ORIGINAL ARTICLE

Disparate rates of acute rejection and donor-specific antibodies among high-immunologic risk renal transplant subgroups receiving antithymocyte globulin induction

Samir J. Patel[1], Wadi N. Suki[2], Jennifer Loucks-DeVos[3], Edward A. Graviss[4], Duc T. Nguyen[4], Richard J. Knight[5], Samantha A. Kuten[1], Linda W. Moore[3], Larry D. Teeter[6], Lillian W. Gaber[7] & A. Osama Gaber[5]

1 Department of Pharmacy, Houston Methodist Hospital, Houston, TX, USA
2 Department of Medicine, Houston Methodist Hospital, Houston, TX, USA
3 Department of Pharmacy, Kansas University Medical Center, Kansas City, KS, USA
4 Houston Methodist Research Institute, Houston Methodist Hospital, Houston, TX, USA
5 Department of Surgery, Houston Methodist Hospital, Houston, TX, USA
6 Department of State Health Services, Houston, TX, USA
7 Department of Pathology, Houston Methodist Hospital, Houston, TX, USA

Correspondence
Dr. Samir J. Patel Pharm.D., BCPS, Department of Pharmacy, Houston Methodist Hospital, 6565 Fannin, DB1-09, Houston, TX 77030, USA.
Tel.: 713-441-2168;
fax: 713-441-1225;
e-mail: spatel2@houstonmethodist.org

SUMMARY

Lymphocyte-depleting induction lowers acute rejection (AR) rates among high-immunologic risk (HIR) renal transplant recipients, including African Americans (AAs), retransplants, and the sensitized. It is unclear whether different HIR subgroups experience similarly low rates of AR. We aimed to describe the incidence of AR and *de novo* donor-specific antibody (dnDSA) among HIR recipients categorized by age, race, or donor type. All received antithymocyte globulin (ATG) induction and triple maintenance immunosuppression. A total of 464 HIR recipients from 2007 to 2014 were reviewed. AR and dnDSA rates at 1 year for the entire population were 14% and 27%, respectively. AR ranged from 6.7% among living donor (LD) recipients to 30% in younger AA deceased donor (DD) recipients. *De novo* donor-specific antibody at 1 year ranged from 7% in older non-AA LD recipients to 32% in AAs. AA race remained as an independent risk factor for AR among DD recipients and for dnDSA among all HIR recipients. Development of both AR and dnDSA within the first year was associated with a 54% graft survival at 5 years and was an independent risk factor for graft loss. Despite utilization of recommended immunosuppression for HIR recipients, substantial disparities exist among subgroups, warranting further consideration of individualized immunosuppression in certain HIR subgroups.

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Key words
antilymphocyte globulin, HLA-antibody post-transplantation, Immunosuppression, rejection

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Introduction

In kidney transplantation, the term "high risk" broadly describes any number of donor or recipient comorbidities or conditions, which may lead to acute rejection (AR) and/or impair long-term allograft and patient survival. African Americans (AA), highly sensitized, and repeat transplant recipients are commonly characterized as "high-immunologic risk" (HIR). As such, these patients are often administered lymphocyte-depleting induction at the time of transplantation to reduce the rate of acute rejection [1–7]. Both antithymocyte

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globulin (ATG) and alemtuzumab have been shown in randomized controlled trials to reduce the incidence of AR in these populations, resulting in 1-year AR rates of approximately 15% [1–4]. It is therefore a widely accepted recommendation that renal transplant recipients considered HIR be given lymphocyte-depleting antibody induction at the time of transplantation [8].

The HIR population itself still encompasses a diverse array of patients based on age, degree of sensitization, or ethnicity, each of which may influence the risk of rejection. Despite these demographic differences, it is common for centers to administer a uniform quadruple-sequential immunosuppressive protocol beginning with lymphocyte-depleting induction to patients categorized as HIR. While it is known that this strategy is effective in reducing AR rates, as demonstrated by the aforementioned studies, it is unclear whether various subgroups of the HIR population experience comparable rates of AR in the context of routine lymphocyte-depleting induction and a uniform maintenance immunosuppressive protocol. Furthermore, the incidence of *de novo* donor-specific antibodies (dnDSA) is not well described specifically in a HIR cohort.

Therefore, the purpose of this study was to describe the incidence and characteristics of both AR and dnDSA in various subgroups of a large, diverse, HIR population receiving ATG induction with triple maintenance immunosuppression and to determine the impact of these events on graft survival beyond the first post-transplant year.

Material and methods

Patient selection

This was a retrospective single-center review of all renal transplant recipients performed from January 2007 through May 2014. HIR adults (≥18 years) who received a living or deceased donor renal transplant or retransplant with ATG induction and triple maintenance therapy with tacrolimus, mycophenolate mofetil, and corticosteroids at discharge were included. "High-immunologic risk" was determined by the presence of at least one of the following criteria: peak panel reactive antibody (PRA) or calculated PRA ≥ 20%, retransplantation, and AA race. Simultaneous pancreas transplant recipients, individuals with pre-existing detectable DSA at the time of transplant, and those with graft loss within the first month post-transplant were excluded from the analysis. This retrospective review was approved by the Houston Methodist Hospital Institutional Review Board.

