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Class and Kinetics of Weakly Reactive Pretransplant Donor-specific HLA Antibodies Predict Rejection in Kidney Transplant Recipients

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Background. The clinical impact of weakly reactive pretransplant donor-specific antibody (DSA) in kidney transplantation is controversial. While some evidence suggests that weakly reactive DSA can lead to rejection, it is unclear which patients are at risk for rejection and whether posttransplant changes in weakly reactive DSA are clinically meaningful. **Methods.** We retrospectively studied 80 kidney transplant recipients with weakly reactive pretransplant DSA between 2007 and 2014. We performed a multivariate Cox regression analysis to identify immunologic factors most associated with risk of biopsy-proven rejection. **Results.** Biopsy-proven rejection occurred in 13 of 80 (16%) patients. The presence of both class I and II DSA before transplant (hazards ratio 17.4, $P < 0.01$) and any posttransplant increase in DSA reactivity above a mean fluorescence intensity of 3000 (hazards ratio 7.8, $P < 0.01$) were each significantly associated with an increased risk of rejection, which primarily occurred within the first 18 months. **Conclusions.** Pretransplant DSA class and DSA kinetics after transplantation are useful prognostic indicators in patients with weak DSA reactivity. These results identify a small, high-risk patient group that warrants aggressive posttransplant DSA monitoring and may benefit from alternative donor selection.

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INTRODUCTION

As many as 25% of kidney transplant recipients are immunologically sensitized and many have donor-specific antibodies (DSA) that are weakly reactive with human leukocyte antigen (HLA) specificities.[1-6] While strongly reactive DSA (mean fluorescence intensity [MFI] >5000) are well recognized to be associated with acute rejection of the kidney and poor long-term graft outcomes,[7-10] the impact of "weakly reactive" DSA (MFI: 1000–3000) is less clear. This dilemma has gained increasing clinical importance as technologies have evolved to permit detection of weakly reactive DSA[11,12] and treatment options such as apheresis have become more widespread.[13,14] While an important paper recently demonstrated excellent short- and intermediate-term outcomes between patients with weakly reactive DSA versus patients without DSA,[15] other studies suggest that weakly reactive pretransplant DSA contributes to inferior long-term outcomes[1,10] and an increased rate of antibody-mediated rejection (AMR).[1,2,10,16] Moreover, the presence of weakly reactive DSA may identify patients with an immunologic memory response that is difficult to control and portend poor outcome. Given the cost associated with treatment of AMR and impact of AMR on long-term outcomes, there remains a need for studies

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which identify risk factors for poor outcomes in patients with weakly reactive DSA. Identification of such patients may help transplant centers (1) refine posttransplant surveillance practices, (2) adjust immunosuppression, or (3) inform donor selection. Although excellent transplant outcomes can clearly be achieved in patients with weakly reactive DSA as established by the work of Adebiyi et al,[15] it may be possible to optimize outcomes further by using paired exchange mechanisms to avoid crossing any level of DSA yet maintain access to rapid transplantation.

What immunologic factors predict outcomes in patients with weakly reactive DSA? While degree of HLA mismatch,[17,18] sensitizing event,[19,20] and class of DSA[2,10,18,21,22] are all associated with worse outcomes overall in kidney transplant recipients, the impact of these risk factors is unknown in patients with weakly reactive DSA. Another potential mechanism to identify high-risk patients is through posttransplant monitoring of DSA reactivity. Given the sensitivity of current solid phase assays, transplant centers can detect and follow changes in posttransplant DSA reactivity and potentially intervene. However, changes in antibody reactivity after transplant are difficult to interpret[23–26] and not well studied in patients with preexisting weakly reactive DSA. Although increases in posttransplant DSA reactivity predict graft dysfunction in patients with *de novo* DSA[27] and DSA persistence in patients with strongly reactive pretransplant DSA is associated with graft loss,[28,29] only 2 studies, both of which used nonstandard immunosuppression, have shown an increased risk of rejection with persistence of weak pretransplant DSA.[6,15] Thus, it is unclear whether posttransplant DSA monitoring has prognostic value in this population and whether clinical care should be altered based on this information.

The primary objective of this study was to address these knowledge gaps by identifying kidney transplant candidates with weakly reactive DSA who are at highest risk for a poor outcome. A secondary objective was to determine whether the kinetics of DSA after kidney transplantation predict poorer transplant outcome. We hypothesized that risk factors for rejection could be identified both pre- and posttransplant. These included degree of class I and II HLA mismatch, antibody class, and sensitizing event in the pretransplant setting and elevations in DSA MFI levels in the posttransplant setting. Using a multivariate model, we found that pretransplant DSA class, in particular the combination of class I and II DSA, was most predictive of rejection and rejection severity. Moreover, posttransplant increases in DSA reactivity were significantly correlated with rejection, and this risk of rejection following DSA MFI increases was most pronounced among patients with the pretransplant combination of class I and II DSA.

