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Preformed Donor-specific Antibodies Against HLA Class II and Graft Outcomes in Deceased-donor Kidney Transplantation

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Background. Many kidney transplant centers in the United States report both HLA class I and II antibodies detected by sensitive solid-phase assays (SPAs) to United Network for Organ Sharing as unacceptable antigens, significantly reducing the compatible donor organ pool and prolonging waiting time for highly sensitized patients. However, the clinical relevance of all detected donor-specific antibodies (DSAs) by SPA is not unequivocal, because fluorescence intensity does not always accurately reflect antibody pathogenicity. Our center does not exclude patients from transplantation based on DSA class II.

Methods. We performed a retrospective analysis in 179 deceased-donor kidney transplant recipients with solely DSA class II before transplant and patients without DSA and compared graft survival, rejection, and clinical outcomes. Patient survival was also compared with matched controls on the waiting list. **Results.** Patients transplanted with DSA class II showed a clear survival benefit compared with matched patients who remained on dialysis or were waitlisted on dialysis/transplanted at 5 years (100%, 34%, and 73%, respectively). After a mean follow-up of 5.5 years, there was no significant difference in death-censored graft survival between transplanted patients without DSA and those with preformed DSA class II (adjusted HR 1.10; 95% confidence interval, 0.41–2.97), although the incidence of rejection was higher in recipients with DSA class II (adjusted HR 5.84; 95% confidence interval, 2.58–13.23; $P < 0.001$). Serum creatinine levels at 1, 3, and 5 years posttransplant did not differ between groups. No predictors of rejection were found, although patients who received basiliximab induction therapy had higher incidence of rejection (100%) compared with those who received antithymocyte globulin (52%). **Conclusions.** We conclude that for highly sensitized patients, deceased-donor kidney transplantation with DSA class II yields a survival benefit over prolonged waiting time on dialysis. Instead of listing DSA class II as unacceptable antigens, an individual approach with further immunologic risk assessment is recommended.

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While over 100 000 patients are currently on the waiting list for deceased-donor kidney transplantation, only ~20 000 kidney transplants are performed yearly in the United States.¹ The presence of anti-HLA antibodies significantly decreases the compatible donor organ pool and prolongs waiting time, especially for patients with a panel-reactive antibody (PRA) titers above 80%. Although the novel allocation policy improved the allocation for these highly sensitized patients,² Gebel et al³ showed that still 25% of patients who are 100% sensitized would not get an offer for a deceased-donor kidney. Therefore, it remains important to critically reevaluate factors that determine immunologic risk before transplant and to avoid exclusion of potential donors due to nonsignificant anti-HLA antibodies.

The complement-dependent cytotoxic crossmatch (CDC-XM) has been the gold standard test to determine immunologic risk since its discovery in 1969.⁴ Development of more sensitive solid-phase assays (SPAs) such as Luminex multiplex arrays enabled the assessment of individual anti-HLA antibodies and the detection of HLA donor-specific antibodies (HLA-DSAs). After its implementation, multiple studies described an adverse effect of DSA before transplant on graft outcome, reviewed in the study by Mohan et al,⁵ which led to the transition toward a conservative protocol regarding pretransplant DSAs in most transplant centers in the United States. Although SPAs unquestionably enhanced the assessment of donor-recipient compatibility, the clinical relevance of all detected DSAs is not unequivocal.⁶ The median fluorescence intensity (MFI) output from SPAs does not always accurately reflect antibody strength or pathogenicity,⁷ which leads to a potential risk of incorrect exclusion of possible kidney donors. Despite this risk, advancements in HLA-incompatible transplantation^{8,9} and improved available treatments for antibody-mediated rejection (AMR),¹⁰ both DSA class I and II are usually reported to United Network for Organ Sharing (UNOS) as unacceptable antigens, excluding any offers with these antigens to reach the patient. For highly sensitized patients, this means that their chance of receiving a kidney offer remains low (lower pool of compatible donors through virtual crossmatch), and prolonged time on dialysis awaits.

Based on the differential expression of HLA class I and II antigens in the donor kidney^{11,12} and the deletion of majority of HLA class II-expressing donor antigen-presenting cells within a few days after transplantation,^{13,14} our center does not exclude potential kidney transplant recipients solely based on the presence of DSA class II at the time of deceased-donor transplant. Therefore, we are able to retrospectively evaluate graft survival, rejection, and function in patients who were transplanted with DSA class II before transplant.

MATERIALS AND METHODS

Patient Population

Between August 2007 and February 2015, 191 patients with ESRD received a deceased-donor kidney transplant at Brigham and Women's Hospital (BWH) in Boston. All patients were retrospectively screened for reported DSA in their most recent serum before transplant. Patients with DSA class II and patients without DSA were included in this study, while patients who tested positive for DSA class I were excluded. A flow diagram of the study population is

shown in Figure S1 (SDC, <http://links.lww.com/TXD/A204>). All patients were followed up to December 2017. Our study was reviewed and approved by the ethical committee of the Partners Human Research Committee at the Brigham and Women's hospital in Boston. Informed consent was waived because of the retrospective nature of the study, and analysis was performed with anonymous clinical data.