Immunosuppressive protocol

Rabbit-ATG (Thymoglobulin®, Genzyme Corporation, Cambridge, MA, USA) was administered at a dose of 1.5 mg/kg intravenous (IV) daily for 3 days, with the first dose initiated intra-operatively prior to reperfusion of the kidney. Dosage adjustments were made based upon white blood cell and platelet counts, per product labeling. In cases of delayed graft function (DGF), defined as hemodialysis within the first post-operative week, an additional 1–2 dosages of ATG were given. Intravenous methylprednisolone 200 mg was initiated intra-operatively, followed by a taper to 30 mg/day of oral prednisone by post-operative day 3, and 5–10 mg/day by post-operative day 90. Mycophenolate mofetil 500 mg was given every 12 hours during ATG administration and then increased to 1 gm every 12 hours at discharge. Tacrolimus was initiated when the serum creatinine declined by one-third of the pretransplant value or at the completion of ATG in the setting of DGF. Goal 12-hour tacrolimus trough levels were 8–10 ng/ml for the first 3 months, 6–8 ng/ml for months 3 through 12, and 4–8 ng/ml thereafter.

Post-transplant immune monitoring

DSA testing at our center has been previously described [9]. Briefly, samples of recipient sera were screened for class I and class II antibodies when clinically warranted for graft dysfunction. Routine dnDSA monitoring was performed at 1, 3, 6, 9, and 12 months, and biannually, thereafter. Single antigen bead technology (LABScreen, One Lambda, Canoga Park, CA, USA) was used for screening of dnDSA against donor HLA antigens utilizing a multichannel flow array (Luminex, Austin, TX, USA). A median fluorescence index (MFI) of greater than 2000 was used as the cutoff for a positive dnDSA. This MFI in our laboratory represented the cutoff at which the signal-to-noise ratio was considered acceptable to confidently declare the presence of DSA. A "strong" dnDSA was defined as having a MFI >8000, while "multiple DSAs" referred to the presence of dnDSA against two or more donor antigens.

Rejection definitions

Percutaneous renal biopsies with ultrasound guidance were obtained in the event of unexplained graft

dysfunction or per protocol for recipients who developed detectable dnDSAs on two separate occasions. Renal biopsy samples were evaluated for adequacy and then triaged for light microscopy, electron microscopy, and immunofluorescence testing. The biopsies were evaluated and reported according to Banff guidelines. Morphologic evidence of tissue injury and the presence of linear C4d binding to peritubular capillaries in the context of positive DSAs were quintessential for making the diagnosis of antibody-mediated rejection (AMR). Microvascular inflammation (i.e., capillaritis and glomerulitis at scores of ≥1) with C4d-positive reaction on immunohistology and a positive DSA serology were manifestations of active/subclinical rejection that were more commonly recognized in protocol biopsies from stable kidneys. C4d-negative AMR was rendered for C4d-negative biopsies that showed other evidence of moderate-to-severe microvascular inflammation with ≥2 scores for capillaritis and glomerulitis. Electron microscopy evaluation was frequently performed particularly for biopsies from grafts that were at or more than 6 months post-transplant.

Statistical analysis

Baseline data were reported as mean ± SD for continuous variables, and as frequencies and proportions for categorical variables. Differences in baseline data across groups of donor type (living versus deceased) were compared using the chi-square and Fisher exact tests for categorical variables and the unpaired t-test or Kruskal–Wallis test for continuous variables as appropriate. We sought to elucidate the effects of age and race on immunologic outcomes. Therefore, after first analyzing the incidence of AR according to the donor type, we then further determined the distribution of age by percentile groupings (5%) for the entire cohort. After this descriptive analysis and determining that younger "middle" age groupings had higher AR risks, we chose a biologically plausible cut point of 40 years of age to represent the naturally occurring dichotomous distribution of age associated with AR in our model. This resulted in living donor (LD) and deceased donor (DD) subgroups consisting of: (i) non-AA recipients aged <40 years, (ii) non-AA recipients aged ≥40 years, (iii) AA recipients aged <40 years, and (iv) AA recipients aged ≥40 years. Cause-specific cumulative incidence for AR and dnDSA was estimated using the method of Fine and Gray [10]. Subdistribution hazard ratios across donor subgroups were reported. Univariate and multivariate Cox proportional hazards models were used to determine the contribution of potential risk factors to the development of AR and dnDSA at 1 year, and graft failure at 5 years. Landmark analysis was also performed in the group of patients whose graft survived after 1 year to determine the impact of AR and dnDSA on the graft survival beyond the first year post-transplant [11]. Only first-rejection episodes were included in the analysis. Variables having P-value <0.2 in the univariate analysis or being considered clinically important were further examined in the multivariate analysis, and a parsimonious model was chosen. All analyses were performed on Stata version 13.1 (StataCorp LP, College Station, TX, USA). A P-value of <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 555 HIR kidney-alone transplants occurred from January 2007 through May 2014. Patients were excluded from the analysis for the following reasons: desensitization and/or pre-existing DSA (n = 70), renal graft loss within the first month (n = 13), and not prescribed tacrolimus or mycophenolate mofetil at discharge (n = 8). None of the graft losses within 1 month were due to hyperacute or acute rejection. This resulted in 464 patients included in the analysis, each receiving ATG induction and a triple maintenance regimen consisting of tacrolimus, mycophenolate, and prednisone. All transplant recipients had at least 1 year of follow-up, and mean overall follow-up was 41 ± 20 months.

