ORIGINAL ARTICLE

Racial differences in incident *de novo* donor-specific anti-HLA antibody among primary renal allograft recipients: results from a single center cohort study

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The findings of the research in this manuscript support the idea that African American renal transplant recipients are at a higher risk of developing de novo donor-specific anti-HLA antibodies (dnDSA). This risk of dnDSA in African American is strongly related to inadequate immunosuppression and receiving a deceased or living-unrelated allograft. Finally, this research shows that although dnDSA leads to poor outcomes, it is not influenced by a recipient's race/ethnicity.

SUMMARY

Controversy exists as to whether African American (AA) transplant recipients are at risk for developing *de novo* donor-specific anti-human leucocyte antigen (HLA) antibody (dnDSA). We studied 341 HLA-mismatched, primary renal allograft recipients who were consecutively transplanted between 3/1999 and 12/2010. Sera were collected sequentially pre- and post-transplant and tested for anti-HLA immunoglobulin G (IgG) via single antigen bead assay. Of the 341 transplant patients (225 AA and 116 non-AA), 107 developed dnDSA at a median of 9.2 months post-transplant. AA patients had a 5-year dnDSA incidence of 35%. This was significantly higher than the 5-year dnDSA incidence for non-AA patients (21%). DQ mismatch (risk) and receiving a living-related donor (LRD) transplant (protective) were transplant factors associated with dnDSA. Within the AA patient cohort, HLA-DQ mismatch, not-receiving a LRD transplant, nonadherence and BK viraemia were the most common factors associated with early dnDSA (occurring <24 months post-transplant). Nonadherence and pretransplant diabetes history were the strong precursors to late dnDSA. Despite the higher rates of dnDSA in the AA cohort, post-dnDSA survival was the same in AA and non-AA patients. This study suggests that DQ matching, increasing LRD transplantation in AA patients and minimizing under-immunosuppression will be key to preventing dnDSA.

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Introduction

Development of immunoglobulin G (IgG) donor-specific anti-human leucocyte antigen (HLA) antibodies (DSA) following transplant is a risk factor for and possible cause of allograft loss [1–4]. *De novo* DSA (dnDSA) is most likely to emerge in the first 2 years post-transplant [5–8]. By 5 years post-transplant, almost one-fifth of all primary renal transplant recipients without preformed DSA become dnDSA positive [5]. dnDSA often first appears when allograft function is stable. However, following dnDSA, many patients will experience rejection episodes and allograft failure.

In the United States, some reports have shown that African American (AA) transplant recipients may be more likely to develop dnDSA than non-AA transplant recipients [7,9,10]. Similarly, reports have shown that AA transplant recipients have poor allograft survival and experience higher rates of acute rejection [11–15]. The reason for the greater propensity for allograft failure among AA transplant recipients is not fully understood. Some reasons that have been given include immunosuppression choice/dosing, socio-economic status and medication nonadherence [16–19]. It is also possible that dnDSA plays a major role. Furthermore, the factors that precede and possibly predispose patients to developing dnDSA may differ or have differing relative impacts on AA and non-AA transplant recipients and need to be further investigated.

In this report, we describe the incidence of and risk for dnDSA in AA transplant recipients. The cohort of patients transplanted at East Carolina University is a well-characterized cohort of AA and non-AA transplant patients with 5–15 years of post-transplant and 3–5 years of post-dnDSA follow-up.

Subjects and methods

Patients

All renal transplant patients who received living-related (LRD), living-unrelated (LURD) or deceased donor (DD) transplants between 3/1999 and 12/2010 were enrolled. All patients underwent a standard pretransplant evaluation. At time of transplant, all patients were tested for reactivity to their donors via complement-dependent cytotoxicity cross-match (XM). Flow cytometric XM was performed on all living-donor transplants. Starting in 2010, all final XM were performed using flow cytometry. Patients' pretransplant sera (both historical in most patients and at the time of transplant in all patients) were tested using LABScreen® single antigen beads to detect alloantibodies that would be considered dnDSA. Tissue typing was performed using both serology and polymerase chain reaction-single-specific-primer (SSP) methods for HLA-A, HLA-B, HLA-DR and HLA-DQ antigens. Patients found to have donor reactive alloantibodies present in circulation (and detected via XM or single antigen bead assay) were excluded.

Study protocol

Testing and the use of patient data were approved by the East Carolina University Brody School of Medicine Institutional Review Board for human studies. All clinical and research activities are consistent with the Principles of the Declaration of Istanbul.

Immunosuppression

Per protocol, patients with a panel reactive antibody (PRA) <20% and without delayed allograft function received anti-interleukin-2 (IL-2) induction, while patients with a PRA >20% or delayed allograft function received rabbit anti-thymocyte globulin induction. Maintenance immunosuppression included a calcineurin inhibitor (CNI), mycophenolate mofetil (MMF) or equivalent, and a prednisone taper. The prednisone taper started at the time of transplant was reduced to 5 mg per day by 1 month post-transplant after which the dose remained at 5 mg/day. Patients primarily received anti-IL-2 induction (79%) (Table 1). The CNI used was tacrolimus in 49% or cyclosporine in 48% of patients. Mycophenolate doses generally ranged between 2 and 3 g/day throughout the post-transplant follow-up. Patients remained on maintenance immunosuppression at similar intensity (regardless of race) throughout the study period. Immunosuppression was only lowered for cases of suspected drug toxicity. No new therapeutic agents were added to specifically treat dnDSA. Additional immunosuppressive agents were added in cases of rejection. Rejection episodes were initially treated with steroids. Rabbit anti-thymocyte globulin was used to treat biopsy-proven acute cellular rejection (TCMR). If the biopsy was consistent with AMR, the patient received additional therapy such as plasmapheresis, rituximab and/or intravenous immune globulin (IVIg).

Nonadherence definition

Nonadherence was defined as missing clinic visits, missing repeated laboratory visits, arriving to the emergency

Table 1. Patient demographics.