As a prerequisite for transplantation, all patients had a negative T-cell CDC-XM before transplantation. B-cell CDC-XM and T- and B-cell flow crossmatches (FCXMs) were not routinely performed.

The choice of induction therapy, antithymocyte globulin (ATG) or basiliximab (Table 1), was based on the recipient's characteristics, including age, history of chronic infection, prior cancer, and immunological risk. Standard maintenance immunosuppressive regimen consisted of tacrolimus, mycophenolate mofetil, and prednisone. Decisions regarding patient management were made by the caring clinician.

Patients' characteristics, estimated glomerular filtration rate, dipstick urinalysis, graft/patient outcomes, and results of biopsies were obtained by the review of patients' charts.

To assess whether patients transplanted with DSA class II had a survival benefit over dialysis, we performed a matched control analysis, based on BWH center-specific Organ Procurement and Transplantation Network data as of July 9, 2018. We matched all DSA class II patients with 1 patient on the waiting list who remained on dialysis and 1 patient who remained on dialysis or received a transplant, based on PRA (100%: exact match; 98%–99%: match in same range; 95%–97%: match in same range; 85%–94%: match within 2 points; 65%–84%: match within 5 points, <65%: match within 10 points), age at last waitlisting (± 5 years), and time on dialysis since last initiation (with date shifting to account for differences in follow-up). Afterward, the best match was based on (in order of priority) diabetes status, race, and gender. Two DSA class II-positive patients could not be matched well because they were 20 and 23 years old at waitlisting and had 9 and 10 years of dialysis before transplant with a PRA of 90% and 100%, respectively. Their best matches in the dialysis only group were eventually a 29-year-old with a PRA of 85% and a 31-year-old with a PRA of 100%, respectively. In the dialysis or transplant group, only the 23-year-old had a diverged match and was matched with a 15-year-old with a PRA of 100%.

HLA Typing and Detection of DSA

HLA typing and assessment of anti-HLA antibodies in patients awaiting kidney transplantation was performed by the Tissue Typing Laboratory at BWH. HLA-A/B/DR/DQ typing was primarily done by serological methods. If HLA typing was obtained by molecular techniques, conversion to serologic equivalents was performed.

The presence of anti-HLA antibodies was assessed by LABScreen Mixed (One Lambda Inc., Canoga Park, CA), analyzed on a Luminex platform. In the event of a positive assay, this was followed by LABScreen Single Antigen Class I/Class II (One Lambda Inc.). A normalized MFI ≥ 3000 for class I or ≥ 1000 for class II is considered positive at our center. Until 2012, posttransplant anti-HLA antibody assessment was only performed in the case of clinical indication (eg, elevation of creatinine and for-cause clinical biopsy). Thereafter, surveillance anti-HLA antibody testing was performed in the first

TABLE 1.
Patient characteristics

Variable	DSA class II negative (n = 148)	DSA class II positive (n = 31)	P
Recipient			
Follow-up, y (mean ± SD)	5.5 ± 2.5	5.7 ± 2.1	0.88
Age, y (mean ± SD)	55 ± 14	48 ± 15	0.01
No. female (%)	56 (38)	19 (61)	0.03
Race, n (%)			0.59
White	67 (45)	12 (39)	
African American	55 (37)	15 (48)	
Hispanic	13 (9)	3 (10)	
Asian	13 (9)	1 (3)	
Cause ESRD, n (%)			0.80
Diabetes mellitus	43 (29)	6 (19)	
Hypertension	9 (6)	2 (6)	
Glomerulonephritis	39 (26)	9 (29)	
Polycystic kidney disease	16 (11)	3 (10)	
Other/unknown	41 (28)	11 (35)	
Previous Tx, n (%)			<0.001
None	132 (89)	13 (42)	
1	11 (7)	17 (55)	
2 or more	5 (3)	1 (3)	
PRA, % (mean ± SD)			<0.001
Class I	14 ± 29	57 ± 42	
Class II	9 ± 24	78 ± 22	<0.001
Donor			
Type of donor, n (%)			0.40
SCD	59 (40)	15 (48)	
ECD	35 (24)	4 (13)	
DCD	54 (36)	12 (39)	
Cold ischemia time, h (mean ± SD)	14.42 ± 6.9	12.52 ± 4.4	0.16
HLA-A/B/DR/DQ mismatch (mean ± SD)	6.0 ± 1.9	6.3 ± 0.9	0.96
Delayed graft function, n (%)	72 (49)	17 (55)	0.56
Induction therapy, n (%)			0.28
Antithymocyte globulin	103 (70)	25 (81)	
Basiliximab	45 (30)	6 (19)	

Statistically significant values are indicated in bold font.

DCD, donor after circulatory death; DSA, donor-specific antibody; ECD, expanded-criteria donor; ESRD, end-stage renal disease; HLA, human leukocyte antigen; PRA, panel-reactive antibody; SCD, standard-criteria donor; SD, standard deviation.

week after transplantation, though further testing was driven by clinical indication.

