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The Value of Protocol Biopsies to Identify Patients With *De Novo* Donor-Specific Antibody at High Risk for Allograft Loss

C. A. Schinstock[1,*], F. Cosio[1], W. Cheungpasitporn[1], D. M. Dadhania[2], M. J. Everly[3], M. D. Samaniego-Picota[4], L. Cornell[5] and M. D. Stegall[1]

[1]*William J. von Liebig Transplant Center, Mayo Clinic, Rochester, MN*
[2]*Joan and Sanford I. Weill Department of Medicine, Cornell Medical College, New York, NY*
[3]*Paul I. Terasaki Foundation, Los Angeles, CA*
[4]*Department of Internal Medicine, Division of Nephrology, University of Michigan, Ann Arbor, MI*
[5]*Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN*
**Corresponding author: Carrie A. Schinstock, Schinstock.carrie@mayo.edu*

De novo **donor-specific antibody (dnDSA) is associated with antibody-mediated rejection (AMR) and allograft loss, yet the allograft histology associated with dnDSA remains unclear. The aim of this study was to examine the allograft histology associated with dnDSA in patients with serial surveillance biopsies. We retrospectively studied adult conventional solitary kidney transplant recipients from October 2007 to May 2014. The definition of dnDSA was new donor-specific antibody (DSA) with mean fluorescence intensity (MFI) >1000. The incidence of dnDSA was 7.0% (54 of 771) over mean follow-up of 4.2 ± 1.9 years. Patients with dnDSA had reduced death-censored allograft survival (87.0% vs. 97.0% no dnDSA, p < 0.01). Moreover, 94% of patients received a biopsy after dnDSA (mean of three biopsies per patient). AMR was present in 25.0% and 52.9% of patients at dnDSA detection and at 1 year, respectively. Patients with both class I and II dnDSA had the highest rate of allograft loss. The higher the sum MFI at dnDSA detection, the higher the incidence of AMR. In conclusion, patients with dnDSA without AMR at time of detection may benefit from a follow-up biopsy within 1 year because AMR can be missed initially. In addition, the dnDSA class and sum MFI at baseline appear to be prognostic. The higher the sum MFI of dnDSA at baseline, the higher the incidence of AMR.**

Abbreviations: AMR, antibody-mediated rejection; BSA, body surface area; CI, confidence interval; cPRA, calculated panel reactive antibody; dnDSA, *de novo* donor-specific antibody; DSA, donor-specific antibody; eGFR, estimated GFR; ESRD, end-stage renal disease; IQR, interquartile range; IVIG, intravenous immunoglobulin; MFI, mean fluorescence intensity; NA, not assessed; OR, odds ratio; SAB, single antigen bead; SD, standard deviation

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Introduction

De novo donor-specific antibody (dnDSA) is a major risk factor for chronic antibody-mediated rejection (AMR) and allograft loss (1–5). The reported incidence of dnDSA varies from 6.2% to 27.8% depending on the cohort studied (2–4,6–9), and up to 24% of allografts fail within 3 years of dnDSA detection (3). Medication nonadherence and previous acute cellular rejection in the setting of class II HLA mismatch are the main risk factors for dnDSA development (2,3,6), yet a subset of transplant recipients develop early dnDSA for reasons that are unclear. Regardless, no available therapy has been proven effective, emphasizing the need for prevention and therapeutic clinical trials.

The problem is that designing a clinical trial to prevent or treat patients with dnDSA is difficult. The number of patients who develop dnDSA is relatively small. Not all patients with dnDSA develop AMR or graft loss, and many patients have stable allograft function for years (6). Including these patients in a clinical trial is not ideal because they would receive unnecessary treatment, which would dilute any treatment effect, thus necessitating a larger trial. Enriching a study population with patients who are the most likely to progress to a meaningful clinical end point is a critical component in the design of an effective clinical trial.

Our goal was to examine serial allograft biopsies in patients with dnDSA to identify a subgroup of patients most likely to progress to allograft failure. We also aimed to identify potentially modifiable risk factors for dnDSA outside of medication nonadherence, acute cellular rejection, and HLA mismatch. We studied a predominantly white living donor kidney transplant population that underwent surveillance donor-specific antibody (DSA) testing and allograft biopsy.

Materials and Methods

This study was approved by the Mayo Clinic institutional review board. We performed a retrospective cohort study of the risk factors and outcomes of our adult solitary conventional kidney transplant recipients who were transplanted between October 2007 and May 2014. We studied only the initial transplant from patients who were retransplanted at our center during the studied time period (n = 5), and we excluded patients if no baseline single antigen bead (SAB) results were available (n = 8), if DSA was not tested after transplant (n = 25), or if the patient had a positive crossmatch and/or DSA was detected with mean fluorescence intensity (MFI) >1000 at the time of transplant (n = 158). Data was collected by chart review. Patients were censored at last follow-up.

Assessment of dnDSA

A SAB solid-phase assay (LABScreen; One Lambda, Canoga Park, CA) was used to identify alloantibody specificities at baseline and after transplant. The definition of dnDSA was any DSA identified after transplant that reached MFI >1000 that was not detected at any time prior to transplant (each patient had at least one SAB test prior to transplant). Our center protocol is to obtain SABs at least yearly when patients are on the kidney transplant waiting list, immediately before transplant, 4 mo after transplant, and yearly after transplant thereafter. SABs are also routinely performed at the time of allograft dysfunction or acute cellular rejection.

Assessment of medication adherence

This information was obtained from the clinical record. We defined medical nonadherence as documented missing labs, unexplained low immunosuppressive drug levels, missed appointments, medications not refilled, or patients who were admittedly nonadherent.

Biopsy assessment

Surveillance biopsies were done at 4, 12, 24, and 60 mo after transplant as standard of care. Biopsies were also performed for allograft dysfunction, proteinuria, or based on provider discretion (i.e. known dnDSA). Kidney biopsy tissue was processed for light microscopy and C4d by immunofluorescence (Bio-Rad Antibodies [formerly AbD Serotec], Raleigh, NC), if indicated.

Light microscopy features of biopsies were scored by slightly modified Banff criteria (10–12). Specifically; acute active AMR was diagnosed in patients with dnDSA if three features were present according to Banff 2013 guidelines: (i) histologic evidence of acute tissue injury including glomerulitis (g >0) and/or peritubular capillaritis (ptc >0), intimal or transmural arteritis (v >0), thrombotic microangiopathy, or acute tubular injury, in the absence of any other apparent cause; (ii) evidence of current or recent antibody interaction with vascular endothelium including C4d ≥2 with immunofluorescence on frozen section and/or g + ptc ≥2; and (iii) serologic evidence of DSAs.