	Entire Cohort	All dnDSA IgG positive	African American			Non-African American			AA DSA+ versus non-AA DSA+ *P*-value*
			dnDSA IgG negative	dnDSA IgG positive	*P*-value	dnDSA IgG negative	dnDSA IgG positive	*P*-value	
Number of subjects	341	107	143	82	–	91	25	–	–
Patient characteristics									
Mean age (SD)	51 (12)	48 (13)	49 (10)	48 (11)	0.48	53 (12)	51 (17)	0.50	0.30
Recipient transplant age <30, *n* (%)	20 (6)	11 (10)	5 (4)	7 (9)	0.11	4 (5)	4 (16)	0.04	0.28
Male, *n* (%)	201 (59)	67 (62)	75 (52)	51 (62)	0.15	59 (65)	16 (64)	0.94	0.99
African American, *n* (%)	225 (66)	82 (77)	–	–	–	–	–	–	–
Cause for end-stage renal disease									
Hypertension	220 (65)	69 (65)	108 (76)	53 (65)	0.07	43 (47)	16 (64)	0.14	0.99
Diabetes	119 (35)	39 (36)	50 (35)	28 (34)	0.87	30 (33)	11 (44)	0.31	0.48
Received pretransplant dialysis, *n* (%)	259 (75)	84 (79)	117 (81)	68 (83)	0.83	58 (64)	16 (64)	0.98	0.04
College graduate- Associate degree or above, *n* (%)	28 (14)	5 (5)	12 (13)	4 (8)	0.40	11 (23)	1 (8)	0.22	0.94
Donor characteristics									
African American donor, *n* (%)	108 (32)	37 (35)	65 (45)	34 (41)	0.57	6 (7)	3 (12)	0.37	0.007
Female donor, *n* (%)	161 (47)	43 (40)	67 (47)	34 (42)	0.43	51 (56)	9 (36)	0.08	0.63
Transplant characteristics									
Deceased donor, *n* (%)	189 (55)	70 (65)	90 (63)	62 (75)	0.06	29 (32)	8 (32)	0.99	<0.001
Living-unrelated donor, *n* (%)	58 (17)	16 (15)	9 (6)	7 (9)	0.52	33 (36)	9 (36)	0.98	<0.001
Living-related donor, *n* (%)	94 (28)	21 (20)	44 (31)	13 (16)	0.01	29 (32)	8 (32)	0.99	0.08
Delayed allograft function, *n* (%)	39 (12)	17 (16)	15 (11)	16 (20)	0.06	7 (8)	1 (4)	0.52	0.12
Mean total HLA mismatch (SD)	3.7 (1.6)	4.9 (1.6)	4.7 (1.8)	5.5 (1.4)	<0.001	4.4 (1.8)	4.9 (1.4)	0.25	0.06
DQ mismatch >0, *n* (%)	272 (80)	97 (91)	102 (71)	76 (93)	<0.001	73 (80)	21 (84)	0.67	0.19
Mean Pretransplant PRA Antibody (SD)	7 (19)	9 (23)	9 (20)	9 (23)	0.99	3 (13)	8 (23)	0.16	0.85
Immunosuppression									
Thymoglobulin induction, *n* (%)	71 (21)	24 (22)	40 (28)	19 (23)	0.68	7 (8)	5 (20)	0.08	0.99
Tacrolimus-based maintenance, *n* (%)	168 (49)	48 (45)	75 (52)	36 (44)	0.29	45 (49)	12 (48)	0.85	0.82
Post-transplant BK virus viraemia history, *n* (%)	23 (7)	15 (14)	5 (3)	13 (16)	0.001	3 (3)	2 (8)	0.31	0.32
Post-transplant nonadherence history, *n* (%)	35 (10)	26 (24)	7 (5)	25 (30)	<0.001	2 (2)	1 (4)	0.62	0.007
Rejection									
At least 1 acute rejection episode, *n* (%)	101 (29)	47 (44)	36 (25)	35 (43)	0.008	18 (19)	12 (48)	<0.001	0.65
Acute rejection in the first 6 months post-transplant	42 (12)	13 (12)	17 (12)	9 (11)	0.84	12 (13)	4 (16)	0.73	0.49

Table 1. Continued.

	Entire Cohort	All dnDSA IgG positive	African American			Non-African American			AA DSA+ versus non-AA DSA+
			dnDSA IgG negative	dnDSA IgG positive	*P*-value	dnDSA IgG negative	dnDSA IgG positive	*P*-value	*P*-value*
First acute rejection episode classification									
TCMR, *n* (% of rejection episodes)	68 (67)	22 (47)	29 (81)	16 (46)	0.009	17 (94)	6 (50)	0.018	0.58
AMR only, *n* (% of rejection episodes)	4 (4)	3 (6)	1 (3)	3 (9)		0 (0)	0 (0)		
Mixed, *n* (% of rejection episodes)	29 (29)	22 (47)	6 (17)	16 (46)		1 (6)	6 (50)		
Patients with >1 acute rejection episode	32 (31)	16 (34)	10 (7)	11 (13)	0.79	6 (7)	5 (20)	0.57	0.52
Allograft function and status									
Months of follow-up†, median (range)	73.1 (1–179)	57 (10–168)	86 (1–179)	58 (12–168)	0.004	76 (10–170)	51 (10–168)	0.18	0.52
Patient death-censored allograft loss, *n* (%)	88 (26)	41 (38)	38 (27)	33 (40)	0.03	9 (10)	8 (32)	0.006	0.62

IgG, immunoglobulin G; AA, African American; SD, standard deviation; HLA, human leucocyte antigen; PRA, panel reactive antibody; dnDSA, de novo donor-specific anti-HLA antibody; TCMR, T-cell-mediated rejection; AMR, antibody-mediated rejection; Mixed, AMR+TCMR.

**P*-value compares African American dnDSA-positive and non-African American dnDSA-positive groups.

†Follow-up time is not censored for death or allograft loss. All *P*-values are for comparison across all three groups listed.

room or clinic with undetectable CNI levels, and/or chart documentation of nonadherence to medication regimens.

Acute rejection

Acute rejection was defined as an increase in serum creatinine of at least 20% above baseline serum creatinine with histologic evidence on renal allograft biopsy. Diagnosis of acute rejection was based on Banff 1997 criteria as revised in 2005 [20,21].