Characteristics of the study population as a whole and stratified by donor type are shown in Table 1. DD transplants made up the majority (78%) of the cohort. As expected, several differences were observed in the characteristics of LD versus DD recipients. LD recipients tended to be younger, were more likely to be preemptive, had shorter durations of pretransplant dialysis and lower PRAs, and had fewer HLA mismatches compared with DD recipients. In addition, there were significantly more Caucasians in the LD group compared with the DD group. In contrast, the proportion of AAs was significantly higher in DD transplants than in LD transplants, whereas Hispanics comprised similar proportions of each group. The most common causes of end-stage renal disease were hypertension (42%), diabetes (13%), failure of a previous allograft (13%), and polycystic kidney disease (7%, data not shown). The mean ATG induction dose administered was 4.9 ± 0.9 mg/kg for the entire cohort, with DD recipients receiving a significantly higher cumulative dose (4.9 ± 1 vs. 4.7 ± 0.8 mg/kg, P = 0.015). DGF occurred in

Table 1. Baseline characteristics stratified by transplant type.

	All patients (n = 464)	Living donor recipients (n = 104)	Deceased donor recipients (n = 360)	P-value (living versus deceased donor recipients)
Age in years, mean ± SD	48 ± 13	46 ± 13	49 ± 13	0.084
Age <40 years, n (%)	126 (27)	31 (30)	95 (26)	0.492
Race, n (%)				
African American	238 (51)	43 (41)	195 (54)	0.021
Caucasian	117 (25)	41 (39)	76 (21)	<0.001
Hispanic	81 (17)	14 (13)	67 (19)	0.211
Male gender, n (%)	222 (48)	53 (50)	170 (47)	0.534
Repeat transplant, n (%)	59 (13)	18 (17)	41 (11)	0.121
Preemptive, n (%)	53 (11)	31 (30)	22 (6)	<0.001
Pretransplant dialysis duration in years, mean ± SD	4.0 ± 3	2.7 ± 3	4.3 ± 3	<0.001
pPRA (%), mean ± SD	46 ± 34	39 ± 30	48 ± 35	0.015
pPRA ≥ 20%, n (%)	335 (72)	71 (68)	264 (73)	0.315
pPRA ≥ 80%, n (%)	111 (24)	10 (10)	101 (28)	<0.001
Donor age in years, mean ± SD	36 ± 15	39 ± 10	36 ± 16	0.052
Expanded criteria donor, n (%)	–	–	36 (10)	–
HLA mismatches out of 6, mean ± SD	4.1 ± 1.7	3.6 ± 1.6	4.3 ± 1.7	<0.001
Cumulative ATG dose in mg/kg, mean ± SD	4.9 ± 0.9	4.7 ± 0.8	4.9 ± 1.0	0.015
Delayed graft function, n (%)	46 (9)	1 (1.0)	45 (13)	<0.001

ATG, antithymocyte globulin; pPRA, peak panel reactive antibody; HLA, human leukocyte antigen; SD, standard deviation.

significantly more DD recipients than LD (13% vs 1%, $P < 0.001$). Maintenance immunosuppression levels and dosages during the first year are shown in Table 2.

Acute rejection

Biopsy-proven AR occurred in 65 (14%) patients within the first year, at a mean of 4 ± 3.1 months post-transplant. Sixty rejections (92%) were diagnosed as cellular, including included borderline changes in 21 (32%), grade 1 in 24 (37%), grade 2 in 13 (20%), and grade 3 in two (3%) cases. AMR without a cell-mediated component was observed in the remaining five (8%) cases. Thirteen (20%) of the rejections were classified as mixed (both cellular and antibody mediated).

DD transplant recipients experienced a significantly higher rate of AR than LD recipients at 1 year (16.1% vs. 6.7%, respectively; $P = 0.009$). Given the low incidence of AR in LD recipients, we combined all LD age and race subgroups into one group (All LD) to depict the incidence of AR at 1 year among various HIR subgroups (Fig. 1). AR rates varied widely, with younger AA DD recipients experiencing a 1-year incidence of 30%, which was significantly higher than the remaining DD recipients combined as well as all LD recipients. In addition, younger AA DD recipients experienced a significantly higher rate of AMR (including both AMR alone and as part of mixed rejections) compared with the remaining DD cohorts (17% vs. 5.5%; $P = 0.008$) and with LD recipients (17% vs. 5.7%; $P = 0.029$). Due to recognizable demographic differences between DD and LD cohorts, risk factors for AR were determined separately for each cohort. Risk factors for AR in the DD population by univariate and multivariate analyses are

Table 2. Immunosuppression through 1 year post-transplant.

	Month 1	Month 3	Month 6	Month 12
Tacrolimus, ng/ml	8.8 ± 3.7	7.9 ± 3.3	7.5 ± 3.3	7.1 ± 4.9
Mycophenolate mofetil, gm/day	1.97 ± 0.6	1.83 ± 0.5	1.62 ± 0.6	1.60 ± 0.6
Prednisone, mg/day	19.7 ± 6.9	10.4 ± 4.7	8.8 ± 3.5	7.5 ± 3.5