Eplet Mismatch Calculation

Low-resolution HLA haplotypes from recipients and donors—HLA-A, B, C, DRB1, and DQB1, determined by serology—were converted into high-resolution alleles using The National Marrow Donor Program's HaploStats Application (www.haplostats.org). The genotype-phased tool uses HLA haplotype frequency information from different countries and ethnically specific populations to determine the most likely alleles of the specified HLA antigen. DRB3/4/5 and DQA1 alleles were determined using EpViX software (www.epvix.com.br), which is based on population frequency databases from The National Marrow Donor Program and the international ImmunoGeneTics information system.

Eplet mismatch was calculated using HLA-Matchmaker software (HLAMatchmaker DRDQDP Eplet Matching version 2.1, www.epitopes.net). Total DR-DQ eplet mismatch

was considered as the sum of DRB1, DRB3/4/5, DQA1, and DQB1 eplet mismatches. For DR eplet mismatch, the number of DRB1 and DRB3/4/5, and for DQ eplet mismatch, the number of DQA1 and DQB1 eplet mismatches was aggregated.

Diagnosis of Acute Rejection

All reported episodes of rejection were biopsy proven. The need for biopsy was determined by the caring clinician, generally after a rise in creatinine ≥ 0.3 mg/dL. Biopsies were reviewed by the Department of Pathology of BWH and graded according to the Banff 2007 classification.¹⁵ Acute AMR was diagnosed when at least 2 of the following characteristics were present: positive C4d staining in peritubular or glomerular capillaries, circulating DSA, and histopathologic changes consistent with AMR.¹⁵ Borderline changes according to the Banff 2007 classification were not considered as acute rejection.

Treatment of Acute Rejection

Patients diagnosed with acute cellular rejection were treated with pulse methylprednisolone 500 mg IV daily for 3 days and in the case of nonresponse, ATG. Acute AMR was treated with pulse methylprednisolone 500 mg IV daily for 3 days and 5 sessions of plasmapheresis followed by intravenous immunoglobulin (IVIg, 100 mg/kg) every other day. In patients with significant kidney dysfunction (glomerular filtration rate decrease $>50\%$), bortezomib 1.3 mg/m² in 4 doses over 10 days was added to the treatment. In the case of no improvement (persistent AMR, persistent positive crossmatch, or failure of DSA to decrease $>50\%$), another 5 sessions of plasmapheresis followed by IVIg were given, combined with a single dose rituximab 375 mg/m².

Statistical Analysis

Statistical analysis was performed using Stata software (StataIC-15, StataCorp LLC). For categorical data, Fisher exact test was used. Continuous data were plotted to assess normal distribution and, when confirmed, analyzed by *t*-test. For nonparametric data, Mann-Whitney *U* test was used. Survival analysis was performed by Kaplan-Meier analysis with statistical difference calculated using the log-rank test. Cox-proportional hazards was used to determine hazard ratios (HRs) and tested using martingale residuals. Tests were 2-sided, and *P*-values <0.05 were considered statistically significant.

RESULTS

Patient Characteristics

Among the 179 patients who were included in this study, 31 patients (17%) had DSA class II in their last pretransplant serum, whereas 148 patients (83%) had no DSA. The mean follow-up was 5.5 and 5.7 years in the DSA-negative and DSA-positive groups, respectively. Recipient and donor characteristics are shown in Table 1. Patients were significantly younger in the DSA class II-positive group ($P = 0.01$), and as expected, the number of females ($P = 0.02$), the number of patients with previous transplants ($P < 0.0001$), and the class I and class II PRA (both $P < 0.0001$) were higher in patients with the DSA class II compared with the no DSA group. There was no significant difference in race, cause of ESRD, type of donor, cold ischemia time, HLA-A/B/DR/DQ mismatch,

delayed graft function, or induction therapy between the DSA class II-negative and -positive patients (Table 1).

Presence of DSA Class II Before Transplant and Survival

Compared with a matched control group of patients who remained on dialysis, receiving a kidney with DSA class II clearly showed a survival benefit over staying on dialysis. Our DSA class II cohort had a 5-year survival of 100%, compared with 34% survival in matched patients who stayed on dialysis and 73% survival in patients who were waitlisted on dialysis/transplanted during that time period (Figure 1). Throughout our study, 23 patients (16%) in the transplanted DSA class II-negative group died with a functioning graft, compared with 2 patients in the DSA class II-positive group (6%).

Graft failure occurred in 25 DSA class II-negative (17%) and in 7 DSA class II-positive patients (23%). Graft failure in patients without DSA was due to a variety of causes (Table S1, SDC, <http://links.lww.com/TXD/A204>). Graft loss in patients with DSA class II was primarily caused by chronic AMR (4 patients), followed by acute humoral rejection, glomerular disease, and allograft thrombosis.