BK Virus (BKPyV) Viraemia testing, diagnosis and management

Plasma BKPyV load was quantified using quantitative real-time polymerase chain reaction (PCR), providing resultant values reported as BKPyV DNA copies per millilitre (copies/ml). BKPyV PCR testing was primarily conducted with the assay from Quest Diagnostics and Focus Diagnostics (Cypress, CA, USA). In patients transplanted through 2006, timing and frequency of screening for BKPyV viraemia post-transplant depended on the treating physician. In patients transplanted from 2007 on, screening for BKPyV viraemia occurred every 6 months post-transplant, with all patients receiving the first testing within 6 months post-transplant. Plasma BKPyV viral load above detectable levels (>500 copies/ml) for more than two separate samples (at least 14 days apart) was considered positive for the study.

Treatment of BKPyV viraemia was based on current recommendations and was managed in a standardized manner, which started with a 25% or more reduction or discontinuation of MMF at the time of initial determination of serial positivity of BKPyV viraemia. Patients were re-assessed weekly and if BKPyV viraemia did not respond, additional reduction of MMF occurred. In all cases, the CNI reduction was done at the time of BKPyV viraemia detection and the degree of reduction varied per patient with respect to the time post-transplant, history of rejection episodes and per the nephrologist's discretion. All patients were maintained on a CNI and prednisone during the course of treatment. In each case in which MMF was discontinued for BKPyV viraemia, the transplant patient received additional treatment with leflunomide (if deemed appropriate) immediately.

Anti-HLA-specific IgG antibody monitoring and testing

Pretransplant sera, from both historical and at-transplant samples, were tested with LABScreen® Single Antigen

Class I and II beads (One Lambda Inc., Canoga Park, CA, USA). Post-transplant patients were routinely monitored for HLA IgG Class I and II antibodies using LABScreen® Mixed beads (One Lambda Inc.) at 1, 3, 6, 9 and 12 months annually, and when clinically indicated. Samples that tested positive on LABScreen® Mixed beads were also tested using LABScreen® Single Antigen Class I and II beads (One Lambda Inc.) to determine antibody specificity. If a patient was found to be positive on LABScreen® Single Antigen, all previous samples tested with the LABScreen® mixed antigen product were tested via the single antigen platform. All LABScreen® single antigen tests were performed according to the manufacturer's protocol using a 1:3 sera dilution. dnDSA in this manuscript are reported at the level of 'low-resolution' typing. 'Low-resolution' typing is DNA-based typing results at the level of the digits comprising the first field in the DNA-based nomenclature (example: A*01).

For mean fluorescence intensity (MFI) reporting in the case of a dnDSA that had multiple single antigen bead alleles (i.e. A2), MFI of the highest allele bead was reported. dnDSA were considered positive if not present at time of transplantation (i.e. MFI <1000) and a post-transplant sera revealed an antibody with a normalized MFI via single antigen bead of 1000 or greater. If the sera were not positive on two consecutive samples, it was considered transient dnDSA.

Statistical methods

All statistical analyses were performed using STATA/MP version 14.1 (College Station, TX, USA). A two-sided *P*-value of less than 0.05 was considered statistically significant. Observations between groups were compared using the Fisher's exact or Chi-square test for categorical variables. Unpaired *t* test, one-way ANOVA or Kruskal–Wallis test were used for continuous variables. Competing-risks regression by the method of Fine and Gray was used to prepare the cumulative incidence functions for dnDSA [22]. Stepwise Cox proportional hazard modelling was used to analyse predictors of dnDSA. Variables with a *P* value less than 0.10 on univariate analysis were included in the multivariate model. Kaplan–Meier analysis was used to determine probability of survival with a log-rank used to compare groups.

Results

During >5 years of post-transplant follow-up of the 341 adult, HLA-mismatched, pretransplant dnDSA-negative, XM-negative, primary renal transplants recipients transplanted between 3/1999 and 12/2010, dnDSA occurred in 107 transplant patients ($n = 341$). Most of the patients developed class II dnDSA alone or with concomitant class I dnDSA (85% for AA and 84% for non-AA patients). The median time to dnDSA was 9.2 months (range 1–123 months). Ninety-nine patients had 3-year and 70 patients had 5-year follow-up from the first date of dnDSA detection (Fig. 1).

dnDSA incidence is higher is in African American renal transplant recipients

The cumulative 5-year incidence of dnDSA in AA transplant recipients was much higher than in non-AA patients (35% vs. 21%, $P = 0.006$, Fig. 2a). Of the HLA-DQ-mismatched patients, AA patients with DD transplants were at a higher risk of developing dnDSA within the first 5 years post-transplant compared to the DD non-AA patients ($P = 0.03$, Fig. 2b). Within the cohort of DQ-mismatched LURD transplants, AA patients were at a higher (but not statistically significant) risk of developing dnDSA when compared to non-AA patients. Both non-AA and AA patients receiving LRD transplants had a similar incidence of dnDSA over the first 5 years post-transplant.

Pre- and early post-transplant patient characteristics differed between AA patients who did and did not develop dnDSA (Table 1). These characteristics also differed between dnDSA-positive AA transplant patients and dnDSA-positive non-AA patients. As compared to AA transplant patients without dnDSA, AA patients who developed dnDSA were more likely to be DQ mismatched and had a higher number of HLA mismatches (based on HLA-A, HLA-B, HLA-DR and HLA-DQ typing). AA patients with dnDSA were also more likely to have episodes of BK viraemia and had a higher rate of medication nonadherence than AA transplant recipients without dnDSA. In non-AA transplant recipients, only young age was shown to be correlated with dnDSA. Between dnDSA-positive patients (AA and non-AA), AA transplant recipients with dnDSA were more likely to have a DD transplant and had a higher rate of medication nonadherence than non-AA patients with dnDSA.

