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## Kidney Transplant with Low Levels of DSA or Low Positive B-Flow Crossmatch: An Underappreciated Option for Highly-Sensitized Transplant Candidates

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## Abstract

**Background**—Avoiding donor specific antibody (DSA) is difficult for sensitized patients. Improved understanding of the risk of low level DSA is needed.

**Methods**—We retrospectively compared the outcomes of 954 patients transplanted with varied levels of baseline DSA detected by single antigen beads and B flow cytometric crossmatch (XM). Patients were grouped as follows: -DSA/-XM, +DSA/-XM, +DSA/low+XM, +DSA/high+XM, and -DSA/+XM and followed for a mean of 4.1±1.9 years (similar among groups, p=0.49).

**Results**—Death-censored allograft survival was similar in all groups except the +DSA/high+XM group, which was lower at 79.1% versus 96.2% in the -DSA/-XM group (p<0.01). The incidence of chronic antibody mediated rejection (CAMR) based on surveillance biopsy was higher with increasing DSA (8.2% -DSA/-XM, 17.0% +DSA/-XM, 30.6%+DSA/low + XM, and 51.2% +DSA/high+XM, p<0.01), but similar in groups without baseline DSA (8.1% -DSA/-XM vs. 15.4% -DSA/+XM, p=0.19). Having a calculated panel reactive antibody (cPRA) ≥80% was independently associated with CAMR (HR 5.2, p=0.03) even when DSA was undetected at baseline. By 2 years posttransplant, the incidence of CAMR was 19.4% in patients with cPRA ≥80% and undetected DSA and negative XM at baseline.

**Conclusion**—Kidney transplantation with low level DSA with or without a low positive XM is a reasonable option for highly sensitized patients and may be advantageous compared to waiting for a negative XM deceased donor. The risk for CAMR is low in patients with no DSA even if the XM is positive. Patients with cPRA ≥80% are at risk for CAMR even if no DSA is detected.

## INTRODUCTION

Performing a kidney transplant in a recipient with donor-specific alloantibody (DSA) is generally associated with an increased risk of antibody mediated rejection (AMR) and subsequent allograft loss<sup>1-7</sup>, but the risk associated with DSA is variable<sup>8-10</sup>. The histologic outcomes following transplantation with low level DSA that is only detected with sensitive testing remain unclear.

Prolonged waiting for a kidney just to avoid low level DSA may not always be in the best interest of the patient. Factors other than the presence of alloantibody impact allograft and patient survival such as preemptive kidney transplantation<sup>11,12</sup>, living versus deceased donor<sup>13</sup>, delayed graft function<sup>14,15</sup>, and recurrent renal disease<sup>16,17</sup> among others. Prolonged waiting on dialysis also carries risk<sup>18,19</sup>. In fact, patients who receive transplants from HLA-incompatible donors have improved survival than matched controls who remain on the waiting list<sup>20,21</sup>. Improved understanding of the risk of low level DSA is needed to improve donor selection and increase access to transplantation for sensitized patients.

The aim of this study was to examine the outcomes of patients with low level DSA and/or low positive XM in the current era of antibody characterization using single antigen beads (SAB) and B-flow cytometric crossmatch (XM). The outcomes assessed were allograft and patient survival, the incidence of early acute *clinical* AMR, and the prevalence of chronic AMR based on surveillance biopsies at 1,2 and 5 years posttransplant. We also assessed the factors associated with chronic AMR.

## MATERIALS AND METHODS

We performed a retrospective cohort study of all adult solitary kidney transplant recipients transplanted since our center has routinely performed sensitive DSA testing with both SAB and B-flow cytometric crossmatch (XM) [October 2007 through May 2014]. We excluded all pediatric, ABO incompatible and dual organ transplant recipients. In patients who received more than 1 transplant during the study time period, the second transplant was excluded from analysis (n=5), and we excluded patients if baseline SAB results were not available (n=9). Data was collected by chart review.