Kaplan-Meier analysis showed no difference in uncensored ($P = 0.86$, data not shown) or death-censored graft survival ($P = 0.38$, Figure 2) between DSA class II-negative and DSA class II-positive patients, with a death-censored 5-year graft survival of 84% and 80%, respectively. We adjusted the outcomes for age at transplant, prior transplants, and gender

(Table 2), because these variables differed between groups in the univariate analysis. Adjusted HR of graft failure was 1.10 (95% confidence interval [CI], 0.41–2.97; $P = 0.86$); however, power is reduced due to our small sample size. Cold ischemia time, donor type, and induction therapy could be important confounders, but due to limited sample size, these variables could not be added to the Cox regression. Therefore, we applied another model to assess HR for graft loss with preformed DSA class II, adjusted for cold ischemia time, donor type, induction therapy, and each of these variables combined with age and gender (data not shown). None of the HR was statistically significant.

Incidence of Rejection in Patients with DSA Class II Before Transplant

Twenty-two patients (15%) without DSA before transplant developed at least 1 episode of rejection during the follow-up (Figure 3A). Significantly more rejection occurred in patients with preexisting DSA class II (19 patients [61%]) ($P < 0.0001$), with an adjusted HR of 5.84 (95% CI, 2.58–13.23; Table 2). In addition, a significant difference was observed in the type of the first episode of rejection between DSA-negative and DSA-positive patients ($P < 0.0001$) (Figure 3B). Cellular rejection was the main type of rejection in DSA class II-negative patients (15 out of 22 patients), while AMR and mixed rejection were predominant in the DSA class II-positive group with 9 and 9 out of 19, respectively. Timing of the first rejection, biopsy scores, and treatment of AMR

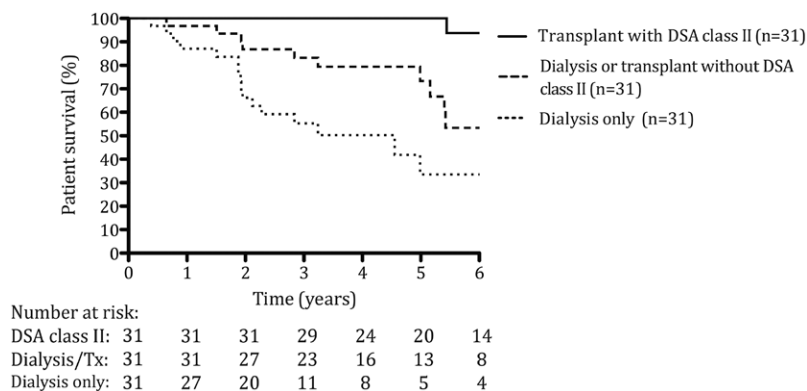


FIGURE 1. Kaplan-Meier survival analysis of patients transplanted with DSA class II compared with 2 matched control groups of patients who remained on dialysis (dialysis only) or who were waitlisted on dialysis and could receive a compatible kidney transplant (dialysis or transplant). DSA, donor-specific antibody; Tx, transplant.

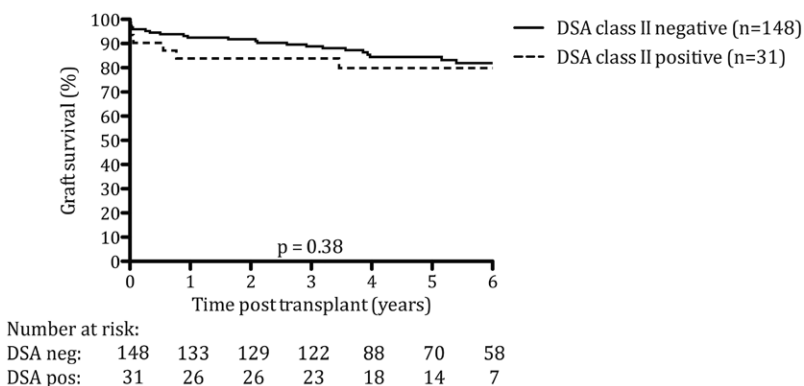


FIGURE 2. Kaplan-Meier death-censored graft survival of recipients without DSA before transplant and recipients with preformed DSA class II. DSA, donor-specific antibody.

TABLE 2.**Adjusted hazard ratios of renal transplant outcomes with preformed DSA class II**

Transplant outcome	Model	Hazard ratio (95% CI) ^a	P
Graft failure	Unadjusted	1.45 (0.62-3.36)	0.39
	Adjusted ^b	1.10 (0.41-2.97)	0.86
Rejection	Unadjusted	6.08 (3.28-11.27)	<0.001
	Adjusted ^b	5.84 (2.58-13.23)	<0.001

^aHazard ratio for outcome with the presence of DSA class II before transplant.

^bAdjusted for age at the time of transplantation, number of prior transplants (0, ≥1), and gender. Statistically significant values are indicated in bold font.

CI, confidence interval; DSA, donor-specific antibody.

in DSA class II-positive patients are shown in Table 3. Most patients with AMR (67%) had their first rejection within the first half-year of transplant. Patients with early AMR who received additional treatment such as Eculizumab, Rituximab, or Bortezomib on top of the standard treatment of plasmapheresis/IVIG did not experience graft loss, while late AMR was usually accompanied by irreversible chronic changes.