MATERIALS AND METHODS

Study Design and Patient Selection

This was a retrospective cohort study that initially included 1318 patients who received a kidney transplant at the University of Pennsylvania (January 2007–June 2014). Approval for the study was obtained from the IRB (protocol #821620) prior to commencement of the study. This study period was selected because (1) solid phase detection methods had been adopted at our center by this time, (2) the existence of weak DSA reactivity did not appreciably alter patient selection or posttransplant care during this time at our institution, and (3) this time period allowed adequate follow-up and reporting of short and intermediate posttransplant outcomes. Patients who were simultaneously transplanted with a liver (n = 13), heart (n = 32), or pancreas (n = 45) were excluded. As the primary focus of our study was to examine outcomes and identify risk factors in only patients with weakly reactive DSA, we excluded patients without pretransplant DSA (n = 536). We further excluded patients with no known sensitizing event (n = 606) given their low frequency of DSA[30] and potential biologic differences from patients with a prior alloexposure which may influence posttransplant outcome. We also excluded patients who received rituximab (n = 6) (**Figure S1, SDC,** http://links.lww.com/TXD/A218). The final cohort thus consisted of 80 patients. All patients received induction immunosuppression (73/80 received rabbit anti-thymocyte globulin [rATG], 6/80 received basiliximab, and 1/80 sequentially received both basiliximab and rATG). Maintenance immunosuppression consisted of steroids, tacrolimus, and mycophenolate mofetil or mycophenolic acid. Twelve patients (15%) received modified cyclosporine. No patients were desensitized. Treatment for cellular-based rejection consisted largely of weight-based doses of thymoglobulin and steroids, while treatment of AMR consisted of intravenous immunoglobulin and plasmapheresis.

HLA Typing and Antibody Assessment

HLA typing of recipients and donors was performed using DNA-based techniques and included HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA, and -DQB. Anti-HLA antibody testing was performed using Luminex single antigen bead assays (SAB). Assays were performed according to the instructions provided by the manufacturer except for the addition of dithiothreitol to sera to reduce interference. Reactivity due to denatured epitopes and nonspecific reactivity detected by the SAB assays were ruled out by careful analysis of the specificity pattern of bead reactivity and the constancy of reactivity among the different Luminex bead assays (One Lambda, Canoga Park, CA), and negative control values were taken into account to normalize the reported MFI. DSA specificities included HLA-A, -B, -C, -DRB1, -DRB3/4/5, -DQA, and -DQB. With the exception of DQA, DP, and allele-specific antibodies, any DSA with MFI value >3000 resulted in the listing of the corresponding antigen as unacceptable in UNet (United Network of Organ Sharing). In our lab, the coefficient of variation in these assays is <20% for low range MFI values (1000–3000 MFI). MFIs between 800 and 3000 were considered weakly reactive and MFIs below 800 were considered insignificant given their proximity to the limit of detection of the assay above the negative controls.

Flow cytometry crossmatches for T and B cells were performed prospectively using a Beckman-Coulter FC500. Cells were treated with Pronase 1 mg/mL and stained with affinity purified $F(ab')_2$ goat anti-IgG and Fcγ, 2.2 moles FITC per mole $F(ab')_2$ (1:160 dilution). In most cases, serum specimens used in the final crossmatch included a current serum draw within 30 days prior to transplantation. At our center, flow cytometry crossmatch reactivity is expressed using a relative ratio of molecules of equivalent soluble fluorescence values of the tested sample over the negative control sample. Interpretation of this ratio was as follows: negative: <1.5; weakly reactive: 1.5–2; positive: >2.

All patients received SAB testing prior to transplant. Patients with individual DSAs <3000 MFI and a negative or borderline cross were transplanted except when (1) the MFI values in the historic sera (in the preceding 4 y) were higher than 3000 and/or (2) a weak DSA <3000 MFI was against a repeat mismatch from a previous transplant. Frequency of posttransplant SAB testing was largely at the judgment of each patient's clinician, although a protocol governing DSA testing for sensitized patients was implemented in 2009. Currently, DSA is monitored at 1, 3, 6, and 12 months.

Biopsies

All biopsies were performed for-cause based on evidence of impaired allograft function, including elevations in creatinine and proteinuria. New onset DSA provoked biopsy in 3 patients but was accompanied by evidence of reduced kidney function in all cases. The diagnosis of rejection was based on the Banff 2007 diagnostic criteria.[31] Acute rejection was categorized as T-cell–mediated rejection (acute cellular rejection [ACR]) and/or AMR. Biopsies were evaluated by a renal pathologist.