The presence of transplant glomerulopathy (cg >0) signified chronic AMR. In this study, patients with acute, active AMR could have cg >0, which is a modification from the Banff 2013 guidelines; therefore, some patients met criteria for acute, active AMR and chronic AMR simultaneously. Electron microscopy was not routinely done in all cases, and it was not used to determine the presence of chronic AMR.

Immunosuppression and treatment protocols

Patients received anti–thymocyte globulin (ATG; Thymoglobulin; Sangstadt, Menlo Park, CA), 1.5 mg/kg per day for four doses; anti-CD25 receptor antibodies (Simulect, Novartis Pharmaceuticals, East Hanover, NJ); or alemtuzumab (Campath; Genzyme, Cambridge, MA) as induction per center protocol. Currently, alemtuzumab is the standard induction agent if the patient age is <65 years, the B cell flow cytometric crossmatch is negative, and no DSA is detected with MFI >2000; anti-CD25 receptor antibodies are given if the patient is ≥65 years with a negative crossmatch; and ATG is given to all other patients. Prior to 2011, alemtuzumab was not part of routine protocol. At that time, ATG was given to all patients unless they were aged ≥65 years and had a negative B cell flow cytometric crossmatch, in which case they received anti-CD25 receptor antibodies.

Standard maintenance immunosuppression consisted of prednisone, tacrolimus, and mycophenolate mofetil in patients who receive induction with ATG and anti-CD25 antibodies. Patients were on a steroid-free immunosuppression protocol if they received alemtuzumab induction (tacrolimus and mycophenolate mofetil only).

Because no therapy has been proven effective for the sustained reduction in DSA, no specific therapy for dnDSA was given outside of routine treatment for acute cellular rejection, mixed acute cellular rejection and AMR, or low immunosuppressive levels. Specifically, 75.9% (42 of 54) patients received no new therapy during follow-up; 11.1% (6 of 54) received thymoglobulin, intravenous immunoglobulin (IVIG), and plasmapheresis for mixed acute cellular rejection and AMR; 9.3% (5 of 54) received corticosteroids for acute cellular rejection alone; and 1.9% (1 of 54) received IVIG therapy alone.

Laboratory monitoring

All patients had their serum creatinine and estimated GFR (eGFR) assessed at least every 3 mo. At yearly intervals, patients had more thorough assessment of their renal function that included iothalamate clearance testing and 24-h urine protein testing.

Statistical analysis

Statistical analysis was performed on JMP v10 (SAS Institute, Cary, NC). For numerical data, groups were compared with the t-test or the Wilcoxon rank sum test, as indicated. Counts and percentages were compared using the Fisher exact test. Odds ratios (ORs) were used and described by their point estimate and corresponding 95% confidence intervals (CIs). Logistic regression was used for multivariate analysis to determine the risk factors for dnDSA. Time-to-event data were summarized for each group using Kaplan–Meier estimates. Cox regression with a time-dependent variable (dnDSA) was used to test between groups (Wald test at the 0.05 level). Testing was two-sided at the 0.05 level. The paired t-test was used to compare paired continuous data, and the McNemar test was used to compare paired proportions.

Results

Demographics

Figure 1 shows a flow chart of the patients in the study. Baseline characteristics are shown in Table 1. Overall mean follow-up was 4.2 + 1.9 years and was similar among those developing dnDSA and those in whom dnDSA was never detected. The incidence of dnDSA was 7.0% during this time frame. In our cohort, the transplant recipients were predominantly white, and 82.3% (637 of 771) received their transplant from a living donor. There was no difference in sex, race, donor type, cause of end-stage renal disease, calculated panel reactive antibody, prior organ transplant, or prior polyomavirus

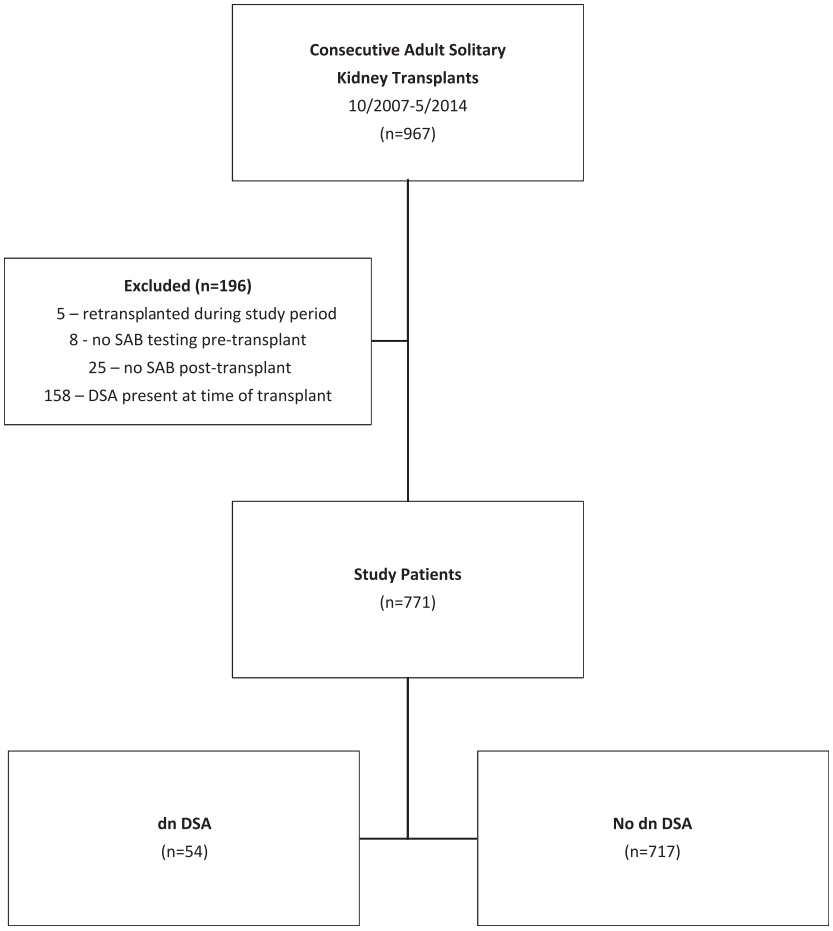


Figure 1: Patients studied. dnDSA, *de novo* donor-specific antibody; DSA, donor-specific antibody; SAB, single antigen bead.

among patients who did or did not develop dnDSA. Patients who developed dnDSA were younger (mean age 48.3 ± 15.6 vs. 53.0 ± 13.8 years, $p = 0.04$) and had more HLA mismatches (mean 4.2 ± 1.5 vs. 3.6 ± 1.9, $p < 0.01$). The majority of patients received thymoglobulin for induction immunosuppression, but there was a higher proportion of patients who received alemtuzumab in the dnDSA cohort than the non-dnDSA cohort (31.5% [17 of 54] vs. 18.2% [130 of 717], $p = 0.03$). There were also more patients in the dnDSA group who had a prior acute cellular rejection episode (35.2% [19 of 54] vs. 15.8% [113 of 717], $p < 0.01$) or had a documented history of medication nonadherence.