DSA Class II Characteristics and Rejection Development

Since not all patients with DSA class II developed rejection, DSA class II-positive patients were divided into subgroups of DSA to assess predictors of rejection (Figure 4). We did not find a difference in rejection-free survival between patients who had 1 DSA before transplant compared with patients with multiple DSAs (Figure 4A), with an unadjusted HR of 1.13 (95% CI, 0.45–2.82; $P = 0.80$). Although Kaplan-Meier curves of sum MFI, divided into low sum MFI (<8000) and high sum MFI (≥8000) seem to separate, with our limited sample size we do not have enough power to detect a

significant difference (Figure 4B) (unadjusted HR 1.27; 95% CI, 0.78–2.08; $P = 0.97$). Similarly, there was no difference in rejection incidence between patients who had solely DR or DQ DSA, or both (data not shown). Finally, a comparison between the sum MFI of DR or DQ DSA between patients who rejected compared with those that did not also failed to show a difference (Figure 4C).

Eplet Mismatch and Rejection Development

Analysis of antibody specificity from highly sensitized patients using the epitopes instead of HLA antigens may allow better understanding of antibody reactivity observed following a sensitizing event, though it is unclear if it may predict a worse outcome in sensitized patients before transplant. Results on eplet mismatches are shown in Table 4. There was no difference in total, DR, or DQ eplet mismatch between patients who developed rejection and patients without rejection ($P = 0.98$, $P = 0.73$, and $P = 0.66$, respectively). In addition, the number of eplet mismatch did not predict graft loss.

Basiliximab Induction and AMR in DSA Class II-positive Patients

T-cell depletion induction therapy is the preferred choice in high-immunological risk patients, though sometimes the history of prior cancer, severe infection, or older age may change the induction choice from ATG to basiliximab. Six patients in the DSA class II-positive group received basiliximab for induction therapy, whereas 25 patients received ATG. Excluding 2 patients who immediately lost their graft due to nonimmunological causes (both received ATG for induction), all 6 patients who received basiliximab developed rejection (100%), compared with 13 out of 23 patients who developed rejection in the recipients who received ATG (57%; $P = 0.07$).

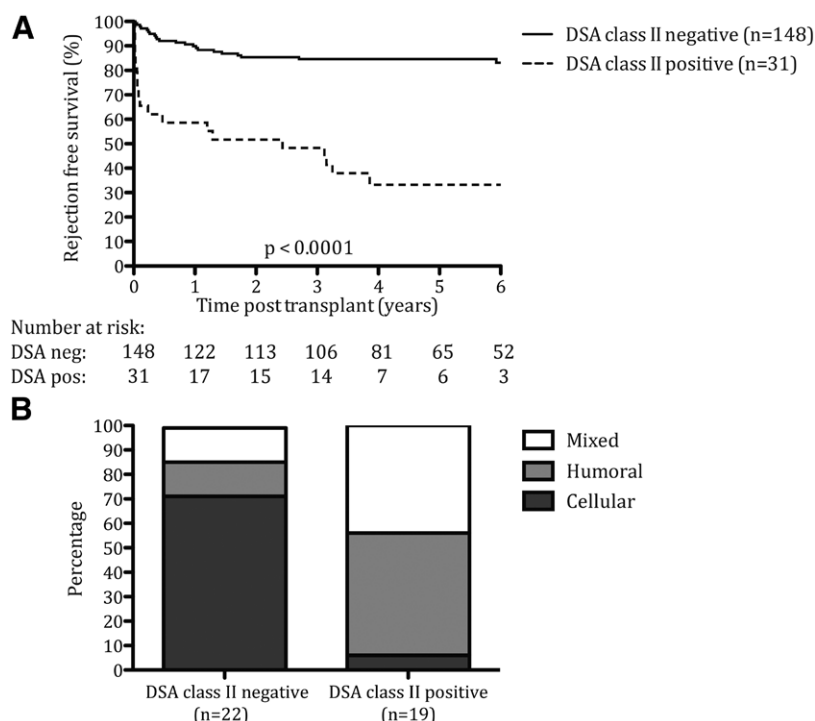


FIGURE 3. Incidence and type of rejection in patients with and without DSA class II. Rejection-free Kaplan-Meier survival analysis of recipients with preformed DSA class II compared with patients without DSA class II (A). Percentage of type of rejection in DSA class II-negative and DSA class II-positive recipients (B). DSA, donor-specific antibody.

TABLE 3. Time to rejection, biopsy scores, treatment, and outcome of patients with preformed DSA class II and posttransplant AMR