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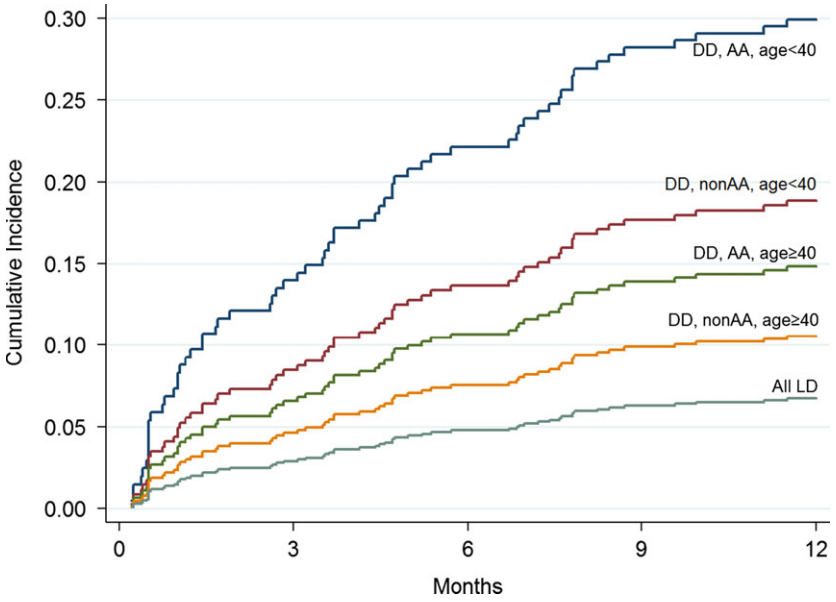

	Sub-hazard ratio	P-value	95% CI	
DD, AA, age<40	5.11	<0.001	2.11	12.38
DD, nonAA, age<40	3.00	0.033	1.09	8.22
DD, AA, age≥40	2.31	0.056	0.98	5.43
DD, nonAA, age≥40	1.60	0.314	0.64	4.01
All LD	(ref)			

Figure 1 Cause-specific cumulative incidence for acute rejection (AR) at 1 year among high-immunologic risk subgroups. Given the low overall incidence among living donor recipients, all living donor recipients are combined into one group (All LD). Abbreviations: AA, African American; CI, confidence interval; DD, deceased donor; LD, living donor.

shown in Tables 3 and 4, respectively. Independent risk factors for AR at 1 year among DD recipients included recipient age <40 years (hazard ratio [HR] 2.01, $P = 0.021$), AA race (HR 1.95, $P = 0.037$, and repeat transplant (HR 2.26, $P = 0.042$; Table 4). No differences were observed in AR rates based on PRA as a continuous variable or presence of moderate (PRA > 20%) or high (PRA > 80%) pretransplant sensitization. In a separate multivariate analysis of LD transplants only, no independent risk factors for AR were identified and only repeat transplant approached significance (data not shown).

Donor-specific antibodies

dnDSAs occurred in 123 (27%) recipients within the first year at a mean of 4.8 ± 4.2 months post-transplant. While dnDSA rates tended to be higher among DD recipients than in LD recipients, this did not reach statistical significance (28.3% vs. 20.1%, $P = 0.09$). Approximately half of the recipients (51%) exhibited multiple dnDSA specificities, that is, 2 or more dnDSA present on a single measurement. The majority (66%) of patients had dnDSAs, which persisted on at least 2 or more separate measurements. Twenty-eight percent of patients developed "strong" dnDSAs with MFIs of greater than 8000. A significant association was observed with the presence of dnDSA and AR within the first year, with 31 (25%) of the dnDSA-positive patients experiencing AR within the first year, compared with 34 (10%) of the dnDSA-negative patients ($P < 0.0001$). Of the 31 patients with both AR and dnDSA, two rejections were derived from protocol biopsies in the absence of graft dysfunction, while 29 were derived from for-cause biopsies. The diagnosis of AR and DSA occurred within close proximity of each other (mean time between AR and dnDSA 0.2 ± 4.7 months). Rejections occurred more often in patients with multiple dnDSA specificities compared with those with a single dnDSA specificity (29% vs. 14%, $P = 0.02$) and in those with stronger MFIs (>8000) compared with weaker MFIs (41% vs. 15%, $P = 0.0006$). There was no difference in the time to dnDSA detection post-transplant among those with (4.3 months) or without (4.9 months) rejection.

Interestingly, among AAs, there was no difference in the rate of dnDSA at 1 year between DD transplant recipients (32%) and LD recipients (35%, $P = 0.70$). This occurred despite the majority of AA LD recipients

Table 3. Risk factors for acute rejection at 1 year among deceased donor kidney recipients.

	No AR (n = 302)	AR (n = 58)	HR	P-value	95% CI	
Age						
18–29	21 (7.0)	11 (19.0)	(ref)			
30–39	50 (16.5)	13 (22.4)	0.57	0.171	0.26	1.27
40–49	77 (25.5)	12 (20.7)	0.36	0.014	0.16	0.81
50–59	76 (25.2)	12 (24.1)	0.41	0.028	0.19	0.91
≥60	78 (25.8)	8 (13.8)	0.24	0.002	0.10	0.61
Age <40 years, n (%)	71 (24)	24 (41)	2.10	0.006	1.24	3.53
Race, n (%)						
African American	158 (52)	37 (64)	(ref)			
Caucasian	66 (22)	10 (17)	0.69	0.291	0.34	1.38
Hispanic	58 (19)	9 (16)	0.68	0.299	0.33	1.41
Other	20 (7)	2 (4)	0.44	0.259	0.11	1.83
Race, African American, n (%)	158 (52)	37 (64)	1.54	0.114	0.90	2.63
Male gender, n (%)	145 (48)	24 (41)	0.81	0.436	0.48	1.37
Repeat transplant, n (%)	31 (10)	10 (17)	1.72	0.120	0.87	3.39
Preemptive, n (%)	19 (6)	3 (5)	0.81	0.727	0.25	2.60
Pretransplant dialysis duration, years, mean ± SD	4.3 ± 3.2	4.6 ± 3.7	1.04	0.355	0.96	1.12
Cold ischemia time, hours mean ± SD	20.7 ± 8.1	19.6 ± 7.5	0.98	0.300	0.95	1.02
pPRA (%), mean ± SD	47.6 ± 34.7	52.0 ± 33.8	1.00	0.397	1.00	1.01
pPRA ≥ 20%, n (%)	211 (70)	41 (71)	1.03	0.926	0.58	1.81
pPRA ≥ 80%, n (%)	81 (27)	16 (28)	1.05	0.878	0.59	1.86
HLA mismatches out of 6, mean ± SD	4.2 ± 1.7	4.6 ± 1.7	1.13	0.162	0.95	1.35
Cumulative ATG dose, mg/kg, mean ± SD	5.0 ± 1.0	4.8 ± 0.8	0.86	0.312	0.65	1.15
Delayed graft function, n (%)	37 (12)	8 (14)	1.20	0.639	0.57	2.52
Donor age in years, mean ± SD	36 ± 16	35 ± 16	1.00	0.929	0.98	1.02