Risk factors for dnDSA

Patients aged >60 years were less likely to develop dnDSA in our cohort based on univariate analysis (OR 0.5 [95% CI 0.3–1.0], $p = 0.04$) (Table 2). Risk factors for the development of dnDSA based on univariate analysis were alemtuzumab induction (OR 2.1 [95% CI 1.1–3.8], $p = 0.03$); HLA mismatches at the A locus (OR 4.5 [95% CI 1.6–12.5], $p < 0.01$), DR locus (OR 3.2 [95% CI 1.3–8.1], $p = 0.01$), and DQ locus (OR 4.6 [95% CI 1.8–11.7], $p < 0.01$); prior acute cellular rejection (OR 2.9 [95% CI 1.6–5.2], $p < 0.01$); and documented medication nonadherence (OR 7.5 [95% CI 3.9–14.2], $p < 0.01$). Independent risk factors for dnDSA determined by multivariate models were DQ mismatch (OR 4.8 [95% CI 2.0–14.3], $p < 0.01$), prior history of acute cellular rejection (OR 2.4 [95% CI 1.3–4.5], $p < 0.01$), and documented medication nonadherence (OR 7.9 [95% CI 3.9–15.4], $p < 0.01$) (Table 2, multivariate model 2).

Characteristics of dnDSA

Overall, 7% of patients developed dnDSA. The mean time to dnDSA detection after transplant was 1.8 ± 1.6 years (Figure 2), and 3.2% (25 of 771) of patients developed dnDSA within 1 year after transplant. Anti–class I DSA alone was present in 9.3% (5 of 54), anti–class II DSA alone was present in 70.4% (38 of 54), and anti–class I and II DSA was present in 20.4% (11 of 54) of patients (Table 3). In total, 29.6% (16 of 54) had anti–class I dnDSA and 90.7% (49 of 54) had anti–class II dnDSA. The dnDSA completely disappeared during

Table 1: Baseline demographics

Characteristic	All patients N = 771	dnDSA n = 54	No dnDSA n = 717	p-value
Age (years), mean ± SD	52.6 ± 13.9	48.3 ± 15.6	53.0 ± 13.8	0.04
18–30 years, n (%)	55 (8.4)	8 (14.8)	57 (8.0)	
>30–40 years, n (%)	78 (10.1)	8 (14.8)	70 (9.8)	
>40–50 years, n (%)	132 (17.1)	8 (14.8)	124 (17.3)	
>50–60 years, n (%)	205 (26.5)	17 (31.5)	188 (26.2)	
>60 years, n (%)	291 (37.8)	13 (24.1)	278 (38.8)	
Race, n (%)				0.46
White	596 (90.3)	45 (83.3)	651 (90.8)	
Hispanic	18 (2.3)	2 (3.7)	16 (2.2)	
Black	31 (4.0)	4 (7.4)	27 (3.8)	
Asian	12 (1.2)	1 (1.9)	11 (1.5)	
American Indian/Pacific Islander	14 (1.8)	2 (3.7)	12 (1.7)	
Donor type, n (%)				0.13
Deceased donor	134 (17.4)	7 (13.0)	127 (17.7)	
Living related donor	295 (38.3)	16 (29.6)	279 (38.9)	
Living unrelated donor	342 (44.4)	31 (57.4)	311 (43.4)	
Sex (male), n (%)	483 (62.6)	30 (55.6)	453 (63.2)	0.31
Cause of ESRD, n (%)				0.73
Diabetes	143 (18.5)	7 (13.0)	136 (19.0)	
Glomerulonephritis	275 (35.7)	19 (35.2)	255 (35.6)	
Hypertension	45 (5.8)	5 (9.3)	40 (5.6)	
Cystic renal diseases	130 (16.9)	9 (16.7)	121 (16.9)	
Other	130 (16.9)	11 (20.4)	119 (16.6)	
Unknown	48 (6.2)	3 (5.7)	45 (6.3)	
cPRA, %, mean ± SD	12.3 ± 27.1	8.9 ± 22.7	12.6 ± 27.3	0.27
Prior solid organ transplant, n (%)	131 (17.0)	13 (24.07)	108 (16.5)	0.19
Induction, n (%)				0.06
Thymoglobulin	388 (50.3)	27 (50.0)	361 (50.4)	
Basiliximab	235 (30.5)	10 (18.5)	224 (31.3)	
Alemtuzumab	147 (19.1)	17 (31.5)	130 (18.2)	
HLA mismatch (>1), n (%)				<0.01
A	573 (74.9)	50 (93.0)	523 (72.9)	
B	530 (82.4)	49 (92.6)	581 (81.0)	
DR	584 (76.4)	49 (90.7)	535 (74.6)	
DQ	535 (69.5)	49 (90.7)	486 (67.8)	
HLA mismatch, mean ± SD	3.3 ± 1.9	4.2 ± 1.5	3.6 ± 1.9	<0.01
Polyomavirus, n (%)[1]	34 (4.4)	5 (9.3)	29 (4.0)	0.08
Acute cellular rejection,[1] n (%)	132 (17.1)	19 (35.2)	113 (15.8)	<0.01
Documented medication nonadherence, n (%)	53 (8.2)	18 (33.3)	45 (6.3)	<0.01
Follow-up (years), mean ± SD	4.2 ± 1.9	4.7 ± 2.0	4.2 ± 1.9	0.06
Follow-up after dnDSA detection (years), mean ± SD	NA	3.2 ± 2.0	NA	NA

cPRA, calculated panel reactive antibody; dnDSA, *de novo* donor-specific antibody; ESRD, end-stage renal disease; NA, not assessed; SD, standard deviation.
[1]Present prior to dnDSA detection.

follow-up in 16.7% (9 of 54) of patients. The distribution of dnDSA MFI is shown in Table 3.