We also compared the incidence of dnDSA in AA and non-AA transplant recipients by nonadherence and BK viraemia to see whether these factors alone were the reasons for the dnDSA racial disparity (Fig. 2c,d). We were unable to find a statistically higher incidence of dnDSA in the AA cohort (over the non-AA) cohort based on nonadherence or BK viraemia. The number of cases in the non-AA cohort was small (for BK viraemia

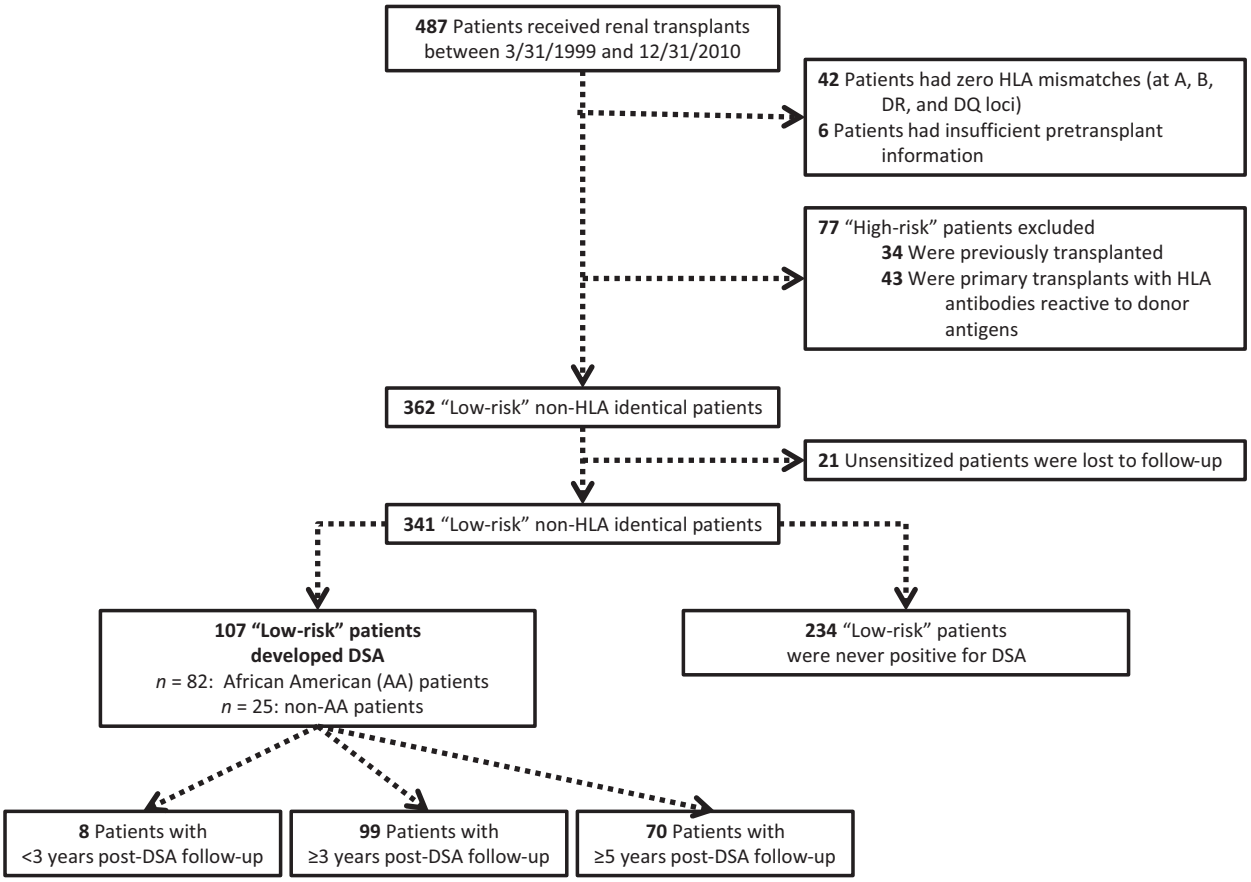

Figure 1 Breakdown of patient selection.

and nonadherence) and the rate of dnDSA was numerically higher for nonadherent AA and BK viraemic AA patients compared to the respective non-AA patient cohorts. It is likely that some of the difference in AA and non-AA patients may be related to these post-transplant events. However, not all of the disparity in dnDSA can be explained by medication nonadherence and/or low immunosuppression following BK viraemia.

Because dnDSAs were significantly more common in AA transplant recipients and because little data exist in this cohort alone, we restricted the Cox regression analyses of predictive variables to data from AA transplant recipients. In the bivariate model of pre- and post-transplant predictors (Table 2), DD transplant recipient, pretransplant history of hypertension, presence of HLA-DR mismatch, presence of HLA-DQ mismatch, documented history consistent with immunosuppression nonadherence and pre-dnDSA development of BKPyV viraemia were predictive for dnDSA development. Conversely, an AA patient receiving a LRD transplant significantly decreased the risk of DSA by 70%. Collectively, these protective and risk factors entered the multivariate model. The multivariate model suggests that the following three variables were the risk factors of dnDSA in AA transplant recipients: pre-dnDSA development of BKPyV viraemia, documented history consistent with immunosuppression nonadherence, and presence of HLA-DQ mismatch. Additionally, receiving a LRD transplant was shown to be dnDSA protective. As DQ mismatch was the strongest predictor of dnDSA, we evaluated the cohort of only DQ-mismatched AA patients to see whether the same variables were also predictive of developing DSA. On multivariate analysis of the DQ-mismatched AA patient cohort, LRD transplant was found to be protective as it had a significantly lower dnDSA rate than LURD and DD transplants. Conversely, documented history consistent with immunosuppression nonadherence and pre-dnDSA development of BKPyV viraemia were associated with a higher risk of dnDSA development in DQ-mismatched AA transplant recipients.