Variables and Measures

Subject level variables are shown in Table 1. Degree of donor-recipient HLA matching was categorized based on HLA class (Class I: A or B, Class II: DR). For Class I, patients were categorized as 0–2 or 3–4 mismatches. For Class II, patients were categorized as 0 or 1–2 mismatches. Serum creatinine values were converted to estimated glomerular filtration rate (eGFR) using the 4-variable Modification of Diet in Renal Disease equation.[32] Rejection was defined as any biopsy-proven AMR or ACR.

Statistical Analyses

Patient characteristics by pretransplant DSA class were analyzed using Kruskal-Wallis tests for continuous variables and the Chi-squared test for categorical variables. The primary outcome variable, rejection-free survival, was considered from time of transplant until biopsy-proven rejection, or censored by loss to follow-up, study termination, or death with a functioning allograft.[33-35]

Patient, graft, and rejection-free survival were analyzed using Kaplan-Meier product limit estimates and the log-rank test for between-group differences. To adjust for covariates, a multivariable Cox regression model was built using backwards elimination assessing variables that met prespecified nominal significance ($P < 0.20$).[36,37] Variables removed from the model were tested for evidence of confounding and effect modification using percent hazard differences (>15%) and likelihood ratio tests ($P \leq 0.05$), respectively.[37] The Groennesby and Borgan test determined final model adequacy and the Schoenfeld test of residuals was used to test the assumption of proportionality of hazards for the final multivariable model. All statistical analyses were performed using STATA 14.0/MP statistical software.[38]

RESULTS

Patient Characteristics

Of the 1318 patients who received a kidney transplant, 80 (6%) met the study inclusion criteria with a pretransplant DSA MFI between 800 and 3000 (**Figure S1, SDC,** http://links.lww.com/TXD/A218). Patient characteristics are described in Table 1. Fifty-eight percent of patients were females, 48% were Black, and the median age was 48 years. Notably, 21% of patients received a living donor and 48% had a preemptive transplant. Immunologically, 9% had a weakly reactive B cell crossmatch, 5% had a weakly reactive T cell crossmatch, 60% were poorly matched to class I HLA (3–4 mismatches), and 84% were poorly matched to HLA-DR (1–2 mismatches), consistent with national trends.[6] Pretransplant sensitizing events were balanced: 28% of patients were sensitized by pregnancy, 30% by transfusion, 21% by prior transplant, and 21% by multiple events. Forty-three percent of patients had weakly reactive class I DSA before transplant, 46% had weakly reactive class II DSA, and 11% had both weakly reactive class I and II DSA.

TABLE 1.

Baseline characteristics

Variable	Total (n = 80)
Age at transplant, median (IQR)	48 (39–56)
Female gender, n (%)	46 (58)
Race, n (%)	
White	30 (38)
Black	38 (48)
Other[a]	12 (15)
Pretransplant diabetes	16 (20)
Highly recurrent primary disease,[b] n (%)	14 (18)
Pretransplant dialysis, median, y (IQR)	0.7 (0–4.6)
Preemptive transplant, n (%)	38 (48)
Living donor, n (%)	17 (21)
Serum creatinine at transplant mg/dL, median (IQR)	6.9 (5.0–8.7)
Sensitization type, n (%)	
Pregnancy	22 (28)
Transfusion	24 (30)
Prior Transplant	17 (21)
Multiple Types	17 (21)
UNOS cPRA, median (IQR)	56 (0–88)
Class I PRA, median (IQR)	11 (0–63)
Class II PRA, median (IQR)	0 (0–70)
Pretransplant DSA Class, n (%)	
Class I	34 (43)
Class II	37 (46)
Class I & II	9 (11)
Degree of class I HLA (A/B) matching, n (%)	
0–2 mismatches	32 (40)
3–4 mismatches	48 (60)
Degree of class II HLA (DR) matching, n (%)	
0 mismatches	13 (16)
1–2 mismatches	67 (84)
B cell flow, n (%)	
Negative	73 (91)
Weakly reactive	7 (9)
T cell flow, n (%)	
Negative	76 (95)
Weakly reactive	4 (5)

[a]Other = Hispanic, Asian, Native American, Pacific Islander, Multiracial.
[b]Focal Segmental Glomerular Sclerosis, Systemic Lupus Erythematosus, Hemolytic Uremic Syndrome.
cPRA, calculated panel reactive antibody; DSA, donor-specific antibody; HLA, human leukocyte antigen; IQR, interquartile range; PRA, panel reactive antibody; UNOS, United Network for Organ Sharing.