Patients	Time to rejection, mo	t	i	g	ah	v	c4d	ptc	cg	ci	ct	cv	DSA at rejection	Treatment	GF, years posttransplant
1	0.2	0	2	3	0	0	2	3	0	0	0	0	Y	S, PP, IVIG	Y (0.06)
2	0.2	2	2	2	2	?	0	1-2	1-2	1	1	2	Y	S, PP, IVIG	Y (0.55)
3	0.3	0	3	1	2	0	3	3	0	0	1	2	Y	S, PP, IVIG	Y (0.77)
4	37	1	1	1	3	0	0	1	2	3	3	3	Y	S ^a	Y (3.46)
5	38	0-1	1	3	1	0	0	1	3	1-2	1-2	2	U	S ^a	Y (6.71)
6	0.3	0	1	1-2	0	0	0	3	1	0	0	0	N	S, PP, IVIG ^c	N
7	0.3	1	1	1	2	1	3	2	0	1	1	2	Y	S, PP, IVIG	N
8	0.4	2	2	0	0	0	3	0-1	0	0	0	0	Y	S, PP, IVIG, BTZ, RTX	N
9	0.6	0-1	0-1	1	2	2	0	2	0	1-2	1	2-3	N	S, PP, IVIG, BTZ, RTX	N
10	0.8	1	1-2	0-1	0-1	0	3	1-2	0	0	0	0-1	Y	S, PP, IVIG	N
11	0.9	1	0-1	0	1	0	0	2	0	0	0-1	1-2	Y	S, PP, IVIG, BTZ, ECZ	N
12	1	0	0	1	0	0	3	1-2	0	0	0	0	Y	S, PP, IVIG	N
13	2	0-1	1	0	2	0	0	0-1	1	2	2	2	Y	S, PP, IVIG	N
14	5	1-2	1	1	2		2	1-2					N	S ^b	N
15	15	2	2	2	2-3	0	3	1-2	2-3	2	2-3	2-3	Y	S, PP, IVIG	N
16	29	0	0-1	2	1	0	0	2	2	1	1	3	N	IVIG	N
17	37	1	0-1	2	3	0	3	2	1	1-2	1-2	2	Y	S, PP, IVIG, BTZ	N
18	46	2	2	0	2	1	3	1-2	0	3	2-3	2	Y	S, PP, IVIG, BTZ	N

^aBiopsy showed severe transplant glomerulopathy, no additional treatment was given.

^bVery limited biopsy, suspicious for AMR, therefore treatment with steroids. Another biopsy 2 weeks later showed no rejection, no further treatment.

^cAt the time of biopsy no DSA, but because of pretransplant DSA and pathology scores, it was considered AMR and treated as described.

ah, arteriolar hyaline thickening; AMR, antibody-mediated rejection; BTZ, Bortezomib; cg, allograft glomerulopathy; ci, interstitial fibrosis; ct, tubular atrophy; cv, fibrous intimal thickening; DSA, donor-specific antibody; ECZ, Eculizumab; g, glomerulitis; GF, graft failure; i, interstitial inflammation; N, No; PP, plasmapheresis; ptc, inflammation of peritubular capillaries; S, Steroid; t, tubulitis; Y, Yes; v, intimal arteritis.

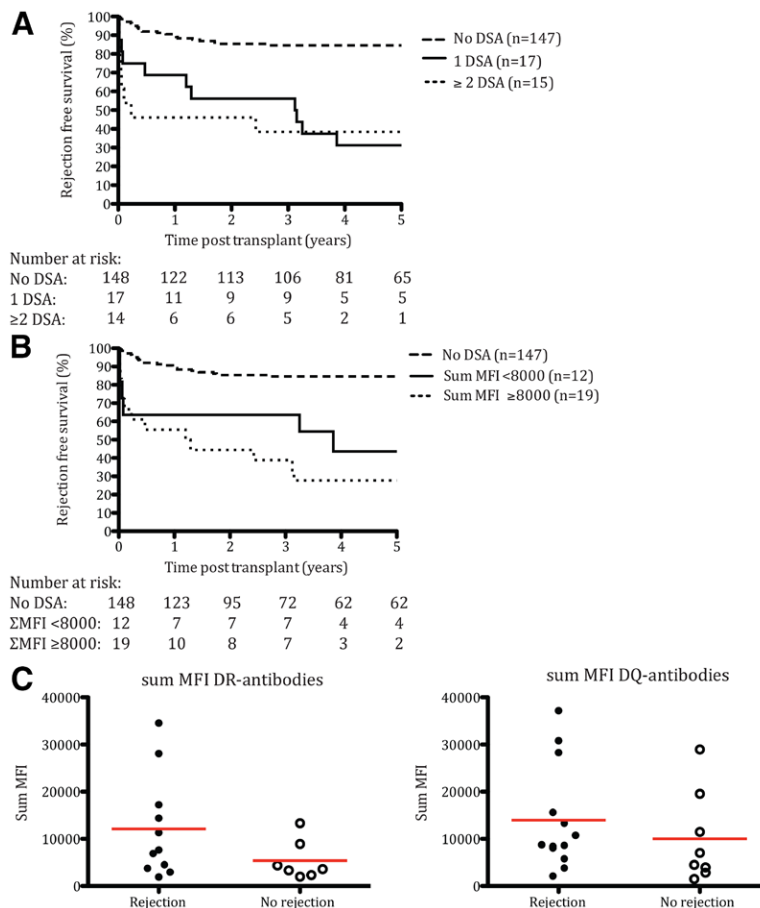


FIGURE 4. Rejection and DSA class II characteristics. Rejection-free survival analysis of deceased-donor recipients according to (A) number of DSA class II and (B) sum MFI of DSA class II. C, Comparison of sum MFI of HLA-DR (DR) and -DQ (DQ) DSA between patients who underwent at least 1 rejection episode (rejection) compared with those who did not reject (no rejection). DSA, donor-specific antibody; MFI, median fluorescence intensity.