AR, acute rejection; ATG, antithymocyte globulin; CI, confidence interval; HLA, human leukocyte antigens; HR, hazards ratio (univariate); pPRA, peak panel reactive antibody; SD, standard deviation.

Table 4. Cox proportional hazard model for risk factors of acute rejection at 1 year among deceased donor kidney recipients (multivariate).

	HR	P-value	95% CI
Age <40 years	2.01	0.021	1.11–3.63
Race AA	1.95	0.037	1.04–3.64
Repeat transplant	2.26	0.042	1.03–4.98

AA, African American; CI, confidence interval; HR, hazards ratio.

having received a kidney from an AA donor (86%) or from a related donor (64%), and despite having fewer HLA mismatches compared with AA DD recipients (3.9 ± 1.6 vs. 4.7 ± 1.3 of 6 mismatches, P = 0.0007). In contrast, non-AA DD recipients experienced a significantly higher rate of dnDSA (24%) compared with non-AA LD recipients (9.8%, P = 0.012). This occurred despite a similar degree of HLA mismatching (3.8 ± 1.6 vs. 3.8 ± 2.0 for DD and LD non-AAs, respectively, P = 0.12). Due to similar rates of dnDSA across AA subgroups, AAs were combined into one group (All AA) to depict the rates of dnDSA at 1 year among HIR subgroups (Fig. 2). The rates of dnDSA varied widely among subgroups, ranging from as low as 7% among non-AA LD transplants ≥40 years to 32% among all AA recipients. Risk factors for dnDSA in the entire population by univariate and multivariate analyses are shown in Tables 5 and 6. Independent risk factors for dnDSA

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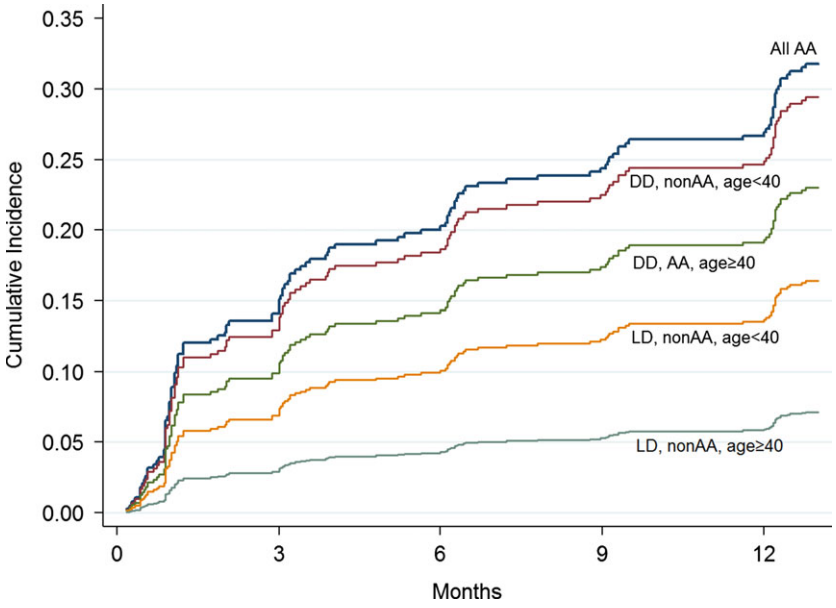


	Sub-hazard ratio	P-value	95% CI	
All AA	5.15	0.005	1.63	16.26
DD, nonAA, age<40	4.69	0.017	1.32	16.73
DD, AA, age≥40	3.53	0.038	1.07	11.58
LD, nonAA, age<40	2.43	0.287	0.47	12.42
LD, nonAA, age≥40	(ref)			

Figure 2 Cause-specific cumulative incidence for *de novo* donor-specific antibody at 1 year among high-immunologic risk subgroups. Given the similarly high incidence of dnDSA among African American (AA) recipients, all AAs are combined into one group (All AA). Abbreviations: AA, African American; CI, confidence interval; DD, deceased donor; LD, living donor.

at 1 year post-transplant among all patients included AA race (HR 1.46, $P = 0.047$), and a greater number of HLA mismatches (HR 1.26 for each mismatch, $P = 0.001$). Male gender on the other hand was associated with a lower risk of dnDSA (HR 0.67, $P = 0.03$; Table 6). Again, no differences were observed in dnDSA rates based on PRA as a continuous variable or the presence of moderate (PRA > 20%) or high (PRA > 80%) pretransplant sensitization.