Early versus late dnDSA in African American renal transplant recipients

We aimed to determine whether some variables were more indicative of early or late dnDSA in AA transplant

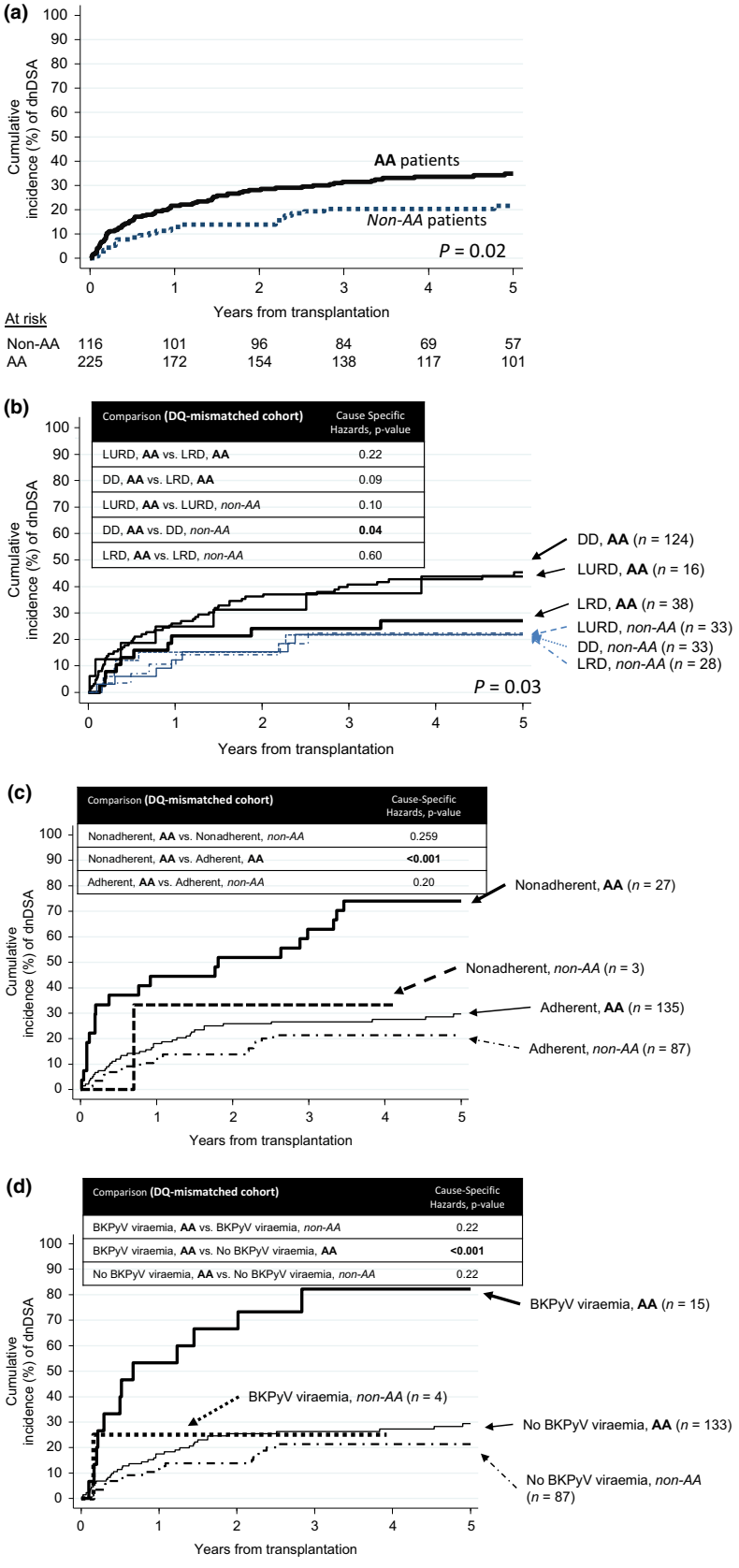

Figure 2 *De novo* DSA incidence stratified by race (in panel a). A further stratification by donor type (in panel b) shows AA living-unrelated and AA deceased donor AA patients are at the highest risk of developing *de novo* donor-specific anti-HLA IgG antibody/antibodies (dnDSA). The risk for dnDSA in the AA cohort is driven primarily by nonadherence (panel c) and BKPyV viraemia (and the associated immunosuppression minimization) as shown in panel d. AA, African American; LURD, living-unrelated donor; LRD, living-related donor; DD, deceased donor.

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Table 2. Bivariate and multivariate analyses of predictors of dnDSA appearance in African American transplant recipients.