We compared patient and allograft survival, cause of allograft loss, early acute *clinical* AMR, surveillance allograft histology, and predictors of chronic AMR among the following 4 groups with increasing DSA: no DSA with >1000 mean fluorescence intensity (MFI) and negative XM [-DSA/-XM (n= 795)]; DSA with >1000 MFI and negative XM [+DSA/-XM (n=53)]; DSA any MFI and positive XM up to mean channel shift of 199 [+DSA/low + XM (n=36)]; and DSA any MFI and positive XM mean channel shift of >200 [+DSA/high + XM (n=43)]. We also studied patients with positive XM without detectable DSA (any MFI) at baseline [-DSA/+XM (n=26)].

### Donor Specific Antibody Assessment

A solid phase assay (LAB screen, One Lambda, Canoga Park, CA, USA) was used to identify baseline alloantibody specificities. An MFI of 1000 was considered positive in patients with negative XM to avoid false positivity from laboratory variability or background. The cPRA was calculated based on MFI >2000 because of center practice.

### Flow Cytometric Crossmatch Testing

B flow cytometric crossmatch testing was used and reported because B cells express both class I and II HLA antigens, and thus this test can detect both anti class I or II alloantibodies. Specifically, a 3 color flow cytometric crossmatch was performed in this study. Fluorescein isothiocyanate (FITC)-conjugated F(ab)'<sub>2</sub> goat antihuman IgG was used to assess alloantibody binding by way of indirect immunofluorescence and 2 other fluorescence parameters (CD3 PerCP, and CD19PE) for identifying T and B cells. Donor cells were treated with pronase (1ug/2ml) for 15 minutes. Next donor cell aliquots (300,000) were mixed with 20 µL of controls or patient serum and incubated for 30 minutes. After washing, fluorescent antibodies were added and incubated for an additional 15 minutes and washed and aliquots acquired on FACS Calibur (BD Biosciences) using a 1024 scale. The interpretation of the flow cytometric crossmatch was performed by directly comparing the fluorescence intensity of donor B lymphocytes after being treated with patient serum to the

fluorescent intensity of donor cells after treatment with a negative control serum. The interpretation was based on the right or bright “shift” of the mean channel fluorescence noted from the negative control and donor sera. A mean channel fluorescence shift greater than 106 for was considered positive for B cells.

Auto-crossmatch testing was performed in some cases when the XM was higher than expected based on DSA results. This test was done by performing the routine T and B-flow cytometric crossmatch using recipient lymphocytes and serum. The most immediate pretransplant or pre-desensitization assessment was considered baseline.

### Biopsy Assessment

Surveillance biopsies are standard of care for all patients transplanted at our center regardless of baseline DSA. Allograft histology from surveillance biopsies taken at 1, 2, and 5 years posttransplant was compared. To determine the overall incidence of acute cellular rejection and chronic AMR, both surveillance and indication biopsies were examined. Kidney biopsy tissue was processed for light microscopy and immunofluorescence for C4d (AbD Serotec).

Light microscopy features of biopsies were scored by Banff criteria<sup>22-24</sup> on all biopsies. Early acute *clinical* AMR was defined as an increase in serum creatinine  $>0.3\text{mg/dl}$ ; increased DSA; and histologic findings of acute, active AMR. Acute, active AMR (Banff 2013) was specifically 1) ptc + g score = 2 or 2) ptc  $>0$  or g  $>0$  and C4d  $>1$ . The presence of cg score  $>0$  signified chronic AMR. Electron microscopy was not routinely done and not used as criteria for chronic AMR. Acute cellular rejection was based on histological criteria only and included borderline acute cellular rejection. Transplant arteriopathy was not compared between groups because of the potential for sampling error and unclear distinguishing histopathologic features between arteriosclerosis from hypertension and transplant arteriopathy<sup>25-27</sup>.

