptc)				
ptc > 0, n (%)	8 (100.0)	7 (50.0)*	19 (90.5)	**.004**[a]
C4d staining				
C4d = 0, n (%)	3 (37.5)	8 (57.1)	13 (61.9)	.49[a]
C4d > 0, n (%)	5 (62.5)	6 (42.9)	8 (38.1)	
Chronic transplant glomerulopathy (cg)				
cg > 0, n (%)	4 (50.0)	4 (28.6)	7 (33.3)	.59[a]
Mesengial matrix expansion (mm)				
mm > 0, n (%)	0 (0)	2 (14.3)	14 (66.7)*	**<.001**[a]
Interstitial fibrosis (ci)				
ci > 0, n (%)	3 (37.5)	8 (57.1)	9 (42.9)	.60[a]
Tubular atrophy (ct)				
ct > 0, n (%)	3 (37.5)	9 (64.3)	7 (33.3)	.18[a]
Total inflammation (ti)				
ti > 0, n (%)	8 (100.0)	12 (85.7)	17 (81.0)	.42[a]
Percentage of total inflammation (%)				
Median (minimum–maximum)	45 (15-70)	50 (0-90)	50 (0-100)	.84[c]
Interstitial fibrosis tubular atrophy (IFTA)				
IFTA > 0, n (%)	2 (25)	5 (35.7)	8 (38.1)	.80[a]
Thrombotic microangiopathy, n (%)	0 (0)	3 (21.4)*	0 (0)	**.035**[a]

DSA, donor specific antibody; HLA, human leucocyte antigen.
[a]Chi-square; [b]1-way ANOVA; cKruskal–Wallis H. *The group from which the statistical difference originates; [+]statistically significant difference between marked groups.

the advanced recipient and donor age and the presence of BKV infection were the only risk factors for graft failure in multivariate analysis [HR = 1.058, CI: 1.014-1.104 (*P* = .010); HR = 1.046, CI: 1.003-1.091 (*P* = .037); and HR = 2.827 CI: 1.165-6.861 (*P* = .022), respectively] (Table 4). Similarly, the advanced recipient and donor age and the presence of BKV infection were independent risk factors for patient survival [HR = 1.107, CI: 1.048-1.170 (*P* < .001); HR = 1.062, CI: 1.009-1.118 (*P* = .022); and HR = 3.970, CI: 1.440-10.949 (*P* = .008), respectively] (Table 5).

DISCUSSION

In this study, the effects of anti-HLA antibody status on graft and patient outcomes were investigated. We did not observe any differences in acute rejection, posttransplant infectious and

Table 4. Factors Affecting Graft Survival

	Univariate Analysis		Multivariate Analysis	
Graft Loss	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Recipient age	1.063 (1.025-1.102)	**.001**	1.058 (1.014-1.104)	**.010**
Living → cadaveric	4.740 (2.146-10.470)	**<.001**	2.694 (0.984-7.375)	.054
Donor age	1.050 (1.010-1.090)	**.013**	1.046 (1.003-1.091)	**.037**
Donor eGFR	0.971 (0.955-0.988)	**.001**	0.996 (0.977-1.015)	.68
BK virus (yes → no)	2.042 (0.902-4.627)	.087	2.827 (1.165-6.861)	**.022**
Urinary tract infection (yes → no)	2.012 (0.912-4.439)	.084	1.493 (0.570-3.912)	.41
Recipient's eGFR at discharge	0.979 (0.961-0.997)	**.023**	0.994 (0.976-1.013)	.54

eGFR, estimated glomerlar filtration rate; HR, hazard ratio.

noninfectious complications, and the patient survival among the study groups. Although median eGFR at 2 years was significantly lower in DSA-positive patients, graft survival was similar between anti-HLA antibody groups. It has been shown that not acute rejection episodes but advanced recipient and donor age and the presence of BKV infection negatively affect both graft and patient survival.