	All dnDSA		DQ DSA*		Early IgG dnDSA		Late IgG dnDSA	
	HR (95% CI) *n* = 225 (82 dnDSA+)	*P*-value	HR (95% CI) *n* = 178 (65 DQ dnDSA+)	*P*-value	HR (95% CI) *n* = 225 (62 dnDSA+)	*P*-value	HR (95% CI) *n* = 225 (20 dnDSA+)	*P*-value
Univariate analysis								
Patient/donor characteristics								
Recipient transplant age <30	1.7 (0.8–3.8)	0.16	2.0 (0.8–4.5)	0.19	1.7 (0.7–4.2)	0.28	2.0 (0.5–8.6)	0.37
Male	1.4 (0.9–2.2)	0.12	1.4 (0.9–2.3)	0.18	1.3 (0.8–2.2)	0.31	1.9 (0.7–4.5)	0.19
Pretransplant hypertension	0.6 (0.4–1.0)	0.05	0.9 (0.5–1.6)	0.74	0.7 (0.4–1.1)	0.13	0.5 (0.2–1.3)	0.19
Pretransplant diabetes	1.0 (0.6–1.5)	0.86	0.7 (0.4–1.2)	0.22	0.6 (0.4–1.1)	0.12	2.9 (1.2–7.2)	0.02
African American donor	0.8 (0.5–1.2)	0.27	1.0 (0.6–1.6)	0.85	0.7 (0.4–1.2)	0.25	0.9 (0.4–2.2)	0.84
Female donor	0.8 (0.5–1.3)	0.45	0.8 (0.5–1.3)	0.39	0.9 (0.5–1.5)	0.79	0.6 (0.2–1.5)	0.29
Pretransplant dialysis history	1.0 (0.6–1.8)	0.95	0.8 (0.4–1.5)	0.47	0.9 (0.5–1.6)	0.63	2.0 (0.5–8.6)	0.36
Transplant characteristics								
Deceased donor	1.6 (1.1–2.6)	0.04	1.5 (0.9–2.7)	0.12	1.5 (0.9–2.7)	0.13	2.0 (0.8–5.2)	0.16
Living-unrelated donor	1.2 (0.5–2.5)	0.70	0.9 (0.4–2.2)	0.88	1.2 (0.5–2.9)	0.72	1.1 (0.3–4.9)	0.87
Living-related donor	0.3 (0.1–0.6)	0.001	0.2 (0.1–0.7)	0.005	0.2 (0.1–0.6)	0.006	0.4 (0.1–1.2)	0.12
Delayed allograft function	1.4 (0.7–2.7)	0.31	1.4 (0.6–2.9)	0.41	1.8 (0.9–3.6)	0.08	1.7 (0.4–4.7)	0.90
HLA-A mismatch >0	1.4 (0.7–3.0)	0.32	1.2 (0.6–2.7)	0.60	1.7 (0.7–4.1)	0.28	1.1 (0.3–3.7)	0.90
HLA-B mismatch >0	1.9 (0.8–4.7)	0.17	1.5 (0.6–3.7)	0.38	2.1 (0.7–6.8)	0.20	1.5 (0.3–6.5)	0.58
HLA-DR mismatch >0	2.2 (1.0–4.8)	0.05	1.1 (0.5–2.5)	0.75	3.7 (1.2–11.7)	0.03	1.1 (0.4–3.2)	0.90
HLA-DQ mismatch >0	4.1 (1.8–9.3)	0.001	–	–	4.3 (1.6–12.0)	0.005	3.5 (0.8–15.2)	0.09
HLA mismatch >4	2.6 (1.6–4.4)	<0.001	1.7 (1.0–3.1)	0.06	2.8 (1.5–5.2)	0.001	2.3 (0.9–5.9)	0.07
Pretransplant PRA >20	0.9 (0.5–1.8)	0.87	0.8 (0.4–1.9)	0.63	1.1 (0.6–2.3)	0.76	0.4 (0.1–3.0)	0.37
Immunosuppression								
Thymoglobulin induction	0.9 (0.6–1.5)	0.79	0.7 (0.4–1.3)	0.25	1.1 (0.6–2.8)	0.48	0.5 (0.2–1.8)	0.31
Tacrolimus maintenance	0.9 (0.6–1.4)	0.71	0.8 (0.5–1.3)	0.37	1.1 (0.7–1.8)	0.74	0.5 (0.2–1.4)	0.17
Post-transplant events								
Acute rejection prior to 6 months post-transplant	1.1 (0.5–2.1)	0.86	0.9 (0.4–2.0)	0.77	1.3 (0.6–2.8)	0.48	0.4 (0.1–3.2)	0.41
Acute rejection episode prior to dnDSA	0.7 (0.4–1.4)	0.32	1.0 (0.5–2.1)	0.93	0.6 (0.3–1.3)	0.20	1.3 (0.4–4.6)	0.70
Nonadherence history	3.3 (2.1–5.3)	<0.001	3.3 (2.0–5.6)	<0.001	2.1 (1.1–3.8)	0.02	11.1 (4.5–27.0)	<0.001
BKPyV viraemia history	3.0 (1.7–5.5)	<0.001	3.0 (1.6–5.8)	0.001	2.7 (1.4–5.3)	0.004	4.5 (1.3–15.4)	0.02
Multivariate analysis								
HLA-DQ mismatch	3.2 (1.3–7.4)	0.007	–	–	3.4 (1.2–9.5)	0.02	–	–
Living-related donor	0.4 (0.2–0.9)	0.03	0.3 (0.1–0.9)	0.036	0.3 (0.1–0.9)	0.04	–	–
Pretransplant diabetes	–	–	–	–	–	–	4.2 (1.7–10.9)	0.003
Nonadherence history	2.9 (1.8–4.6)	<0.001	2.9 (1.7–4.9)	<0.001	1.8 (1.1–3.4)	0.04	14.3 (5.7–35.5)	<0.001
Pre-dnDSA BKPyV viraemia history	2.6 (1.4–4.8)	0.002	2.6 (1.4–5.1)	0.004	2.3 (1.2–4.6)	0.02	–	–

IgG, immunoglobulin G; HLA, human leucocyte antigen; PRA, panel reactive antibody; dnDSA, de novo DSA; BKPyV, BK virus.

*Risk factors for DQ –DSA were evaluated in the HLA-DQ-mismatched cohort (*n* = 178).

Table 3. Early and Late DSA characteristics in African American dnDSA-positive patients.

	Early dnDSA (≤24 months from transplant)	Late dnDSA (>24 months from transplant)	*P*-value
Number of subjects	62	20	
Patient characteristics			
Mean age (SD)	47 (12)	49 (11)	0.55
Recipient transplant age <30, *n* (%)	5 (8)	2 (10)	0.79
Male, *n* (%)	38 (61)	13 (65)	0.77
African American, *n* (%)	62 (100)	20 (100)	–
Cause for end-stage renal disease			
Hypertension	41 (66)	12 (60)	0.62
Diabetes	16 (26)	12 (60)	0.005
Transplant characteristics			
Deceased donor, *n* (%)	48 (77)	14 (70)	0.50
Living-unrelated donor, *n* (%)	5 (8)	2 (10)	0.78
Living-related donor, *n* (%)	9 (15)	4 (20)	0.55
Delayed allograft function, *n* (%)	14 (22)	2 (10)	0.21
DQ mismatch >0, *n* (%)	58 (94)	18 (90)	0.60
Mean Pretransplant PRA Antibody (SD)	11.1 (26)	1.8 (6.7)	0.11
Immunosuppression			
Thymoglobulin induction, *n* (%)	16 (26)	3 (15)	0.21
Tacrolimus-based maintenance, *n* (%)	31 (50)	5 (25)	0.05
Post-transplant BK virus viraemia history, *n* (%)	10 (16)	3 (15)	0.88
Post-transplant nonadherence history, *n* (%)	14 (23)	11 (55)	0.006
dnDSA characteristics			
Class I alone	9 (15)	3 (15)	0.842
Class II alone	33 (53)	12 (60)	
Both	20 (32)	5 (25)	
Acute rejection characteristics			
At least 1 acute rejection episode, *n* (%)	30 (49)	5 (25)	0.07
Acute rejection in the first 6 months post-transplant, *n* (%)	8 (13)	1 (5)	0.33
Acute rejection occurred prior to dnDSA development, *n* (%)	8 (29)	3 (15)	0.81
Median months from dnDSA to the first acute rejection (range)†	7.6 (0–141.8)	1.9 (0–3.8)	0.42
First acute rejection episode classification			
TCMR, *n* (% of rejection episodes)	14 (23)	2 (11)	0.16
AMR only, *n* (% of rejection episodes)	3 (5)	0 (0)	
Mixed, *n* (% of rejection episodes)	14 (23)	2 (11)	
% of first acute rejection cases with a TCMR BANFF grade (IIA or IIB) or above	13%	25%	0.51
% of acute rejection patient's with a C4d-positive biopsy finding*	39%	50%	0.68
Allograft function and status			
Patient death-censored allograft loss, *n* (%)	23 (37)	10 (50)	0.31

*Eight patients with rejection were not tested for C4d positivity (percentages out of the following sample sizes: early dnDSA, $n = 23$; late dnDSA, $n = 4$).