### Immunosuppression and treatment protocols

Patients received ATG (Thymoglobulin Sangstad, Menlo Park Ca, 1.5mg/kg/d for 4 doses; anti-CD25 receptor antibodies (Simulect, Novartis Pharmaceuticals, East Hanover, NJ); or alemtuzumab (Campath, Genzyme, Cambridge, MA) as induction per center protocol. ATG was predominantly used for induction, especially in patients with known DSA. Anti-CD25 receptor antibodies were predominantly used in patients greater than age 65 with negative XM. The standard maintenance immunosuppression consisted of prednisone, tacrolimus, and mycophenolate mofetil in patients who received induction with ATG or anti-CD25 antibodies. Tacrolimus and mycophenolate mofetil were used alone in patients induced with alemtuzumab.

Some patients were included in desensitization studies during this studied time period. Eculizumab was given for desensitization from June 2008 through October 2011 for patients with XM mean channel shift of  $\geq 200$ . The specific details of that desensitization protocol has been described previously<sup>28</sup>. Patients treated with eculizumab after October 2011 were enrolled in another clinical trial in which the criteria for enrollment was to have a XM positive at any level. Another desensitization trial with bortezomib was started in 2008. This

was given to a subset of patients with cPRA>90% and a XM >300 against their original intended donor<sup>29</sup>.

Treatment for early acute *clinical* AMR included plasmapheresis, intravenous immunoglobulin, and occasional eculizumab. No patients received rituximab or splenectomy for acute *clinical* AMR. Because no therapy is proven effective for chronic AMR, we did not routinely provide therapy for patients with isolated AMR beyond 30 days posttransplant.

### Laboratory monitoring

At yearly intervals, patients had assessment of their renal function that included iothalamate clearance and 24-hour urine protein testing.

### Statistical Analysis

Statistical analysis was performed on JMPv10. (SAS, Cary, NC). For numerical data, groups were compared with the t-test or the Wilcoxon rank sum test as indicated. The reference group for statistical comparisons was the –DSA/-XM group. The Cochran Armitage Test for Trend was used to compare ordinal data when applicable. The Fisher's exact test was used to compare counts and percentages. Cumulative incidences were described by Kaplan–Meier estimates. Time-to-event data were compared with the log-rank test. Both univariate and multivariate Cox Regression models were performed to examine the risk factors for chronic antibody mediated rejection. Factors that had a p-value <0.05 were included in the multivariate model. Hazard ratios (HRs) were described by their point estimate and corresponding 95% confidence intervals. Testing was two-sided at the 0.05 level.

## RESULTS

### Patient demographics

Patient demographics are shown in Table 1. Compared to –DSA/-XM recipients, patients with +DSA/ high +XM and –DSA/ + XM were younger ( $p<0.01$ ) and more likely to be female ( $p<0.01$ ). All patients were predominantly Caucasian and recipients of living donor grafts. They were also similar in respect to the etiology of their renal disease, HLA matching, and follow-up.

Induction and/or maintenance immunosuppression therapy was different among the groups ( $p<0.01$ ). More patients in the –DSA/-XM group received basiliximab for induction (with tacrolimus, mycophenolate mofetil, and prednisone for maintenance) or alemtuzumab for induction (with tacrolimus and mycophenolate mofetil for maintenance) as compared to the other groups. Patients in the +DSA/high+XM group were more likely to have had a prior solid organ transplant [53.5% (23/43),  $p<0.01$ ] and dialysis prior to transplant [79.1% (34/43),  $p=0.04$ ].

The proportion of highly sensitized patients (cPRA  $\geq 80$ ) was progressively higher in the groups with increasingly DSA ( $p<0.01$ ). The mean XM channel shift was  $147.9\pm 37.1$  in the +DSA/low +XM group;  $283 \pm 67.7$  in the + DSA/high +XM group, and  $163.1\pm 56.2$  in the –DSA/+XM group. Few patients had an auto-B flow XM performed: 19.4% (7/36) in the +DSA/low + XM group, 11.6% (5/43) in the DSA+/high +XM group, and 30.8% (8/26) in

the -DSA/+XM group. Of the auto-B flow XM tests that were performed, few were positive: [14.3%,(1/7) in the +DSA/low+XM group; 20.0%, (1/5) in the +DSA/high+XM group; and 25.0% (2/8) in the -DSA/+XM group,  $p=0.87$ ].