Allograft survival

Patient and overall allograft survival were similar among patients who did or did not develop dnDSA, as shown in Figure 3; however, patients with dnDSA had reduced death-censored allograft survival ($p = 0.01$). Actuarial death-censored allograft survival was 87.0% in patients who developed dnDSA and 97.0% in patients who did not develop dnDSA ($p = 0.01$). The mean time to death-censored allograft failure after dnDSA detection was 1.6 ± 1.7 years.

Progression of clinical and subclinical AMR

In total, 160 biopsies were obtained from 94.4% (51 of 54) of the studied patients at the time of dnDSA detection or afterward (mean of three biopsies per patient). Biopsies were obtained at the time of dnDSA detection in 74.1% (40 of 54) of patients. At 1 year after dnDSA detection, biopsies were obtained in 68.0% (34 of 50) of the surviving patients with a functioning allograft.

Table 2: Risk factors for *de novo* donor-specific antibody

	Univariate		Multivariate model 1		Multivariate model 2	
Risk factor	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
Age (years)						
18–30	2.0 (0.9–4.5)	0.12	–	–	–	–
>30–40	1.6 (0.7–3.5)	0.24	–	–	–	–
>40–50	0.8 (0.4–1.8)	0.85	–	–	–	–
>50–60	1.3 (0.7–2.3)	0.43	–	–	–	–
>60	0.5 (0.3–1.0)	0.04	0.7 (0.3–1.4)	0.31	–	–
Induction						
Thymoglobulin	1.0 (0.6–1.7)	1.0	–	–	–	–
Basiliximab	0.5 (0.2–1.0)	0.05	–	–	–	–
Alemtuzumab	2.1 (1.1–3.8)	0.03	1.5 (0.7–2.9)	0.26	–	–
HLA mismatch (>1)						
A	4.5 (1.6–12.5)	<0.01	2.5 (0.82–9.5)	0.14	–	–
B	2.2 (0.9–5.6)	0.10	–	–	–	–
DR	3.2 (1.3–8.1)	<0.01	1.5 (0.5–5.2)	0.48	4.8 (2.0–14.3)	<0.01
DQ	4.6 (1.8–11.7)	<0.01	3.5 (1.4–10.7)	0.01	–	–
HLA mismatch	NA	–	1.1 (1.3–0.9) per mismatch	0.42	–	–
Acute cellular rejection[1]	2.9 (1.6–5.2)	<0.01	2.6 (1.3–5.1)	≤0.01	2.4 (1.3–4.5)	<0.01
Documented medication nonadherence	7.5 (3.9–14.2)	<0.01	6.4 (3.1–13.3)	<0.01	7.9 (3.9–15.4)	<0.01

CI, confidence interval; OR, odds ratio.
[1]Prior to *de novo* donor-specific antibody formation.

Table 3: *De novo* donor-specific antibody characteristics

	Class I (alone)	Class II (alone)	Classes I and II	Class I (total)	Class II (total)
% (n)	9.3% (5)	70.4% (38)	20.4% (11)	29.6% (16)	90.7% (49)
Mean ± SD	1668.21 ± 1411.5	3612.01 ± 3425.1	4348.21 ± 5040.6	2075.81 ± 2211.1	3949.51 ± 4903.6
Median (IQR)	1492 (1000–2708.5)	1923 (1274.5–4749.5)	2800.5 (1435–4881.5)	1462 (1083.75–2552)	2176 (1103–4117)

IQR, interquartile range; SD, standard deviation.

At the time of dnDSA detection, 20.0% (8 of 40) of the biopsies met Banff criteria for acute cellular rejection (borderline grade or higher); 25.0% (10 of 40) of the biopsies met criteria for acute, active AMR; and 7.5% (3 of 40) of the biopsies showed chronic AMR (with concomitant acute, active AMR) (Figure 4). The prevalence of acute, active AMR and chronic AMR increased to 52.9% (18 of 34) and 38.2% (13 of 34), respectively, by 1 year following dnDSA detection ($p = 0.04$ and $p = 0.02$, respectively), whereas the prevalence of acute cellular rejection was unchanged (20.0% [8 of 40] vs. 14.7% [5 of 34], $p = 0.76$) (Figure 4).

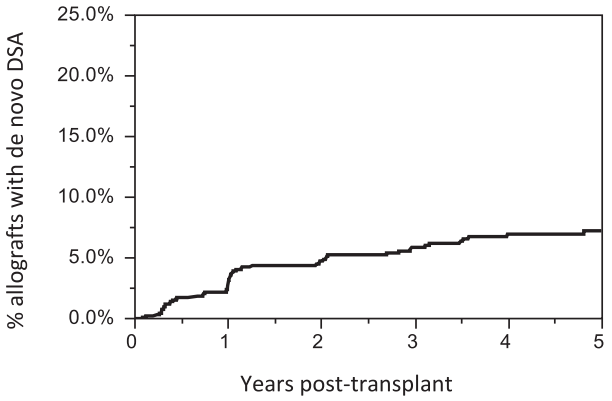


Figure 2: Time to dnDSA detection. The mean time to dnDSA detection after transplant was 1.8 ± 1.6 years. At our center, surveillance testing for dnDSA is performed at 4 mo after transplant and yearly thereafter. Testing for dnDSA is also obtained for clinical indication (i.e. acute cellular rejection). dnDSA, *de novo* donor-specific antibody.

Overall, 65.2% (30 of 46) of the surviving allografts had biopsies ≥2 years after dnDSA detection. Acute, active AMR was present in 33.3% (10 of 30) and chronic AMR was present in 16.7% (5 of 30) of those remaining allografts. Only three patients (10% of patients who received a biopsy >1 year after dnDSA detection) had newly detected acute, active AMR that was not present at baseline or 1 year after dnDSA detection.