Multivariate Cox Regression Analysis of Factors Associated With Rejection

To determine which factors were associated with rejection among patients with weakly reactive pretransplant DSA, we

developed a multivariable Cox regression analysis using variables meeting nominal significance ($P < 0.20$) in a univariate analysis. Variables in the analysis included DSA class, sensitizing event, degree of HLA matching, race, gender, diabetes status, and increase in posttransplant DSA to >3000 MFI. After controlling for the multiple variables, the combination of class I and II pretransplant DSA and an increase in posttransplant DSA to >3000 MFI were both significantly associated with risk of rejection in the multivariate regression (class I and II: hazards ratio [HR] 14.4, $P < 0.01$; DSA >3000: HR: 7.8, $P < 0.01$; Table 2) as were Black race and pretransplant diabetes. A history of multiple sensitizing events was associated with a reduced risk of rejection (HR: 0.06, $P = 0.046$; Table 2), the reason for which is unclear. The degree of neither class I ($P = 0.14$) nor class II ($P = 0.96$) HLA matching was significantly associated with rejection rates (Table 2).

Patient and Graft Outcomes

Overall, 10 of 80 (13%) patients died and 9 of 80 (11%) patients had graft failure during study follow-up. Because pretransplant DSA class and posttransplant DSA MFI elevations were the only immunologic variables associated with acute rejection, we investigated their effect on patient outcomes. After a median follow-up of 5.5 years, there was no difference in overall survival (OS), kidney graft survival (GS), or kidney function at 1 year as measured by eGFR based on pretransplant DSA class (OS: $P = 0.46$, **Figure S2A, SDC,** http://links.lww.com/TXD/A218; GS: $P = 0.27$, **Figure S2B, SDC,** http://links.lww.com/TXD/A218; eGFR: $P = 0.30$, Table 3) or posttransplant increases in DSA (OS: $P = 0.11$, **Figure S2C, SDC,** http://links.lww.com/TXD/A218; GS: $P = 0.72$, **Figure S2D, SDC,** http://links.lww.com/TXD/A218; eGFR: $P = 0.44$, data not shown).

Effect of DSA Class on Kidney Graft Rejection

To further understand the relationship between pretransplant antibody class and initial rejection, we analyzed the frequency and timing of rejection events based on DSA class. There were no differences in baseline patient characteristics when patients were grouped by DSA class except patients with both class I and II DSA were more likely to have a weakly reactive B cell crossmatch ($P = 0.02$, **Table S1, SDC,** http://links.lww.com/TXD/A218). However, a weakly reactive B cell crossmatch was not associated with an increased risk of rejection ($P = 0.18$, data not shown). Patients with both class I and II DSA had more than triple the risk of acute rejection compared with patients with class I or class II alone (class I: 9%, class II: 14%, class I and II: 56%, $P < 0.01$), coinciding with reduced rejection-free survival ($P < 0.01$, Figure 1; patient HLA details: **Table S2, SDC,** http://links.lww.com/TXD/A218). The elevated risk of acute rejection was due to increases in the incidence of both AMR and ACR (AMR: $P < 0.01$, ACR: $P = 0.02$, Table 3). The majority (54%) of rejection events occurred within the first 12 months across all groups and 80% of rejections in the highest-risk patients (those with class I and II DSA) occurred within the first 18 months. Because we did not perform protocol biopsies, we speculated that the differences in rejection rates might be due to different biopsy rates based on pretransplant DSA class. However, there was no difference in the rate of for-cause biopsy across antibody classes ($P = 0.10$, Table 3).

TABLE 2.

Multivariable Cox regression analysis of factors associated with risk of rejection

Variable	HR	95% CI	*P*
Pretransplant DSA Class			
Class I	Reference		
Class II	1.00	0.20-4.96	0.99
Class I & II	17.44	2.56-118.75	<0.01
Sensitization Type			
Blood transfusion	Reference		
Pregnancy	0.39	0.03-5.76	0.49
Prior transplant	0.21	0.01-5.80	0.36
Mixed types	0.06	0.01-0.96	0.046
Degree of class I HLA (A/B) Matching, n (%)			
0–2 Mismatches	Reference		
3–4 Mismatches	0.30	0.06-1.51	0.14
Degree of class II HLA (DR) matching, n (%)			
0 mismatches	Reference		
1–2 mismatches	0.94	0.07-12.07	0.96
Development of Posttransplant DSA MFI > 3000	7.81	1.80-33.81	<0.01
Race			
White	Reference		
Black	22.76	1.58-327.37	0.02
Other[a]	1.90	0.06-61.14	0.72
Gender			
Males	Reference		
Females	0.78	0.05-11.35	0.86
Pretransplant diabetes	12.51	2.12-73.77	<0.01

[a]Other = Hispanic, Asian, Native American, Pacific Islander, Multiracial.

P-value of <0.05 is considered statistically significant.