Most patients in the +DSA/high + XM group received desensitization, but it was uncommon in the other groups including the +DSA/low + XM group (Table 1). Specifically, in the +DSA/high + XM group: 7.0% (3/43) received plasmapheresis and intravenous immunoglobulin (IVIG) alone, 81.0% (35/43) received eculizumab, plasmapheresis, and IVIG as part of clinical trial, and 14.3% (7/43) received bortezomib pretransplant. In the +DSA/low+XM group, 5.6% (2/36) received eculizumab, plasmapheresis, and IVIG as part of clinical trial for desensitization. No other patients in the +DSA/low+XM group received desensitization therapy prior to or at the time of transplant (Table 1).

### DSA characteristics

The proportion of patients with anti-class I DSA alone was similar among the groups with DSA detected at baseline (Figure 1). More patients in the +DSA/high+XM group had both anti class I and II DSA at baseline [27.9% (12/43) versus 3.7% (2/53) in the +DSA/-XM group and 11.1% (4/36) in the +DSA/low+XM group,  $p<0.01$ ]. The mean sum MFI of anti-Class I, anti-Class II, and anti-Class I+II was also highest in the +DSA/high +XM group,  $p<0.01$ .

### Patient and Allograft survival

Patient survival was similar in all groups over mean follow-up of  $4.1\pm 1.9$  years ( $p=0.82$ ) [actual patient survival: 93.4% -DSA/-XM; 96.3% +DSA/ -XM; 97.2% +DSA/low+ XM; 97.7% +DSA/high + XM and 92.3% -DSA/+XM] (Figure 2). Overall uncensored graft survival also was similar,  $p=0.06$  [actual overall allograft survival: 90.2% -DSA/-XM; 83.0% +DSA/ -XM; 94.4% +DSA/low + XM; 76.7% +DSA/high +XM, and 88.5% -DSA/+XM] (Figure 2). However, death-censored allograft survival was lower in the +DSA/high+ BFXM group (79.1%;  $p<0.01$ ), but similar in other groups [actual death censored allograft survival: 96.2% -DSA/-XM; 86.8% +DSA/-XM; 97.2% +DSA/low + XM; and 96.2% -DSA/+XM] (Figure 2).

Patient survival was also similar when the patients were grouped by the class of DSA present at baseline,  $p=0.29$ , (Figure 3). Both overall and death-censored allograft survival were the lowest when both anti-class I and II were present at baseline,  $p<0.01$ . Also, patients with only anti-class II DSA at baseline had inferior death-censored allograft survival compared to patients with only anti-class I or undetected DSA at baseline (Figure 3). Death-censored allograft survival was 70.6% in patients with both anti Class I and II, 87.1% if only anti-Class II was present, 92.9% if only anti class I was present, and 96.2% if DSA was undetected,  $p<0.01$ . Death-censored allograft survival was similar among patients who received eculizumab and/or bortezomib in the +DSA/low+XM and +DSA/high+XM groups (See Supplemental Table 1).

Chronic AMR was identified *prior to allograft loss* in 75.0% (6/8) of the cases of allograft failure in the +DSA/high+XM group (Table 2). Chronic AMR was also identified *prior to graft loss* in 16.7% (5/30) of the cases of allograft failure with -DSA/-XM; 37% (3/8) of

loses with +DSA/-XM, and 100.0% (1/1) of loses in the +DSA/-XM group. No patients in the +DSA/low+XM group had documented chronic AMR prior to allograft loss, and the 1 allograft failure in the +DSA/-XM group was in the setting of de novo DSA.

### Early acute *clinical* antibody mediated rejection

Early acute *clinical* AMR occurred in 16.3% (7/43) of patients in the +DSA/high +XM group, which was higher than the -DSA/-XM group [0.9 % (7/795),  $p < 0.01$ ] (Table 3). The incidence of early acute clinical AMR was 5.5% (2/36) in the +DSA/low+XM group, which was numerically higher than the -DSA/-XM group but this did not reach statistical significance,  $p = 0.05$ . No patients in the +DSA/-XM group and 3.9% (1/26) of patients in the -DSA/+XM group had early acute clinical AMR, which was similar to the -DSA/-XM group ( $p = 1.0$  and  $p = 0.22$ , respectively). No allografts were lost in the first year posttransplant from early acute *clinical* AMR.