TABLE 4.**Eplet mismatch in patients with DSA class II before transplant, with and without episode of rejection**

ePmm, mean \pm SD	Rejection, n = 19	No rejection, n = 10	P
Total	47 \pm 17	47 \pm 21	0.98
DR	20 \pm 14	20 \pm 9	0.73
DQ	25 \pm 9	27 \pm 14	0.66

DSA, donor-specific antibody; ePmm, eplet mismatch.

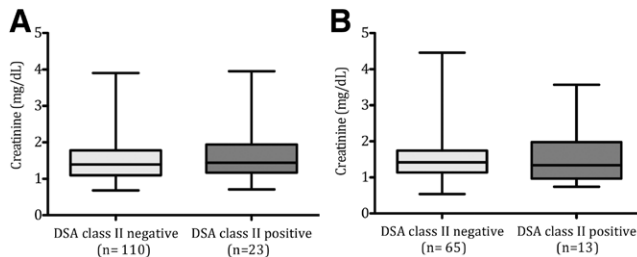


FIGURE 5. Graft function of patients transplanted with and without DSA class II. Creatinine levels at 3 (A) and 5 years (B) posttransplant. DSA, donor-specific antibody.

Anti-HLA Testing Early Posttransplantation

Posttransplant assessment of anti-HLA antibodies was performed in 27 DSA class II-positive patients, because 2 patients had immediate graft failure and 2 other patients who were transplanted before 2012 did not have a clinical indication for anti-HLA antibody assessment. Among these 27 patients with preformed DSA, 5 patients were DSA negative after transplantation. Remarkably, 4 of these patients developed rejection (80%). Preformed DSA persisted posttransplant in 12 patients, out of whom 6 developed rejection (50%). Ten patients developed de novo DSA in addition to their preexisting DSA, while 9 patients in this group developed rejection (90%).

Allograft Function in Patients with Preformed DSA Class II

One-, 3-, and 5-year posttransplant allograft functions were assessed by serum creatinine level in patients with a functioning graft. Allograft function did not differ between preformed DSA class II-negative and -positive patients (Figure 5). One year posttransplant, 129 DSA class II-negative patients had a mean creatinine level of 1.53 ± 0.57 mg/dL, compared with a mean of 1.67 ± 0.88 mg/dL in 26 DSA class II-positive patients ($P = 0.94$). Three years posttransplant, mean creatinine values were 1.49 ± 0.53 and 1.64 ± 0.75 mg/dL, respectively ($P = 0.54$). At 5 years posttransplant, mean creatinines were 1.57 ± 0.78 mg/dL in DSA class II-positive and 1.51 ± 0.62 mg/dL in DSA class II-negative patients ($P = 0.94$).

DISCUSSION

In this study, we investigated medium-term graft survival, episodes of rejection, and clinical outcomes after deceased-donor kidney transplantation in patients who were transplanted with preformed DSA class II. We found a clear survival benefit in patients transplanted with DSA class II over matched control patients who stayed on dialysis, or patients

who remained on dialysis or received a transplant. Comparing patients transplanted with DSA class II with patients transplanted without DSA before kidney transplantation, surprisingly, we did not find a significant difference in graft survival, although the incidence of rejection was 4 times higher in DSA class II-positive patients, mainly because of AMR. In patients with a functioning graft, no difference was found in creatinine levels between DSA class II-negative and -positive patients at 1, 3, and 5 years posttransplant.

The assessment of separate classes of DSA is of interest because of the difference in HLA antigen expression in the donor kidney. HLA class I is expressed on every nucleated cell, including renal tubular and endothelial cells, which are major cell targets of rejection in the kidney. HLA class II is traditionally only found on antigen-presenting cells or, in stress or inflammatory situations, on proximal tubule cells and kidney microvascular endothelium, while large renal vessels are usually devoid of HLA class II molecules.^{11,12} Thus, injury that can be incurred by antibodies against different classes have different pathophysiologic mechanisms, and outcomes and should be assessed separately.