CI, confidence interval; DSA, donor-specific antibody; HLA, human leukocyte antigen; HR, hazards ratio; MFI, mean fluorescence intensity.

TABLE 3.

Posttransplant outcomes by pretransplant DSA class

Variable	Total (n = 80)	Class I (n = 34)	Class II (n = 37)	Class I and II (n = 9)	*P*
Any rejection, n (%)	13 (16)	3 (9)	5 (14)	5 (56)	<0.01
Acute cellular rejection, n (%)[a]	11 (14)	3 (9)	4 (11)	4 (44)	0.02
AMR, n (%)[a]	4 (5)	0 (0)	1 (3)	3 (33)	<0.01
Any cause biopsy, n (%)	32 (40)	15 (44)	11 (30)	6 (67)	0.10
Posttransplant DSA with MFI >3000, n (%)	13 (16)	3 (9)	7 (19)	3 (33)	0.17
Posttransplant DSA Class with MFI >3000, n (%)	n = 13	n = 3	n = 7	n = 3	0.02
Class I	4 (31)	3 (100)	1 (14)	0 (0)	
Class II	8 (62)	0 (0)	6 (86)	2 (67)	
Class I and II	1 (8)	0 (0)	0 (0)	1 (33)	
eGFR at 1 y, median (IQR)	57 (44–74)	58 (42–72)	60 (45–82)	53 (43–67)	0.30

[a]Two patients had both acute cellular and AMR.
P-value of <0.05 is considered statistically significant. Acute cellular rejection includes borderline rejection by Banff criteria.
AMR, antibody-mediated rejection; DSA, donor-specific antibody; eGFR, estimated glomerular filtration rate; IQR, interquartile range; MFI, mean fluorescence intensity.

Patients with class I and II DSA who experienced rejection were more likely to require hospitalization (class I = 33%, class II = 20%, class I and II = 100%, $P = 0.03$) and required more intensive immunosuppression (**Table S3, SDC,** http://links.lww.com/TXD/A218). Importantly, tacrolimus levels did not differ by DSA class (**Table S4, SDC,** http://links.lww.com/TXD/A218) and there were no medication changes preceding rejections. Three patients had transient leukopenia prior to rejection, one of whom had a mild infection, and 2 other patients had mild infections prior to rejection (**Table S4, SDC,** http://links.lww.com/TXD/A218). Exclusion of one patient with documented non-adherence did not change the results (data not shown).

DSA Dynamics and Kidney Graft Rejection

To better understand the effects of an increase in posttransplant DSA reactivity, we quantified posttransplant DSA MFI dynamics and the relationship between DSA dynamics, DSA class, and rejection. Overall, the strength of DSA increased above 3000 MFI after transplant in 13 of 80 (16%) patients, with 8 of 13 (62%) increases occurring in the first 90 days and 12 of 13 (92%) occurring within 18 months (Figure 2). These increases were associated with a significantly increased risk of rejection (Figure 3A). The greatest risk of rejection occurred in patients whose DSA MFI increased more than 4-fold after transplant, although this result did not achieve statistical significance (>4-fold: 5/8, <4-fold: 1/5, $P = 0.13$) (data not shown). The majority (67%) of patients with rejection after a DSA MFI increase required inpatient treatment, although this did not differ from patients whose rejection was not associated with an increase in DSA MFI (**Table S5, SDC,** http://links.lww.com/TXD/A218). Interestingly, the frequency of posttransplant DSA increases did not differ significantly based on the class of pretransplant antibody, although there was a trend toward more increases in DSA MFI in patients with class II or both class I and II DSA (class I: 9%, class II: 19%, class I and II: 33%, $P = 0.11$, Table 3). This suggests that the effect of a DSA MFI increase differed based on the pretransplant DSA class.

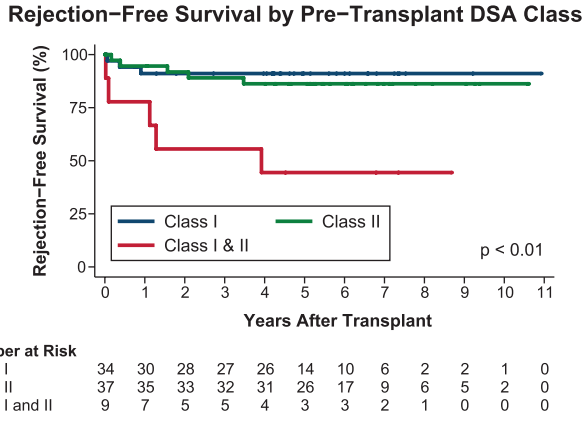

FIGURE 1. Effect of pretransplant DSA class on rejection-free survival. Rejection-free survival of patients grouped by the class of their pretransplant DSA. "Number at risk" indicates the number of patients in each group who are alive at each time point and therefore at risk for death over the ensuing time period. DSA, donor-specific antibody.