Graft loss and impact of both AR and dnDSA occurrence within the first year on graft survival

Renal allograft survival rates for the entire cohort were 97%, 89%, and 80% at 12, 36, and 60 months, respectively, post-transplant. A total of 69 graft losses occurred throughout the follow-up period, of which 19 were due to acute rejection or interstitial fibrosis/tubular atrophy associated with recurrent/chronic rejection ("immunologic" graft losses). Of the 19 transplant recipients with immunologic graft losses, 13 occurred in AAs compared with two in Caucasians, three in Hispanic recipients, and one in a patient of Asian ethnicity. Immunologic graft losses accounted for 10.5% of failed grafts among Caucasians compared *with* 34% in non-Caucasians ($P = 0.038$), and 53% of failed grafts *n* recipients <40 years of age, compared with 20% of graft losses in recipients ≥40 years ($P = 0.016$). Among patients aged ≥60 years, the immunologic graft failure rate was 11%.

Thirty-one patients developed both AR and dnDSA within the first post-transplant year. The presence of AR and dnDSA detection within the first year was associated with lower graft survival at 5 years post-transplant (54%) compared with dnDSA without AR (75%, $P = 0.053$), AR without dnDSA (76%, $P = 0.145$) and neither occurrence (85%, $P = 0.001$) within the first year (Fig. 3). Cox proportional hazard modeling identified both AR and dnDSA within the first postoperative year (HR 3.64, $P = 0.001$), DGF (HR 2.27, $P = 0.011$), and DD (HR 2.52, $P = 0.021$) as independent predictors of graft loss at 5 years (Table 7).

Discussion

Lymphocyte-depleting antibody induction therapy is currently recommended in renal transplant recipient

Table 5. Risk factors for donor-specific antibodies at 1 year in all patients.

	No DSA (n = 341)	DSA (n = 123)	HR	P-value	95% CI	
Age in years, mean ± SD						
18–29	29 (8.5)	13 (10.5)	(ref)			
30–39	57 (16.7)	27 (22.0)	0.67	0.314	0.30	1.47
40–49	86 (25.2)	32 (26.0)	0.50	0.078	0.23	1.08
50–59	85 (24.9)	32 (26.0)	0.52	0.098	0.24	1.13
≥60	84 (24.6)	19 (15.5)	0.27	0.005	0.11	0.68
Age <40 years, n (%)	86 (25)	40 (33)	1.79	0.022	1.09	2.95
Race, n (%)						
African American	161 (47)	77 (63)	(ref)			
Caucasian	98 (29)	19 (16)	0.65	0.160	0.35	1.19
Hispanic	59 (17)	22 (18)	0.67	0.278	0.33	1.38
Other	23 (7)	5 (4)	0.36	0.158	0.09	1.49
Race, African American, n (%)	161 (47)	77 (63)	1.63	0.057	0.99	2.68
Male gender, n (%)	172 (50)	50 (41)	0.77	0.298	0.47	1.26
Repeat transplant, n (%)	45 (13)	14 (11)	1.60	0.128	0.87	2.95
Preemptive, n (%)	42 (12)	11 (9)	0.61	0.287	0.24	1.52
Pretransplant dialysis duration, years, mean ± SD	3.9 ± 3.3	4.4 ± 3.2	1.03	0.343	0.96	1.11
pPRA (%), mean ± SD	45.9 ± 33.7	47.2 ± 34.1	1.01	0.120	1.00	1.01
pPRA ≥ 20%, n (%)	239 (70)	83 (68)	1.15	0.615	0.67	1.98
pPRA ≥ 80%, n (%)	80 (24)	29 (24)	1.19	0.539	0.68	2.07
HLA mismatches out of 6, mean ± SD	4.0 ± 1.8	4.7 ± 1.3	1.22	0.022	1.03	1.45
Cumulative ATG dose, mg/kg, mean ± SD	4.9 ± 1.0	4.8 ± 0.9	0.90	0.458	0.68	1.19
Delayed graft function, n (%)	37 (11)	9 (7)	1.31	0.473	0.63	2.75
Donor age in years, mean ± SD	37 ± 15	33 ± 15	0.99	0.487	0.98	1.01
Deceased donor	258 (76)	102 (83)	2.53	0.020	1.16	5.55

ATG, antithymocyte globulin; CI, confidence interval; DSA, donor specific antibodies; HLA, human leukocyte antigen; HR, hazards ratio (univariate); pPRA, peak panel reactive antibody; SD, standard deviation.