†Excluded cases in which acute rejection appeared prior to dnDSA.

patients. Early dnDSA was defined as dnDSA detected in the first 24 months post-transplant ($n = 62$). Late dnDSA was defined as dnDSA appearing after 24 months post-transplant ($n = 20$). Generally, patient characteristics did not differ between the early and late AA dnDSA groups (Table 3). The rates of acute rejection, C4d-positive acute rejection and class of dnDSA were similar irrespective of time of dndSA onset.

For early dnDSA, the Cox regression multivariate model for predictors of dnDSA was similar to what was found for the entire AA dnDSA cohort. The impact of pre-dnDSA development of BKPyV viraemia and documented history consistent with immunosuppression nonadherence were slightly lower compared the entire dnDSA cohort. For late dnDSA, fewer variables were associated with late dnDSA development. The final

multivariate model differed from the early dnDSA models. Only end-stage renal disease due to diabetes and nonadherence were predictors for late dnDSA. Most importantly, nonadherence history increased the risk of late dnDSA by 14 times compared to patients with early or no dnDSA.

Actual survival 3 and 5 years from time of first detection of dnDSA

Of those with dnDSA, allograft loss was more common than in those without dnDSA (38% vs. 20%, respectively, $P < 0.001$). Of the 107 primary renal transplant recipients with dnDSA, only eight had not crossed the 3-year post-dnDSA time point. The remaining 99 were included in the actual 3-year post-dnDSA survival analysis. Of these 99, 70 had adequate follow-up for the 5-year post-dnDSA survival analysis. Figure 3 shows the actual 3- and 5- year post-dnDSA survival. Within 1 year of dnDSA detection, 9% of allografts failed. By 3 years post-dnDSA, the allograft failure rate was 28% (Fig. 3a). By 5 years post-dnDSA, 39% of allografts failed (Fig. 3b). Between AA and non-AA transplant recipients, the 3-year post-dnDSA allograft survival found to be similar between AA and non-AA dnDSA-positive patients (Fig. 3c). This suggests that once dnDSA develops, the course to failure is decided by factors other than patient's race (or race related genetics).

Discussion

Our study aimed to investigate potential racial disparities regarding dnDSA. Some reports have indicated that being AA may be a risk factor for allograft loss or DSA [7,10]. Our study confirmed that AA patients are indeed at a higher risk of developing dnDSA. By 1 year post-transplant, 21% of AA renal transplant patients developed dnDSA compared to 10% in non-AA transplant patients. By 5 years post-transplant, the dnDSA incidence is 36% in AA and 21% in non-AA transplant