To elucidate how pretransplant DSA class affected the outcome of DSA MFI increases, we compared the rate of rejection in patients with DSA MFI elevations within each pretransplant DSA class. Surprisingly, elevation of DSA MFI after transplant was associated with an increased risk of rejection only for patients with pretransplant class II or both class I and II DSA (HR for rejection given DSA >3000 vs DSA <3000: class I = 0 [0–0], class II = 8.2 [1.4–49.5], class I and II = 13.5 [1.4–135.2], Figure 3B–D). This corresponded to a difference in which antibody class increased between patients with different pretransplant DSA classes. Specifically, patients with existing class I were more likely to have significant increases in class I DSA MFI posttransplant, and patients with existing class II or both class I and II were more likely to have increases in class II DSA MFI after transplant ($P = 0.02$, Table 3). When we examined all patients regardless of pretransplant antibody class, we found that elevations of class II or both class I and II DSA MFI significantly increased the risk of rejection compared with elevations in class I alone (Figure 3E, $P < 0.01$).

DISCUSSION

While the transplantation of patients with weak DSA reactivity and a negative crossmatch has been shown to have acceptable short- and intermediate-term outcomes,[15] this practice is associated with an increased risk of AMR and potential risk of long-term graft loss. In this study, we investigated the immunologic risk factors associated with rejection among patients with weakly reactive pretransplant DSA, and we examined the prognostic utility of posttransplant DSA

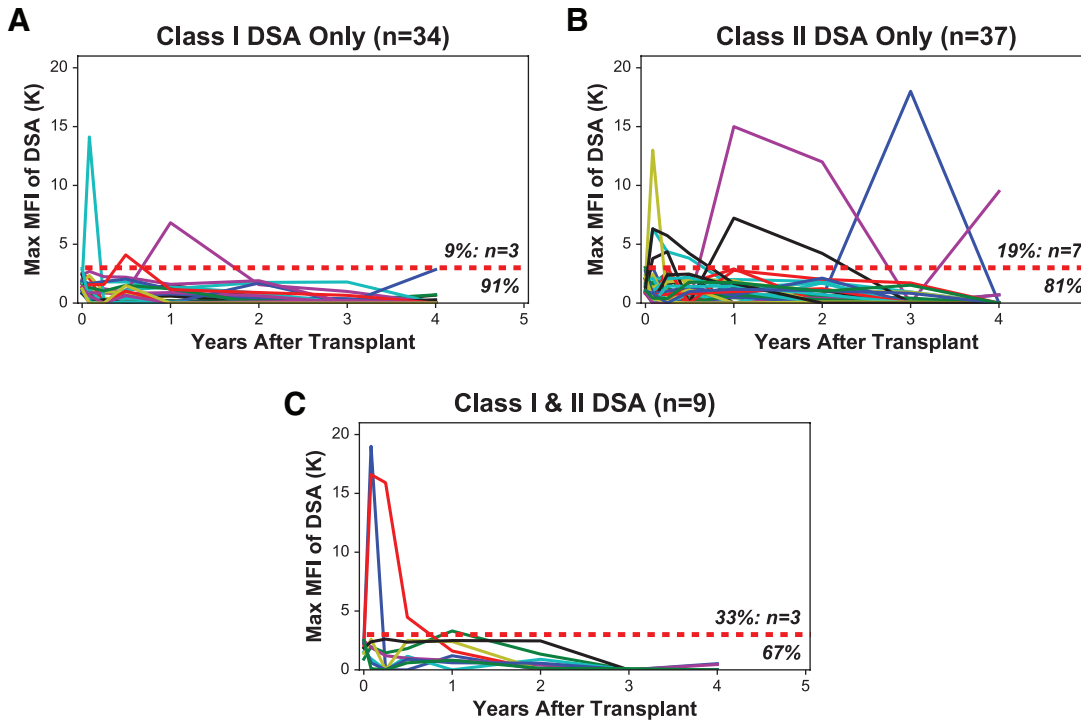

FIGURE 2. Dynamics of pretransplant DSA evolution. Each line represents a single patient. MFI values shown are of the DSA with the highest MFI at each timepoint. Only DSA specificities that were present at low MFI values prior to transplant are shown. Red dotted line represents 3000 MFI. Percentages and counts indicate the percentage and number of patients whose DSA increased above 3000 MFI or remained below 3000 MFI throughout follow-up. A, Patients with only class I DSA prior to transplant. B, Patients with only class II DSA prior to transplant. C, Patients with both class I and class II DSA prior to transplant. DSA, donor-specific antibody; MFI, mean fluorescence intensity.

monitoring. We found that the combination of weakly reactive class I and II DSA at the time of transplant is associated with an increased risk of acute rejection requiring intensive treatment, mainly within the first 18 months. We also found that posttransplant increases of this low pretransplant DSA to >3000 MFI were associated with an increased rate of acute rejection. Notably, the effect of these increases was most pronounced in patients with class II or class I and II DSA.