### Antibody mediated rejection detected on surveillance biopsy

There was a clear pattern of increased microvascular inflammation and chronic AMR when a higher level of DSA was detected at baseline (Figure 4). This pattern was present at 1, 2, and 5 years ( $p < 0.01$  at 1, 2 and 5 years Cochran Test for Trend comparing -DSA/-XM, +DSA/+XM, +DSA/low+XM, and +DSA/high+XM groups). At 1 year, the prevalence of peritubular capillaritis was only 4.3% (28/647) in the -DSA/-XM group and 50.0% (19/38) in the +DSA/high+XM group. At 5 years, it was present in 3.0% (7/235) of -DSA/-XM group and 43.8% (7/16) in the +DSA/high+XM group. Chronic AMR was present in 2.0% (13/661) of the allografts in the -DSA/-XM group and 26.3% (10/38) of the allografts in the +DSA/high+XM group at 1 year. By 5 years, this increased to 7.2% (17/237) in the -DSA/-XM group and 56.3% (9/16) in the +DSA/high+XM group. In contrast, no difference in microvascular inflammation (peritubular capillaritis or glomerulitis) or chronic AMR was detected between the -DSA/-XM group and the -DSA/+XM group at 1, 2 or 5 years ( $p > 0.05$  at 1, 2 and 5 years for peritubular capillaritis, glomerulitis, and chronic AMR) (Figure 4).

### Acute cellular rejection

The rate of acute cellular rejection detected on surveillance biopsy was similar among all groups at 1 and 2 years (Table 4). At 5 years posttransplant, the rate of acute cellular rejection was similar among patients in the -DSA/-XM group and the +DSA/low+XM, +DSA/high+XM, and -DSA/+XM groups. The prevalence of acute cellular rejection on 5 year surveillance biopsies was higher in the +DSA/-XM group 21.4% (3/14) than the -DSA/-XM reference group at 2.1% (5/238),  $p < 0.01$ ; but the overall number of patients in the +DSA/-XM group who reached the 5 year posttransplant biopsy time point was small.

The overall incidence of acute cellular rejection (determined by examining all biopsies performed) during mean follow-up of  $4.1 \pm 1.9$  years was similar in all groups as compared to the -DSA/-XM group. The incidence was the following: 17.2% (137/795) -DSA/-XM reference, 15.1% (8/53) +DSA/-XM,  $p = 0.85$ ; 8.3% (3/36) +DSA/low + XM,  $p = 0.25$ ; 25.6% (11/43) +DSA/high+XM,  $p = 0.16$ ; and 23.1% (6/26) in the -DSA/+XM group,  $p = 0.43$ .

## Allograft function

Patients in the +DSA/high + XM group had a lower iothalamate GFR compared to the -DSA/-XM group at 1, 2, and 5 years as shown in (Figure 5) [46.9±16.7 vs. 58.7±17.7ml/min/BSA at 1 year, p<0.01; 47.7 ± 12.4 vs. 56.8±17.1 ml/min/BSA at 2 years, p<0.01; and 41.9±11.1 vs 54.7 ±17.2 ml/min/BSA at 5 years, p=0.02]. Proteinuria was also higher in the +DSA/high +XM group at 1 year compared to the -DSA/-BFXM group, [0.5±1.2mg/dl vs. 0.2±0.6, p<0.01], but similar at 2 and 5 years. Despite the differences between +DSA/high+XM and -DSA/-XM groups, the iothalamate GFR and proteinuria were similar among the other groups at 1,2 and 5 years posttransplant.