It has been known since the 1970s that the presence of anti-HLA antibodies before transplantation is a risk factor for ABMR and poor graft outcome.[12,13] Patients with high immunological risk receive stronger immunosuppressive therapies, including desensitization and induction therapy to minimize the negative effects of presensitization.[14] ATG and IVIG induction significantly reduced the frequency of biopsy-proven TCMR and active ABMR in the first 6 months in DSA-positive patients.[15] In the multicenter study with 10.694 living donor KTx recipients, 1-year graft and patient survival rates of CDC-XM and FC-XM-negative and Luminex-positive patients were similar to CDC-XM, FC-XM, and Luminex-negative patients, while the risk of graft loss and death increased in CDC-XM- or FC-XM-positive patients.[16] Although pretransplant DSA had no negative impact on graft survival in the setting of negative FC-XM, Kwon et al demonstrated that the risk of active ABMR increased and 1- and 3-year rejection-free graft survival rates decreased in recipients with pretransplant class II DSA MFI ≥5000 and negative FC-XM test.[17-18] In this study, patients who had desensitization treatment were excluded and only patients who had induction treatment with basiliximab were included, reasonably suggesting that the recipients at high risk of rejection need pretransplant desensitization or strong induction treatment. The University of Wisconsin shared its experience with Luminex-based desensitization protocols for living and deceased kidney donors.[19] Considering the strength of pretransplant DSA (MFI 101-500, 501-1000, and 1001-3000), recipients were stratified in 5 desensitization protocols, which included ATG induction, PP, and IVIG in the highest risk group. Even the incidence of ABMR and TCMR was higher in the desensitization group, rejection rates in highly sensitized recipients were lower than 25% and 1-year kidney function, graft and patient survival were similar to those without desensitization. We did not observe any significant difference between DSA groups for acute rejection, graft, or patient survival, apparently reflecting the successful utilization of more aggressive immunosuppression in high-risk recipients.

Table 5. Factors Affecting Patient Survival

	Univariate Analysis		Multivariate Analysis	
Patient Survival	**HR (95% CI)**	***P***	**HR (95% CI)**	***P***
Recipient age	1.097 (1.051-1.146)	**<.001**	**1.107 (1.048-1.170)**	**<.001**
Living → cadaveric	4.681 (1.935-11.324)	**.001**	2.069 (0.633-6.765)	.23
Donor age	1.059 (1.014-1.107)	**.010**	**1.062 (1.009-1.118)**	**.022**
Donor eGFR	0.971 (0.953-0.990)	**.003**	1.000 (0.976-1.024)	.99
BK virus (yes → no)	2.456 (1.003-6.016)	**.049**	**3.970 (1.440-10.949)**	**.008**
Urinary tract infection (yes → no)	2.416 (0.987-5.913)	.053	2.122 (0.662-6.797)	.21
Recipient's eGFR at discharge	0.977 (0.957-0.998)	**.030**	0.996 (0.973-1.019)	.73

eGFR, estimated glomerular filtration rate; HR, hazard ratio.

As we have found that acute rejection and graft survival were not affected by preformed anti-HLA antibodies, other factors such as complement-binding capacity and IgG subclasses of the DSAs and non-HLA antibodies (such as antibodies against angiotensin-type 1 receptors, anti-AT1R) may also help to refine the pretransplant stratification of rejection risk. C1q-binding and IgG3 posttransplant DSA were strongly associated with allograft failure.[20,21] Pretransplant anti-AT1R have been shown to increase the risk of ABMR, allograft failure, and microvascular inflammation.[22,23] Pretransplant serum concentrations of the soluble CD30 molecule, a member of the tumor necrosis factor receptor superfamily, have also been shown as predictive markers for tubulitis and ABMR in the graft.[24,25]

An increased risk of infectious complications is expected in these patients due to overimmunosuppression. Kim et al determined that CDC-XM-/FC-XM-positive and CDC-XM-negative/FC-XM-positive recipients, who had undergone pretransplant desensitization, experienced higher rates of urinary tract infections, bacteremia, intraabdominal infections, herpes zoster, *Pneumocystis jirovecii* pneumonia, and cytomegalovirus (CMV) viremia than CDC-XM-/FC-XM-negative controls.[26] In a series of 254 KTx recipients, pretransplant DSA greater than 500 MFI and ATG induction were associated with a greater risk of BKV or CMV infection.[27] We found no association between DSA groups and infectious complications. Clinicians should tailor the immunosuppressive therapy to balance side effects such as the risk of allograft rejection, infection, and malignancy.