Our study is the first to quantify the significance of posttransplant DSA dynamics specifically in patients with weakly reactive pretransplant DSA. Although multiple studies have shown that persistence of pretransplant DSA is associated with rejection[4,15,28,39] and graft loss,[29,40] these studies are complicated by either high pretransplant DSA MFI[4,15,29] or a strong correlation between strength of pretransplant DSA reactivity and posttransplant DSA persistence.[28,39,40] Moreover, bead-based epitopes are heterogeneous in their ability to measure true antibody level or antibody avidity to cell membrane-bound HLA, complicating interpretation of serial measurements, and these studies have not taken into account posttransplant DSA dynamics. Here, we have shown that even in patients who are tightly controlled for DSA strength before transplant, DSA MFI values increased after transplant in 13 of 80 (16%) patients and these dynamic increases were associated with an 8-fold increased rejection. Importantly, the risk of rejection following a DSA MFI increase appears higher in patients with class II or combined class I and II and occurs early after transplant. These results support close posttransplant follow-up of patients with weakly reactive DSA and may identify patients who would benefit from increased immunosuppression or a biopsy, even without clinical signs of rejection.

Our results add to a growing body of literature that demonstrates the importance of DSA class as a predictor of kidney graft outcomes. Multiple studies have now demonstrated that the presence of both class I and II DSA prior to transplant is associated with an increased risk of AMR or graft loss.[2,18,21,28,39,40] These studies, however, did not measure DSA strength and included strongly reactive DSA with highly heterogeneous MFIs. Moreover, the generalizability of these studies is unclear due to significant variance in induction immunosuppression regimens and, in one case, the use of nonstandard induction immunosuppression.[18] By selecting only patients with pretransplant DSA <3000 MFI, we are able to clearly demonstrate that DSA class is a significant risk factor even when the strength of DSA reactivity is tightly controlled. Moreover, despite our small sample size, patients with weakly reactive class I and II DSA had a 56% chance of rejection within the first 18 months and all rejection episodes required intensive inpatient treatment. Thus, our study is the first to show that antibody class is a predictor of acute rejection in patients with weakly reactive DSA, even in patients who receive potent lymphocyte-depleting induction immunotherapy with rATG.

Important limitations of this study include its small sample size, short follow-up period, lack of protocol DSA measurements, and retrospective design. Despite these limitations, we were able to identify variables (ie, posttransplant DSA class and kinetics) which identify at-risk patients. Additional limitations include the study of an uncommon event with negligible impact on short and intermediate outcomes (ie, the low rate of rejection in patients with weakly reactive DSA). However, we believe that the importance of weakly reactive DSA will be well recognized by any transplant center looking