The novel ability to assess individual anti-HLA antibodies and DSA before transplantation was followed by multiple studies that showed worse graft outcomes in patients transplanted with preformed DSA. A meta-analysis of 7 retrospective cohorts in 2012 showed an almost doubled risk of AMR and an increased risk of graft failure in patients who were DSA-positive.⁵ DSA class I and class II were not separated in most of these studies. Because of this reported increased risk, most transplantation centers in the United States started reporting both DSA class I and class II to UNOS as unacceptable antigens and have not changed these protocols since, despite newer studies that provided nuances regarding subtype of DSA. Caro-Oleas et al¹⁶ suggested a more important role of preexisting DSA class I in graft failure, as patients with only DSA class I or patients who had both classes of DSA had worse graft survival compared with patients with only DSA class II. A recent large study in the Netherlands with subanalysis of 3237 deceased-donor transplantations and 187 patients with DSA class II before transplant showed a similar observation, where in the first 5 years after transplant, mainly preformed DSA class I or both classes had an unfavorable effect on graft survival.¹⁷ Other studies conversely reported DSA class II to be more detrimental compared with class I,^{18,19} or solely found a reduced graft survival in patients that had DSA against both HLA classes.^{20,21} Unfortunately, the comparison of studies is difficult because of disparities between MFI cutoff values, immunosuppression therapy, study population, and follow-up. In general, however, death-censored 5-year graft survival for patients with preformed DSA class II in our study and these studies is above 75%. Because the alternative for highly sensitized patients is often not transplant, but prolonged waiting time on dialysis,³ transplantation with DSA class II might be a better option for selected patients, in particular for those with elevated PRAs. Comparing our DSA class II-positive cohort with patients on dialysis, we indeed found a reduced mortality rate even if the option of receiving a transplant was taken into account, which is in accordance with the survival benefit found in studies on HLA-incompatible live donors.⁸ To avoid the increased risk of AMR, it is important to assess in each individual patient how likely the chance is that they will receive another kidney

offer within reasonable time, compared with their risk of further deterioration on dialysis. Caution should also be exercised when high titers of DSA class II antibodies are detected. HLA class II antigens can be expressed by the endothelium and tubules of the donor kidneys under inflammatory conditions,¹¹ which in combination with high DSA class II levels could possibly lead to hyperacute rejection.

We found a 4-fold increase in rejection in patients with preformed DSA class II, with an increased incidence in the first 6 months after transplant. Interestingly, it is often described that AMR has an adverse effect on graft survival and clinical outcomes,²²⁻²⁴ which does not reflect our results on 5-year graft survival. This difference may partially be explained by timing and intensity of treatment of AMR. During a part of the previously mentioned studies, treatments such as plasmapheresis, rituximab, and bortezomib were not yet implemented routinely. Over the past decade, these treatments have improved outcomes after AMR,^{25,26} in particular for early AMR post-transplant.²⁷⁻³¹ The difference in timing of AMR may play an important role in our survival outcomes, because most of the AMR episodes occurred in the first 6 months after transplant. Patients with early AMR who were aggressively treated with Bortezomib, Eculizumab, or Rituximab in addition to plasmapheresis/IVIG/steroids did not experience graft loss. Biopsies of patients with late AMR (>3 years after transplant) showed in most cases irreversible chronic changes. Another reason for the good survival outcomes despite the high rate of AMR could be our follow-up time, which might be not long enough for a significant difference in graft survival to emerge.¹⁷ Nonetheless, clinical outcomes 3 and 5 years posttransplant in patients with a functioning graft were not affected by the high rate of early AMR in DSA class II-positive patients.

Besides separating classes of DSA, other methods have been proposed to identify nonsignificant DSA, such as MFI values, IgG3 subclass, C1q-binding of DSA,^{22,32-35} or degree of eplet mismatch.³⁶⁻³⁸ We did not find a higher sum MFI to be predictive for the occurrence of rejection. Neither was the type nor number of DSA, though the subgroup analyses are limited in our cohort due to low number of patients in each group and therefore low power. We also did not find DSA class II eplet mismatch to be predictive for rejection or graft loss. Although studies have suggested that specific eplets may have a higher immunogenicity compared with others,^{39,40} at this point too little is known about specific class II epitopes to be assessed in this cohort. C1q-binding and IgG3 subclass were not analyzed in this study.

It is notable that all 6 DSA class II-positive patients who received basiliximab for induction therapy developed rejection. Patients received basiliximab in the cases of a history of cancer or severe infection, although it is well established that in high-risk patients, ATG has a decreased incidence of acute rejection compared with basiliximab.^{41,42} These results underline that patients with DSA class II are high-risk patients and therefore should likely be treated with depletion-based induction therapy despite older age, history of infections, or cancer.⁴³

The main limitation of our cohort is the small sample size, due to the fact that for every patient a transplantation with least immunologic risk is pursued, and therefore not many patients are transplanted with DSA class II. Combined with the single-center design and retrospective nature of our cohort, this entails the risk of selection bias and confounding factors. Furthermore, B-cell CDC-XM and FCXM were

not routinely performed at the time of transplant, while few studies have suggested that DSAs detected by SPA only impact graft survival when also the FCXM is positive.^{19,44-46} Although our center did not perform FCXM in patients of our cohort, these previous studies suggest an opportunity to further assess immunologic risk in patients with DSA class II, such as with FCXM, instead of automatically reporting the antigen to UNOS and excluding kidney offers that may potentially yield good outcomes.

In conclusion, our results emphasize that for highly sensitized patients, DSA class II detected by SPA should not automatically exclude kidney transplantation, because there might be a survival benefit over dialysis, and in our limited cohort, 5-year graft outcomes are comparable to transplanted patients without DSA. Individual approach with further assessment of immunologic risk, likelihood of identifying another compatible donor with current PRA, and strict post-transplant monitoring of patients are indicated because of the higher rate of AMR. Where exactly to draw the line in terms of listing clinically relevant unacceptable HLA antigens and HLA antibody characteristics that best estimates risk remains an area of ongoing research.

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