The individual Banff scores at the time of and at 1 year after dnDSA detection are presented in Figure 5. The prevalence of moderate glomerulitis numerically increased in the year following dnDSA detection, but this did not reach statistical significance (12.5% [5 of 40] up to 32.3% [11 of 34],

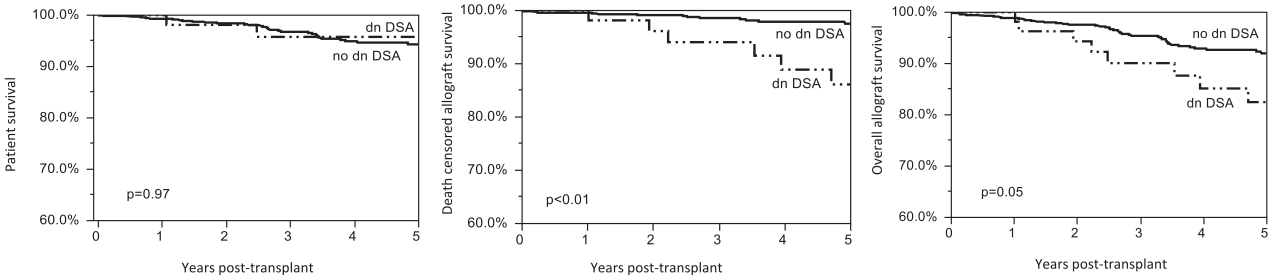
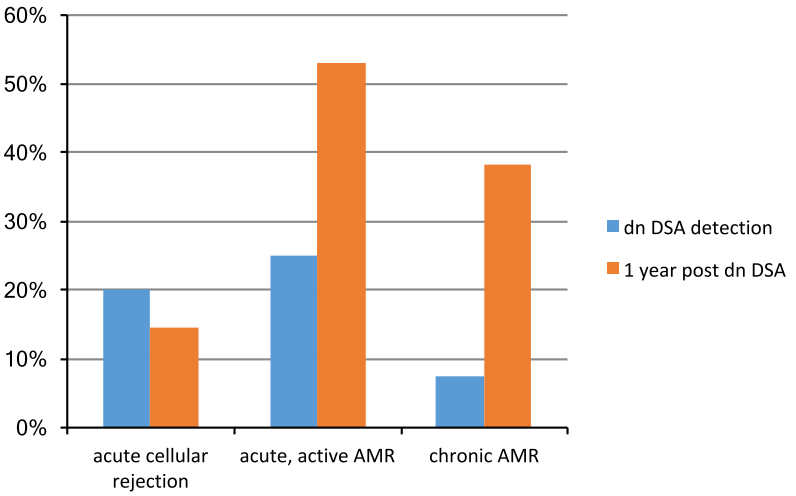


Figure 3: Patient and allograft survival. Patients with dnDSA had reduced death-censored allograft survival (p = 0.01). At the end of follow-up, actuarial death-censored allograft survival was 87.0% in patients who developed dnDSA and 97.0% in patients who did not develop dnDSA (p = 0.01). Cox regression with a time-dependent variable (dnDSA) was used to compare groups (Wald test at the 0.05 level). dnDSA, *de novo* donor-specific antibody.



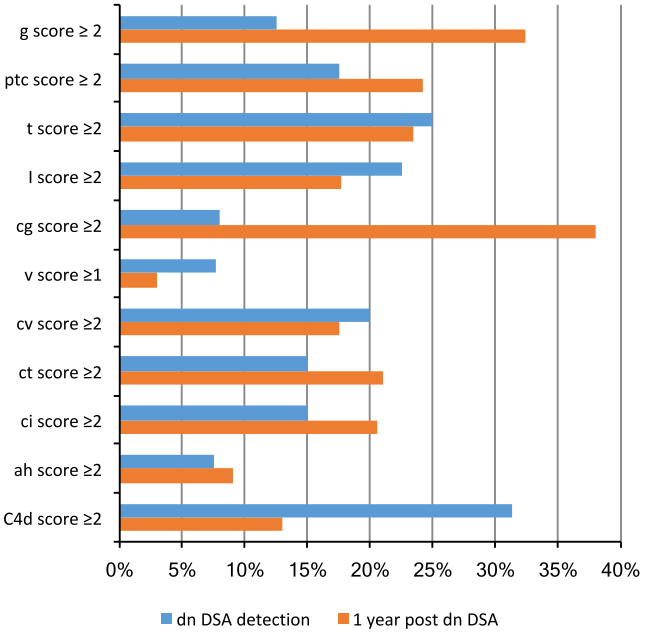
	Acute cellular rejection %(n)	Acute, active AMR %(n)	Chronic AMR %(n)
Time of Detection N=40	20.0(8)	25.0(10)	7.5(3)
1 year N=34	14.7(5)	52.9(18)	38.2(13)
P-value	P=0.76	P=0.04	P=0.02

Figure 4: Allograft rejection at dnDSA detection and at 1 year. The prevalence of acute cellular rejection remained similar at 1 year after dnDSA detection, but there was increased acute, active AMR and chronic AMR. The definition of acute, active AMR was Banff scores (i) ptc + g >2 or (ii) ptc >0 or g >0 and C4d >1. Chronic AMR was present if the Banff transplant glomerulopathy score was cg >0. All patients with chronic AMR had concomitant acute, active AMR. McNemar paired analysis was used to compare serial biopsies. AMR, antibody-mediated rejection; dnDSA, *de novo* donor-specific antibody.

p = 0.07). The prevalence of peritubular capillaritis, tubulitis, acute interstitial inflammation, endothelialitis, chronic vascular lesions, interstitial fibrosis, tubular atrophy, arteriolar hyalinosis, and C4d positivity was unchanged in the year following dnDSA detection, whereas the prevalence of chronic AMR (Banff cg >0) increased (7.5% [3 of 40] up to 38.2% [13 of 34], p = 0.02) (Figure 5).