Loupy et al evaluated kidney allograft biopsies of 1016 recipients. Microvascular inflammation (g > 0 and/or ptc > 0), C4d staining, transplant glomerulopathy (cg > 0), interstitial inflammation, and tubulitis (i > 0 and t > 0) were more common in DSA-positive patients than DSA-negative patients. As patients with complement-binding DSA had the highest risk of graft loss, interstitial fibrosis and tubular atrophy (IFTA) score >2 and coexistence of glomerular and peritubular inflammation and transplant glomerulopathy (g > 0 + ptc > 0 + cg > 0) were also risk factors of graft loss (HR = 2.22 and HR = 2.26, respectively).[21] Glomerulitis (g), ptc, cg, and IFTA scores were higher in DSA-positive recipients both at 3 months and 1-year protocol biopsies.[28] Subacute ABMR at 3 months was related to worse kidney functions at 1 year. In our study, the biopsies from DSA-positive recipients showed higher ptc scores than nDSA-positive recipients but similar to anti-HLA antibody-negative recipients. Recipients with microvascular inflammation (g > 0 and/or ptc > 0) in anti-HLA antibody-negative group may be related to non-HLA antigens. It is known that C4d staining has 95% sensitivity and 96% specificity for the presence of DSA.[29,30] Probably due to the fact that the biopsies in our study were not protocol biopsies and only 21 of 38 patients suspected of acute rejection had PRA, we found no difference in C4d staining between the DSA groups. The similarity of acute and chronic histopathologic changes between DSA groups may have led to similar graft function and survival in our study.

Similar to European and US data, we established that older KTx recipients have significantly reduced graft and patient survival compared to younger recipients.[28,31] Five-year patient survival was 75% in KTx recipients aged 30-49 years, whereas only 61% for those over 65 years of age.[32] In a cohort of 99 860 KTx recipients, the risk of delayed graft function and graft failure was increased with advanced donor age.[33] In our study, 35.5% of donors were aged over 55, and increased donor age decreased graft and patient survival in multivariate analyses.

We demonstrated no relationship with opportunistic infections and DSA groups, but BKV infection was an independent risk factor for both graft and patient loss. Vasudev et al showed that biopsy-proven BKV nephropathy was a complication that occurred at a median time of 11 months after KTx and caused graft loss in 46% of recipients.[34] However, early BKV detection, with the implementation of routine screening protocols, is essential to prevent graft dysfunction and loss.[35] The cornerstone of BK viremia management remains the reduction of immunosuppressive medications without triggering rejection. Since it has been shown that persistent BK viremia was associated with the development of class II de novo DSAs,[36] the immunological risk of the patient has to be considered in the adjustment of immunosuppression.

Our study had several limitations. First, its retrospective design may cause selection and information bias. Second, the study cohort consisted of a limited number of recipients with heterogeneous characteristics and a short follow-up period. Extending the follow-up period will allow a better evaluation of survival analyses and complications. Third, our center does not perform protocol biopsies. Fourth, our center does not routinely perform HLA typing for DRB3/4/5 and DP antigens and DSA against these antigens and monitor posttransplant DSA which can affect clinical outcomes after KTx.

In summary, the data suggest that pretransplant DSA should not be considered as an obstacle for KTx. A detailed evaluation of the immunological risk before transplantation and individualization of immunosuppressive treatment will be effective in the success of graft and patient survival.

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