## Risk Factors for Chronic Antibody Mediated Rejection

The overall incidence of chronic AMR was 11.7% (111/953) during follow-up (determined by examining all biopsies obtained). The incidence correlated with increasing DSA at baseline and was the following: 8.2% (65/795) -DSA/-XM, 17.0% (9/53) +DSA/-XM, 30.6% (11/36) +DSA/low + XM, and 51.2% (22/43) in the +DSA/high+XM group, p<0.01 (Cochran Test for Trend) (Figure 6). However, the incidence of chronic AMR was similar in the -DSA/-XM and -DSA/+XM groups (8.2% (65/795) vs. 15.4% (4/26), p=0.19). Chronic AMR was similar among patients who received ecilizumab and/or bortezomib in the +DSA/low+XM and +DSA/high+XM groups (See Supplemental Table 1).

Early acute *clinical* AMR (HR 4.1, p<0.01), acute cellular rejection (HR 3.0, p<0.01), cPRA 80% (HR 3.8, p<0.01), class I DSA alone (HR 2.6, p<0.01), class II DSA alone (HR 3.5, p<0.01), class I and II DSA (HR 6.4, p<0.01), sum of Class I and II DSA (HR 1.8, p<0.01), +DSA/low + XM (HR 3.7, p<0.01) and +DSA high + XM at baseline (HR 7.3, p<0.01) were risk factors for chronic AMR in the univariate Cox proportional hazards model (Table 5). After adjustment in the multivariate model, the only independent risk factors for chronic AMR were acute cellular rejection (HR 2.3, p<0.01) or having DSA and a high + XM (HR 2.8, p=0.03) at baseline.

Among the patients with identified DSA at baseline (groups +DSA/-XM, +DSA/low+XM, and +DSA/high+XM), the only factor associated with chronic AMR was the sum MFI of DSA (Table 5). We examined sum MFI of as an ordinal variable (MFI <5000, 5000 to <10,000, 10,000 to <15,000, 15,000 to <20,000, and > 20,000). Essentially, for every increase in the sum MFI of DSA by 5000 MFI, the incidence of chronic AMR increased by 50% (HR 1.5, p<0.01). This association was also present when the +DSA/high + XM group was examined alone (data not shown, HR 1.4, p<0.02).

In patients with undetected DSA at the time of transplant (-DSA/-XM and -DSA/+XM groups); acute cellular rejection, number of HLA mismatches, and cPRA 80% were independently associated with chronic AMR (HR 3.6, p<0.01; HR 1.3, p<0.01, and HR 4.5, p<0.01; respectively) (Table 5).

## Outcomes of the highly sensitized (cPRA 80%)

Because having a cPRA 80% was identified as a risk factor for chronic AMR, we specifically examined the allograft survival and histologic outcomes in patients with cPRA



80%. Only patients with undetected DSA baseline were studied to avoid the confounding influence of known DSA. Patient, overall allograft, and death-censored allograft survival between patients with or without cPRA 80% was similar; but the histology was different.

As early as 1 year posttransplant, a higher proportion of allografts had microvascular inflammation in the cPRA 80% group [peritubular capillaritis 13.6% (6/44) vs. 3.9 % (24/610)  $p<0.01$ ; glomerulitis 9.1 % (4/44) vs 1.9%(12/622)  $p<0.01$ ]. By 2 years, more microvascular inflammation and chronic AMR was present in patients with cPRA 80% [peritubular capillaritis 16.1% (5/31) vs. 3.9 % (18/461)  $p<0.01$ ; glomerulitis 16.1 % (5/31) vs. 3.5 % (16/464)  $p<0.01$ ; chronic AMR 19.4% (6/31) vs. 4.7% (22/464),  $p<0.01$ ]. The number of patients with baseline cPRA 80 that had a protocol 5-year biopsy was small, but we found numerically higher peritubular capillaritis, glomerulitis, and chronic AMR; but this did not reach statistical significance [peritubular capillaritis 13.3%(2/15) vs. 2.3%(5/218),  $p=0.07$ ; glomerulitis 6.7%(1/15) vs 4.1%(7/210)  $p=0.49$ ; and chronic AMR 20.0%(3/15) vs. 6.8% (15/220),  $p=0.10$ ].