Allograft function

At the time of dnDSA detection, the mean iothalamate clearance was 55.0 ± 20.4 mL/min per body surface area (BSA), and at 1 year after detection, it decreased to 52.5 ± 18.6 mL/min per BSA (p < 0.01, paired t-test) (Figure 6). At the end of follow-up (a mean of 3.2 ± 2.0 years following dnDSA detection), the mean iothalamate GFR



	Time of Detection n=40* %(n)	1 year post N=34* %(n)	P-value
Glomerulitis g score ≥ 2	12.5(5)	32.3(11)	P=0.07
Peritubular capillaritis ptc score ≥ 2	17.5(7)	24.2(8)	P=0.68
Tubulitis t score ≥ 2	25.0(10)	24.2(8)	P=1.0
Acute interstitial inflammation i score ≥ 2	22.5(9)	17.6(6)	P=0.68
Chronic AMR cg score ≥ 0	7.5(3)	38.2(13)	P=0.02
v score ≥ 1	8.0(3)	2.9(1)	P=1.0
cv score ≥ 2	20.0(8)	17.5(7)	P=1.0
Tubular atrophy ct score ≥ 2	15.0(6)	21.2(7)	P=0.45
Interstitial fibrosis ci score ≥ 2	15.0(6)	21.2(7)	P=0.72
Arteriolar hyalinosis ah score ≥ 2	7.5(3)	3(8.8)	P=1.0
C4d score ≥ 2	31.3(5/16)	13.0(3/23)	P=0.23

Figure 5: Allograft histology at dnDSA detection and at 1 year. The prevalence of chronic AMR increased in the year following the detection of dnDSA. McNemar paired analysis was used to compare serial biopsies. AMR, antibody-mediated rejection; dnDSA, *de novo* donor-specific antibody.

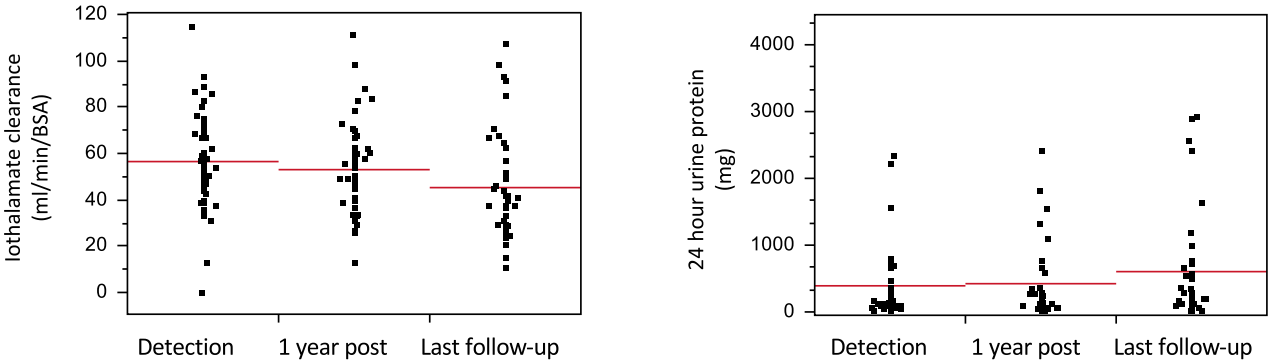


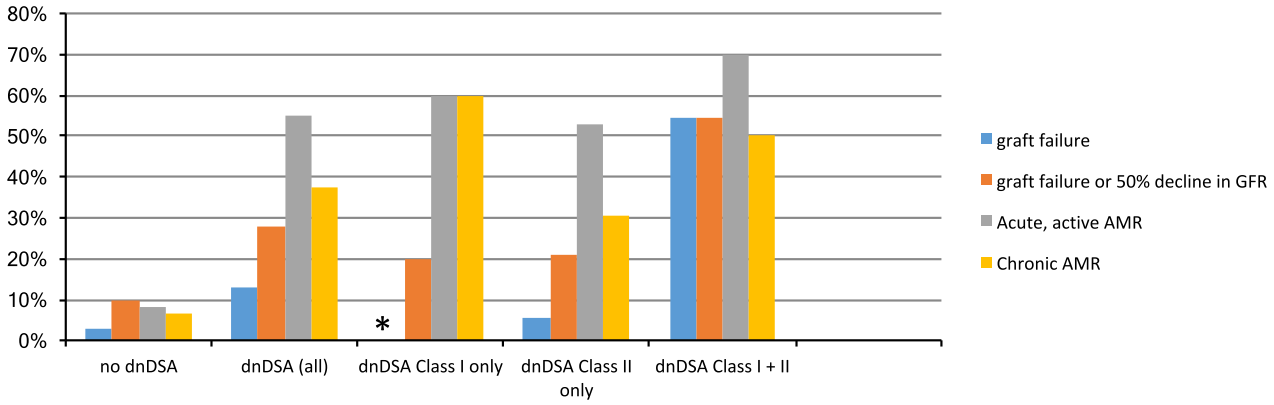
Figure 6: Allograft function and proteinuria when dnDSA detected and at follow-up. At the time of dnDSA detection, the mean iothalamate clearance was 55.0 ± 20.4 mL/min per BSA, and at 1 year after detection, it decreased to 52.5 ± 18.6 mL/min per BSA ($p < 0.01$, paired t-test). During the entire follow-up following dnDSA detection (mean 3.2 ± 2.0 years), mean iothalamate GFR decreased to 44.5 ± 21.7 mL/min per BSA ($p = 0.01$). During the same follow-up, mean 24-h urine proteinuria increased from 391.4 ± 865.5 mg to 603.6 ± 1035.8 mg, but this did not reach statistical significance ($p = 0.24$). BSA, body surface area; dnDSA, *de novo* donor-specific antibody.

decreased to 44.5 ± 21.7 mL/min per BSA ($p = 0.01$, paired t-test). The 24-h urine proteinuria increased from 391.4 ± 865.5mg to 603.6 ± 1035.8 mg, but this did not reach statistical significance ($p = 0.24$, paired t-test).

Which patients with dnDSA developed allograft failure or reduced eGFR?

Patients with dnDSA were followed 3.2 ± 2.0 years after dnDSA detection, and their outcomes were compared with those of patients without dnDSA. Patients with dnDSA had increased incidence of allograft failure (13.0% [7 of 54] vs. 2.9% [21 of 717], $p < 0.01$); the composite end point of graft failure and/or 50% reduction in eGFR (27.8% [15 of 54] vs. 9.6% [69 of 717], $p < 0.01$); acute, active AMR (54.9% [28 of 51] vs. 8.1% [57 of 702], $p < 0.01$); and chronic AMR (37.2% [19 of 51] vs. 6.8% [48 of 703], $p < 0.01$) compared with those patients without dnDSA (Figure 7).