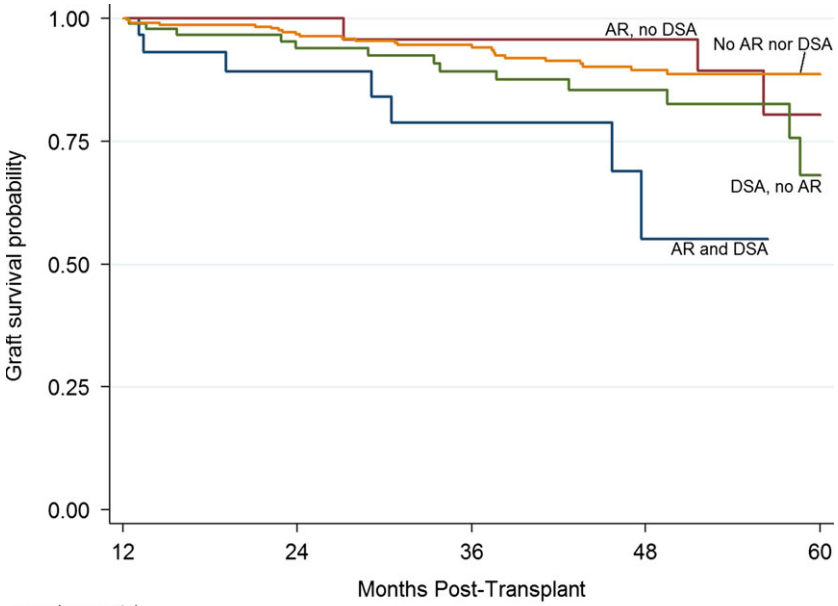
Table 6. Cox proportional hazard model for risk factors for donor-specific antibodies at 1 year among all patients (multivariate).

	HR	P-value	95% CI	
Race AA	1.46	0.047	1.01	2.12
Male gender	0.67	0.030	0.46	0.96
HLA mismatch	1.26	0.001	1.10	1.45

AA, African American; CI, confidence interval; HLA, human leukocyte antigen; HR, hazards ratio.

patients at high risk of rejection [8]. Successful reduction in AR rates in HIR patients has been described in several recent prospective randomized trials, indicating 1-year AR rates as low as 6–15% [1–4]. While differences in long-term graft survival have not been observed based on a specific induction agent, AR is known to be associated with an increased risk of long-term graft failure, thus providing the impetus for widespread use of depleting agents, particularly in high-risk recipients [8,12].

In the current study, we re-examined the incidence and risk factors for AR and dnDSA in a large, diverse, HIR population with a high proportion of AAs in the context of uniform, ATG-based immunosuppression. The 1-year AR incidence of 14% in our study corroborates the favorable results seen in aforementioned studies in HIR patients utilizing lymphocyte-depleting induction. In comparison, AR rates of up to 27% have previous been reported in HIR patients receiving non-depleting induction [1,2]. However, a novel observation from our study is that the AR incidence varied widely, nearly fivefold, among various subgroups of the HIR population. Specifically, younger (<40 years) AA DD recipients experienced a 1-year AR rate of 30%, nearly twice the rate seen in other DD subgroups and almost five times the rate in LD recipients, despite the use of recommended immunosuppressive measures. The low incidence of AR among LD, despite HIR designation as defined by race and sensitization, is most likely



Number at risk

447	353	273	183	84

	HR	P-value	95% CI	
AR and DSA	4.36	0.001	1.86	10.20
AR, no DSA	1.16	0.810	0.35	3.85
DSA, no AR	1.96	0.051	1.00	3.85
No AR nor DSA	(ref)			

Figure 3 Landmark analysis demonstrating graft survival probability over time according to the presence or absence of acute rejection (AR) and/or *de novo* donor-specific antibodies (dnDSA) development within the first post-transplant year. Model only includes those with graft survival through the first post-transplant year. Types of rejection for AR, no DSA group: ACR only, 29; and mixed, 2; and for AR and DSA group: ACR only, 15; mixed, 11; and AMR only, 5.

Table 7. Cox proportional hazard model for risk factors for graft loss at 5 years, adjusting for the presence or absence of acute rejection and/or *de novo* donor-specific antibodies within the first post-transplant year.

	No GF (*n* = 401)	GF (*n* = 63)	HR	*P*-value	95% CI	
AR-DSA						
No AR or DSA	274 (68.3)	33 (52.4)	(ref)			
AR and DSA	22 (5.5)	9 (14.3)	3.64	0.001	1.73	7.66
AR, no DSA	28 (7.0)	6 (9.5)	1.68	0.241	0.70	4.02
DSA, no AR	77 (19.2)	15 (23.8)	1.61	0.128	0.87	2.96
Age <40 years, *n* (%)	110 (27.4)	16 (25.4)	0.88	0.649	0.50	1.55
African American, *n* (%)	203 (50.6)	35 (55.6)	1.13	0.621	0.69	1.86
Male gender, *n* (%)	195 (48.6)	27 (42.9)	0.81	0.420	0.49	1.34
Repeat transplant, *n* (%)	53 (13.2)	6 (9.5)	0.70	0.410	0.30	1.63
Pretransplant dialysis duration in years, mean ± SD	4.0 ± 3.3	4.1 ± 3.3	1.00	0.665	0.94	1.09
pPRA ≥ 80%, *n* (%)	91 (22.7)	18 (28.6)	1.33	0.309	0.77	2.30
HLA mismatches out of 6, mean ± SD	4.1 ± 1.7	4.2 ± 1.7	1.04	0.615	0.89	1.21
Delayed graft function, *n* (%)	34 (8.5)	12 (19.1)	2.27	0.011	1.21	4.26
Donor age in years, mean ± SD	36 ± 15	36 ± 16	1.00	0.802	0.98	1.01
Deceased donor	304 (75.8)	56 (88.9)	2.52	0.021	1.15	5.53

AR, acute rejection; CI, confidence interval; DSA, donor specific antibodies; GF, graft failure; HLA, human leukocyte antigen; HR, hazards ratio; pPRA, peak panel reactive antibody; SD, standard deviation.

attributable to fewer immunologic risks compared with DD recipients such as DGF, ischemic injury, HLA mismatching, and time on dialysis (Table 1). Thus, while the overall rate of rejection appears acceptable and consistent with current literature, a large discrepancy in AR among HIR subgroups is masked, even in the setting of potent immunosuppression.