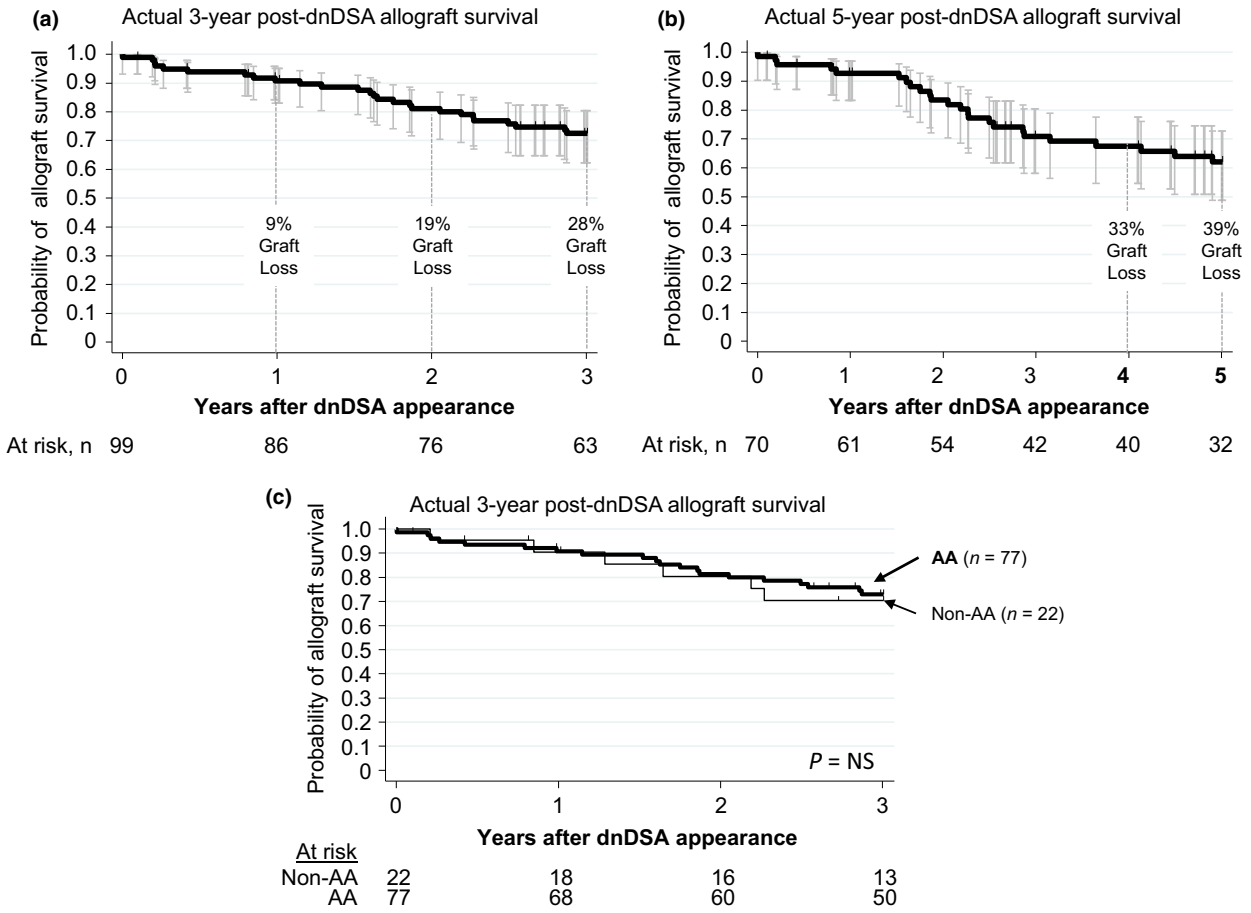

Figure 3 Allograft survival in *de novo* donor-specific anti-HLA IgG antibody/antibodies (dnDSA) patients. In panel a, actual 3-year post-IgG dnDSA allograft survival is shown. In panel b, actual 5-year post-IgG dnDSA allograft survival is shown. *De novo* DSA positive allograft survival is race by shown in panel c.

recipients. This means that AA transplants patients are 1.7 times more likely to develop dnDSA. Despite being at higher risk of developing dnDSA, AA patients were at the same risk of allograft failure once dnDSA developed. Overall (with both AA and non-AA) allografts fail at an annual rate of 7–9% in the first 3 years post-dnDSA detection. By 3- and 5 years post-dnDSA, 28% and 39% of transplant patients experienced allograft failure, respectively.

Finding a higher risk of dnDSA in AA renal transplant patients is important. New research/practice changes are needed to address this problem. One way to address this issue would be to use to a higher level of dnDSA monitoring and a higher level of immunosuppression in all AA renal transplant patients. However, broad approaches are not always the best answers. Our study indicates the risk of dnDSA in AA patients is strongly linked to a few pre- and post-transplant risk factors. On the pretransplant side, we again see that DQ mismatch is one of the leading factors associated with dnDSA. Based on this finding across multiple studies [5–8,23–25], DQ mismatches should be considered in the allograft allocation algorithm. It is also reasonable to do more monitoring for dnDSA and target higher CNI levels in DQ-mismatched patients to prevent dnDSA. We have previously reported that not all DQ are the same when it comes to dnDSA development potential [5,23,26]. DQ7 mismatch has the highest rate of dnDSA followed by DQ2 and DQ8 [26]. Therefore, we may be able to selectively rank DQ mismatches when selecting an appropriate donor or choosing a monitoring/immunosuppressive strategy.

Also of interest, based on our study findings, is the benefit of a LRD in the DQ-mismatched AA transplant patient. One possible reason for the benefit is the AA patient receiving a LRD renal transplant has a better support network and has a strong reason to protect the organ (responsibility to keep a family member organ). Even though nonadherence was slightly lower in the LRD AA patient, previous studies have a shown the potential for a higher risk of nonadherence in living-related donor renal transplants largely as a result of the patient misconception that LRD recipients need less immunosuppression [27]. Further studies are needed to see determine why there is a dnDSA benefit in AA patients receiving a LRD organ. If we are to trust that LRD is a better way to go to prevent dnDSA, then we need to focus on educating AA patients on dialysis about living donation benefits. The rate of living-donor transplant among AA patients is substantially lower than the living-donor transplant rate for Caucasian patients [28,29]. Education of the AA community could help reduce the disparity in living-donor transplant rates, and this may lower dnDSA development.

It is clear that a main dnDSA risk factor is under-immunosuppression. This can come in the form of immunosuppression minimization, discontinuation of immunosuppression due to side effects/viral infections or patient medication nonadherence [24,30–33]. In our study, approximately 1/3 of the dnDSA cases were preceded by a documented case of immunosuppression reduction (secondary to BKPyV viraemia) or nonadherence. The overall correlation supports our previous more detailed analysis of DSA and BKPyV viraemia with the East Carolina University cohort [32]. More details regarding the interplay between BKPyV viraemia, immunosuppression changes and dnDSA are included in this previous report. Relative to time post-transplant, we found a mixed picture of BK viraemia (with immunosuppression reduction) and some nonadherence early (in the first 2 years post-transplant) when dnDSA is most common. However, our study shows the correlation between nonadherence and dnDSA is strongest in late (>2 years post-transplant) dnDSA development. This finding alone is significant given that in the United States, patients relying on Medicare lose drug coverage after 3 years post-transplant [34]. Changes in drug coverage policy may be a way to limit nonadherence and to prevent dnDSA in some patients.

To address nonadherence, a multifaceted approach to monitor for nonadherence with electronic monitoring systems (electronic pill bottles/pill boxes) and periodic education and surveys may be needed. Given one of our study's limitation is that we did not have a prospective system to asses nonadherence, further research is clearly needed to understand the breadth of nonadherence and the true correlation between dnDSA and the immunosuppression dosing. In situations where immunosuppression minimization occurs, more aggressive monitoring may be necessary. It has been shown in cases of tacrolimus monotherapy weaning and in cases of BK viraemia that dnDSA monitoring and re-escalation of immunosuppression can lead to reversal of dnDSA [32,35].

Overall, this is the largest report of dnDSA in AA patients. Given the findings of a heightened risk for dnDSA among AA patients, we should develop studies to understand this problem. Despite the possible implications of these findings for an AA primary renal transplant patient, our data are still only the tip of the iceberg in understanding this disparity of dnDSA in AA. Race and ethnicity are just broad identifiers. It is likely

that this is not the true factor as to why there is a higher risk of dnDSA in AA patients. Further studies are needed to determine whether factors such as CYP450 polymorphisms play a role in tacrolimus metabolism or other polymorphism/genetics may account for a higher risk for development of dnDSA in AA transplant recipients.

Authorship

PB, CEH, CM and SAK: treated the patients. LMR, MJE, AQM, GD and KPB: collected the data. LMR and MJE: conducted the HLA typing/antibody testing. MJE: analysed the data and wrote the manuscript.

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