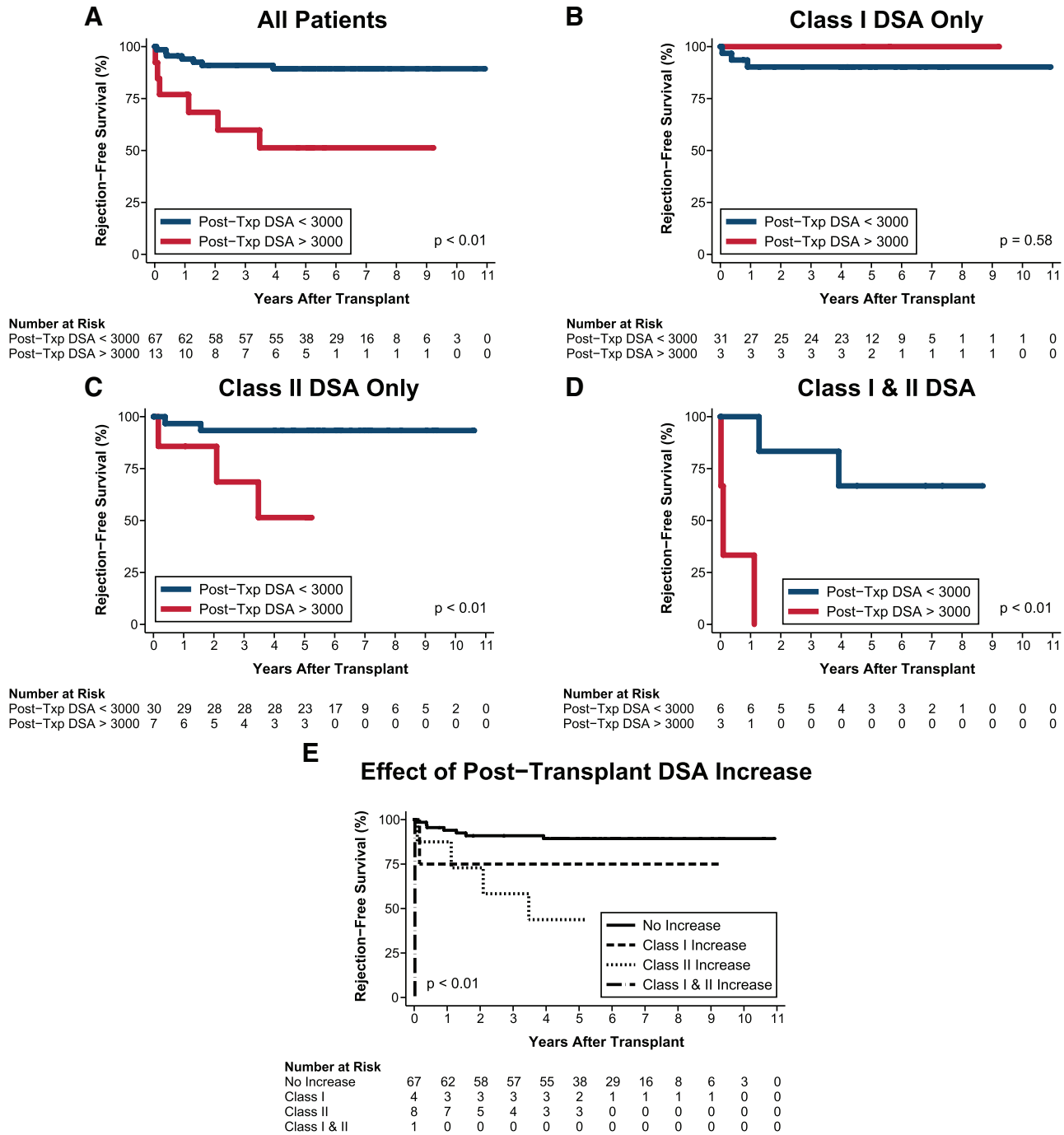

FIGURE 3. Effect of increases in posttransplant DSA reactivity on rejection-free survival. A–D, Rejection-free survival based on whether DSA ever increased to >3000 MFI after transplant among (A) all patients or patients whose pretransplant DSA was to (B) only class I, (C) only class II, or (D) both class I and class II. E, Rejection-free survival of all patients based on the class of DSA that increased after transplant, regardless of the class of DSA that was present prior to transplant. "Number at risk" indicates the number of patients in each group who are alive at each time point and therefore risk for death over the ensuing time period. DSA, donor-specific antibody; MFI, mean fluorescence intensity.

to optimize long-term outcomes and reduce costs associated with treatment of AMR. Another potential limitation of our study is an inability to contextualize the impact of weakly reactive class I DSA with respect to patients without DSA, as we did not include patients without pretransplant DSA. However, this issue has been addressed very well by Adebiyi et al.[15] Moreover, as 93% of our patients received induction therapy with rATG, we were unable to comment on the optimal pretransplant therapy regimen in these patients. Of note, repeating the analysis excluding patients who received basiliximab did not result in any difference in the results or their interpretation (data not shown). Future work that prospectively evaluates induction regimens in these patients will be high value. Finally, we were unable to evaluate the role of either the isotype or C1q-binding affinity of HLA antibodies in our study, both of which may have prognostic value.[29,41-43]

Overall, among kidney transplant recipients with weakly reactive DSA and a negative or borderline crossmatch, we have shown that the presence of both class I and II DSA prior to transplant and subsequent increases in posttransplant DSA

above 3000 MFI are significant risk factors for early rejection. Our study successfully identifies a patient subset at risk for escalating therapeutic intervention and addresses a topic of on-going significance to the transplant community.[1,2,10,15,16] Strengths of this study include its detailed clinical information, representative patient demographics, and quantitative DSA measurements. Importantly our patient cohort is demographically very similar to the US population as a whole and included a significant proportion of Black patients,[6] enhancing its generalizability to the US kidney transplant population. Although patients with weakly reactive DSA of both classes are rare, their rejections were common and severe. We expect that this information will prompt individual centers to consider donor selection in these patients as well as posttransplant monitoring and immunosuppression practices in these at-risk patients. Additional work is necessary to determine how best to clinically manage elevations in DSA reactivity in these at-risk patients, as intensification of conventional T cell–directed immunosuppression and/or the addition of antibody-depleting therapies may help prevent rejection episodes.

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