The incidence of graft failure and the composite end point was similar among patients with only class I, only class II, and no dnDSA detected during follow-up



	no dnDSA	dnDSA (all)	P-value†	dnDSA (class I only)	P-value†	dnDSA (class II only)	P-value†	dnDSA (class I +II)	P-value†
Graft failure	2.9% (21/717)	13.0% (7/54)	P<0.01	0% (0/5)	P=1.0	5.3% (2/38)	P=0.35	54.6% (6/11)	P<0.01
Graft failure or 50% decline in eGFR	9.6% (69/717)	27.8% (15/54)	P<0.01	20.0% (1/5)	P=0.40	21..1% (8/38)	P=0.05	54.6% (6/11)	P<0.01
Acute, active AMR	8.1% (57/702)	54.9% (28/51)	P<0.01	60.0% (3/5)	P<0.01	52.8% (19/36)	P<0.01	70.0% (7/10)	P<0.01
Chronic AMR	6.8% (48/703)	37.2% (19/51)	P<0.01	60.0% (3/5)	P<0.01	30.6% (11/36)	P<0.01	50.0% (5/10)	P<0.01

Figure 7: Allograft failure, eGFR decline, and AMR in patients with and without dnDSA. Graft failure; the composite end point of graft failure and/or 50% reduction in GFR; acute, active AMR; and chronic AMR were higher in patients with dnDSA. Patients with both classes I and II dnDSA had the highest rate of graft loss and 50% decline in eGFR. Overall, 94.4% (51 of 54) of patients received a biopsy. The mean follow-up after dnDSA detection was 3.2 ± 2.0 years. *No patients with class I dnDSA only lost their allografts during follow-up. †All statistical comparisons were with the no dnDSA group. AMR, antibody-mediated rejection; dnDSA, *de novo* donor-specific antibody; eGFR, estimated GFR.

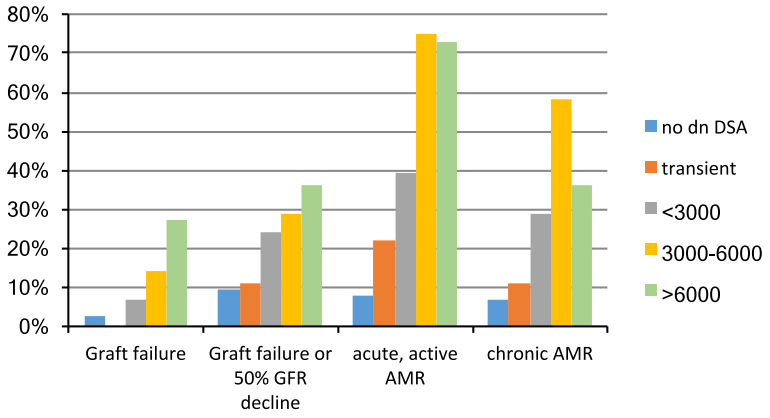
(Figure 7). In contrast, incidence of both end points was higher in patients with both class I and II dnDSA detected. Graft failure and the composite end point occurred in 54.6% (6 of 11) of patients with both class I and II dnDSA ($p < 0.01$ compared with no dnDSA) (Figure 7).

In contrast, the incidence of acute, active AMR and chronic AMR was higher in patients with dnDSA regardless of the class of dnDSA present (Figure 7). The incidence of acute, active AMR was 60.0% (3 of 5), 52.8% (19 of 36), and 70.0% (7 of 10) in patients with class I, class I, and classes I and II dnDSA, respectively ($p < 0.01$ for all classes). Chronic AMR was detected in 60.0% (3 of 5), 30.6% (11 of 36), and 50.0% (5 of 10) of patients with only class I, only class II, and both class I and II dnDSA, respectively ($p < 0.01$ for all classes).

No patients lost their allograft during follow-up if their dnDSA completely disappeared. The rates of the composite end point; acute, active AMR; and chronic AMR were similar in patients with transient dnDSA compared with patients without dnDSA (Figure 8).

There were numeric trends toward increased allograft failure and the composite end point in patients with a higher total sum MFI at baseline, but this did not reach statistical significance ($p = 0.09$ and $p = 0.44$, respectively; Cochran test for trend). Patients with a total MFI 3000–6000 and >6000 had higher rates of acute, active AMR compared with patients with total MFI >3000 at baseline ($p = 0.03$, Cochran test for trend). Specifically the rate of acute, active AMR was 39.3% (11 of 28) in patients with MFI <3000 at baseline, 75.0% (8 of 11) in patients with MFI 3000–6000 at baseline, and 72.7% (8 of 11) with MFI >6000 at baseline. The rate of chronic AMR was similar in patients regardless of baseline total dnDSA MFI (Figure 8).

Of all patients with dnDSA, only those with histologic evidence of acute, active AMR either at DSA detection or on subsequent biopsy had an increased incidence of graft failure and/or 50% reduction in eGFR (Figure 9). Overall, 21.4% (6 of 28) of the allografts failed in patients with



	no dnDSA	Transient	P-value*	MFI <3000 at baseline	MFI 3000-6000 at baseline	MFI >6000 at baseline	P-value†
Graft failure	2.9% (21/717)	0.0% (0/9)	P=1.0	6.9% (2/29)	14.3% (2/14)	27.3% (3/11)	P=0.09
Graft failure or 50% decline in eGFR	9.6% (69/717)	11.1% (1/9)	P=0.6	24.1% (7/29)	28.6% (4/14)	36.4% (4/11)	P=0.44
Acute, active AMR	8.1% (57/702)	22.2% (2/9)	P=0.17	39.3% (11/28)	75.0% (9/12)	72.7% (8/11)	P=0.03
Chronic AMR	6.8% (48/703)	11.1% (1/9)	P=0.48	28.6% (8/28)	58.3% (7/12)	36.4% (4/11)	P=0.4

Figure 8: Allograft function and histology stratified by dnDSA MFI. The rates of allograft loss; the composite end point; acute, active AMR; and chronic AMR were similar in patients with transient dnDSA compared with patients without dnDSA. A higher sum MFI of dnDSA at baseline was associated with higher rates of acute, active AMR ($p = 0.03$, Cochran test for trend). *Comparison between outcomes in patients with no dnDSA and with transient dnDSA. †Cochran test for trend comparison outcomes in patients with dnDSA MFI <3000, 3000–6000, and >6000 at baseline. AMR, antibody-mediated rejection; dnDSA, *de novo* donor-specific antibody; eGFR, estimated GFR; MFI, mean fluorescence intensity.