Our data also indicated that 26% of HIR patients developed dnDSA within the first year. This figure is higher than recent reports which may reflect differences in the populations studied, criteria for determination of a positive dnDSA, and the frequency of dnDSA monitoring [13–16]. Consistent with other reports, dnDSA was associated with rejection, particularly when dnDSAs against multiple donor antigens were present and with higher strengths of the immunodominant dnDSA [16,17]. Confirming our previous report in both low- and high-risk patients, and those of Cooper *et al.*, the presence of both AR and dnDSA was associated with reduced graft survival [9,15]. One important difference, however, in the current study we found is that AR and dnDSA need only to occur within first year to be associated with a fourfold greater risk of graft loss at 5 years compared with those free from both events. This finding has several implications. First, it underscores the negative impact of these combined events on renal allograft survival even in the setting of potent immunoprophylaxis; second, it emphasizes the fact that reduction in AR alone may be insufficient as a surrogate when investigating immunosuppressive regimens targeted at the HIR population. Lastly, this finding identifies a group of HIR patients who may require more intense induction immunosuppression, such as combined T- and B-cell-targeted therapies, and/or more frequent surveillance extending beyond the first year of transplant. In addition, this subgroup may present a population suited for further research into the area of noninvasive biomarkers of allograft rejection [18].

African American transplant recipients have been known to experience inferior allograft survival rates due to both immunologic and nonimmunologic factors [19–21]. This holds true even in recent studies under contemporary maintenance immunosuppression [22–24]. Our study confirms that even in the setting of uniform ATG induction, AA race was an independent risk factor for AR among DD recipients. On the other hand, AA race was not a risk factor for rejection among the LD cohort. This may have been a result of the greater degree of HLA-matching AA LD recipients, although counterintuitive with this idea was our finding that dnDSAs were found in nearly equal proportions of living and DD AA recipients. The high AR rate in younger AAs suggests that perhaps our cumulative dose of ATG may not have been adequate for this subgroup or that they may have be more sensitive to weaning of maintenance agents performed in the entire population. Concordant with the latter point are studies emphasizing the importance of adequate exposure to maintenance agents among HIR recipients [25,26]. However, the fact that AA race was also an independent risk factor for dnDSA may support a role for additional therapies targeted at preventing or reducing dnDSA in this subgroup. Results of trials evaluating B-cell-targeted therapies in addition to ATG, such as those recently reported by Ejaz, *et al.*, will be of particular interest as our data identify a subgroup of patients at greater risk of AR and dnDSA even in the absence of pretransplant donor-specific sensitization [27].

Younger age was identified as an independent risk factor for AR at 1 year among DD recipients. Moreover, immunologic graft losses occurred at twice the rate in younger recipients. This emphasizes the importance of age, which although has been associated with rejection, is not typically included in as part of HIR designation in immunosuppression trials. Furthermore, as the effects of lymphocyte depletion begin to diminish toward the end of the first year, younger patients may remain at a heightened risk of rejection particularly in the setting of habitual weaning of immunosuppressants, dose reductions during concomitant infections, and in the setting of medication nonadherence, which is a known barrier to long-term allograft survival in the younger transplant population [28,29].

As a retrospective, observational study, our study is subject to limitations. Whereas we did not anticipate any particular subgroup to have had major reductions in immunosuppression, we could not control for individual patient dose modifications that may have occurred due to adverse effects, infection, or nonadherence. Patients with pre-existing DSA were excluded from the analysis, given that several LD recipients were desensitized with additional antibody-targeted therapies (while most DD recipients were not) and that many of these patients had early rejections, thus potentially confounding our AR incidence. A separate evaluation of this subset of patient under ATG induction is worthy of study, nonetheless. While biopsies in dnDSA-positive patients were protocol-driven, we lacked protocol biopsy data in dnDSA-negative patients with otherwise stable graft function and were therefore unable to detect cases of subclinical rejection. Finally, as C1q testing was not performed at our center during this time, we were unable to comment on

any potential associations between C1q-positivity and clinical outcomes. However, some studies suggest that C1q positivity is independently driven by the strength (MFI) of the dnDSA and may not be necessary [30].

While immunologic outcomes in HIR recipients are reportedly mitigated by the use of lymphocyte-depleting antibody induction, our experience demonstrates that a seemingly acceptable rate of rejection masks large disparities in rejection rates among HIR subgroups. Younger, AA DD recipients in particular continue to exhibit remarkably higher rates of rejection than other subgroups, and AA recipients as a whole exhibit high rates of dnDSA during the first post-transplant year. These findings may argue for further delineation of HIR patients and a more individualized approach to immunosuppression in certain subgroups of this population.

Authorship

SJP: participated in the performance of the research, data analysis, and writing of the paper. WNS: participated in the writing of the paper. JLD: participated in the performance of the research and writing of the paper. EAG: participated in the writing of the paper and data analysis. DTN: participated in the writing of the paper and data analysis. RJK: participated in the writing of the paper. SAK: participated in the performance of the research and writing of the paper. LDT: participated in the data analysis. LWM: participated in the writing of the paper. LWG: participated in the writing of the paper. AOG: participated in the writing of the paper.

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Conflicts of interest

The authors declare no conflicts of interest.

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