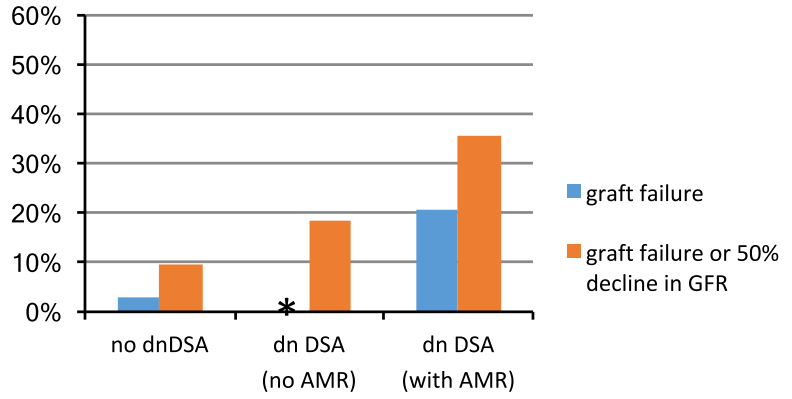
dnDSA and AMR, whereas none failed in patients with dnDSA and no AMR ($p < 0.01$ for dnDSA plus AMR vs. no dnDSA; $p = 1.0$ for dnDSA without AMR vs. no dnDSA). The incidence of graft failure and/or 50% reduction in eGFR occurred in 35.7% (10 of 28) of patients with dnDSA and AMR, 17.3% (4 of 23) of those with dnDSA and no AMR, and 9.6% (69 of 717) of patients with no dnDSA ($p < 0.01$ for dnDSA plus AMR vs. no dnDSA; $p = 0.26$ for dnDSA without AMR vs. no dnDSA).

Discussion

The presence of dnDSA is associated with AMR and allograft loss, but most patients actually have a functioning allograft for the first few years after dnDSA detection. In this study, patients with both anti–class I and II dnDSA had the highest rate of graft loss and the composite end point of graft loss and/or 50% reduction in eGFR. More than half of this small subgroup of patients had allograft failure within 3.2 years following dnDSA detection. We also found that, regardless of the class of dnDSA present, only those patients who developed AMR (i.e. microvascular inflammation) had allograft failure or the composite end point of allograft failure or 50% eGFR, even when the vast majority of the AMR episodes in our cohort were subclinical. No patients in the dnDSA group without AMR had allograft loss.

The use of protocol biopsy allowed us to better understand the progression to AMR after dnDSA. When dnDSA was detected, only 25.0% had histologic findings of acute, active AMR, but the incidence increased to 52.9% by 1 year after dnDSA detection. Consequently, patients without histologic evidence of AMR at the time of dnDSA detection may benefit from a follow-up biopsy within 1 year after dnDSA detection because AMR may be missed on the initial biopsy. Finding new AMR on biopsies performed >1 year after dnDSA detection was unusual, which suggests that some patients with dnDSA never develop AMR. This deserves further study.

Although the SAB output is semiquantitative, the sum MFI of dnDSA at baseline has some prognostic value: The higher the sum MFI at baseline, the higher the incidence of acute, active AMR. In addition, patients whose dnDSA completely disappeared during follow-up had similar rates of graft failure; the composite end point of graft failure and



	no dnDSA	dnDSA no AMR	P-value†	dnDSA AMR	P-value†
Graft failure	2.9% (21/717)	0% (0/23)	P=1.0	21.4% (6/28)	P<0.01
Graft failure or 50% decline in eGFR	9.6% (69/717)	17.3% (4/23)	P=0.26	35.7% (10/28)	P<0.01

Figure 9: Acute, active AMR and dnDSA associated with allograft failure and eGFR decline. *No dnDSA patients without AMR lost their allograft during follow-up. †All statistical comparisons were to the no dnDSA group. AMR, antibody-mediated rejection; dnDSA, *de novo* donor-specific antibody; eGFR, estimated GFR.

50% decline in eGFR; acute, active, AMR; and chronic AMR as those without dnDSA. Moreover, dnDSA completely disappeared in only a small number of patients—a phenomenon that also deserves further study.

Other studies on this subject have also reported detailed histologic findings following dnDSA, but most biopsies were obtained at the time of dnDSA detection (2) or for allograft dysfunction (3,9). The incidence of AMR at the time of dnDSA detection was lower than that reported in other cohorts (6,9), but that was likely because biopsies performed in our cohort were mainly performed for surveillance and not for allograft dysfunction. Our results are particularly informative because we studied biopsies at more than one time point (mean of three biopsies per patient). We also confirmed many previously reported findings. Like de Kort et al, we found that the presence of microvascular inflammation (acute, active AMR in our cohort) was associated with allograft failure (9). The overall incidence of dnDSA in our cohort was also consistent with that previously reported (2,3,6,13), and we found that DQ mismatch, prior medication nonadherence, and acute cellular rejection were linked to the development of dnDSA (2,3,6).

The main limitation of our study was the relatively short follow-up. We reported the most comprehensive histologic follow-up after dnDSA, but the same patients did not have biopsies at all time points, which limited our ability to truly describe the evolution and timing of light microscopic findings. We also did not assess the impact of epitope mismatches on dnDSA development (14) or the effect of DSA characteristics such as titer, IgG subclasses, or C1q (15,16) on prognosis. Finally, we used a 50% reduction in eGFR as a clinical outcome, although it is not currently approved by the U.S. Food and Drug Administration for clinical trials in transplantation (17–19).

These data reemphasize the importance of developing effective therapy either to prevent dnDSA formation or to treat its consequences; however, designing a clinical trial to study this is difficult. We believe a prevention trial is unreasonable, given the relatively low incidence of dnDSA. As others have already discussed (6), a multicenter effort with thousands of patients would be required to adequately power a study, and many patients would be treated unnecessarily. Even a treatment trial in patients with identified dnDSA would require a prolonged multicenter effort. The best approach may be to enroll dnDSA patients with acute, active AMR into a treatment trial because this is a large subset of patients at the greatest risk of graft failure. This approach would minimize the number of patients treated unnecessarily and

maximize the potential to detect a meaningful effect from a potential therapeutic agent.

In conclusion, the development of dnDSA is associated with a progressive increase in antibody-mediated injury in more than half of patients within 1 year of detection. The patients who ultimately developed AMR were at high risk of graft failure and/or 50% reduction in GFR. Nevertheless, there are potentially modifiable risk factors including ensuring medication adherence and avoiding HLA mismatch, especially at the DQ locus.

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Disclosure

The authors of this manuscript have no conflicts of interest to disclose as described by *American Journal of Transplantation*.

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Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1: Individual diagnostic criteria for all cases of acute, active antibody-mediated rejection.