## DISCUSSION

The current study provides new insight into the outcomes of kidney transplants in recipients with evidence of pretransplant DSA. In a cohort comprised mainly of Caucasian living donor transplant recipients who did not receive desensitization therapy, the death-censored actuarial allograft survival was similar among patients with no identified DSA and low level DSA (with or without a low positive B flow XM) at mean follow-up of 4.1 years. Histologic evidence of antibody mediated injury detected by surveillance biopsy was increased in patients with DSA, but renal function was preserved during follow-up. The increased histologic evidence of antibody mediated injury may lead to inferior allograft survival for some patients in the long-term, but the benefits of avoiding dialysis for select patients with limited transplant options likely outweighs the risk of transplantation with low level DSA.

The current kidney allocation system provides greater access to transplantation for sensitized patients. However, our data suggests that waiting for a negative crossmatch deceased donor kidney may not always be advantageous. In our cohort, highly sensitized patients (cPRA 80%) were at risk for antibody mediated injury within a year posttransplant even when no DSA was detected at baseline. The option of a kidney transplant in the setting of low level DSA might actually be as good or better than waiting on dialysis for a negative crossmatch cadaveric transplant, especially when considering the possibility of receiving a preemptive transplant from a living donor (either in a paired donor system or with the patient's original donor). The overall half-life of a cadaveric transplant in the US is roughly 9 years as compared to 14 years for a living donor kidney, thus factors for graft loss beyond those immunologic should be considered<sup>30,31</sup>. Further study of the outcomes of those highly sensitized patients who received deceased donor organs resulting from the new allocation system is needed to better understand the consequences of the policy change.

Another important finding from our study was that patients with undetected DSA and an isolated positive XM (pronase treated) had outcomes comparable to patients with no DSA and a negative B flow cytometric XM. The cause of the positive XM in these cases is

unclear. Longer term follow-up and the study of potential non-HLA antibodies is needed in this group, but we believe that transplantation should be at least considered in these cases. Patients with identified DSA and high XM had the worst outcomes, but it is worth emphasizing that about half of these patients did not have chronic AMR at roughly 5 years posttransplant. Also, the incidence of early acute clinical AMR was only 16.3% with the use of eculizumab, plasmapheresis, and IVIG. Therefore, transplantation in the setting of DSA and high XM could be an option for a select subgroup of highly sensitized transplant candidates who have not yet benefited from paired donation programs or the new allocation system. In general, patients with anti-class II DSA had inferior allograft survival in our cohort and others<sup>5,32</sup>, therefore the avoidance of anti-class II may be a reasonable approach if a +DSA/high + XM transplant is planned. Further study is needed to better understand the DSA characteristics associated with chronic AMR and allograft loss.

Although the role of desensitization was not directly studied in our patient population, our results suggest that the practice of routine desensitization in patients with low level DSA positivity with or without low level positive flow cytometric warrants further study. The incidence of early acute clinical AMR in patients with low positive XM who were not desensitized was comparable if not lower than reported in previous studies in which routine plasmapheresis, intravenous immunoglobulin, or rituximab was used<sup>7,3334</sup>. The impact of desensitization therapy on chronic AMR in the absence of early acute AMR is also unclear. We do not recommend that current desensitization strategies be completely abandoned. Instead, we recommend further study on this subject because of the potential risks associated with desensitization.

This study had limitations including its nonrandomized retrospective design, relatively short follow-up, and that many patients of low immunologic risk were not treated with maintenance prednisone. Longer follow up might show that the subclinical histological findings of AMR on surveillance biopsy years might translate into decreased graft survival at 10 years and beyond as has been shown in other studies<sup>35-37</sup>. Early single antigen bead kits had limited DPB1 and DQA1 antigen coverage, and so it is possible that DSA towards these antigens was missed in some patients which could have impacted results. However, since 2011 we have been routinely looking for DSA towards these antigens. We also do not have long term DSA data or detailed antibody information such as prozone, titer, C1q, or subclasses—all of which have been shown to risk stratify patients at risk for chronic AMR<sup>9,38-40</sup>. Another important limitation is that our cohort was mainly Caucasian living donor transplant recipients. It is not known whether our results can be extrapolated to a minority or deceased donor transplant population.

In conclusion, kidney transplantation with low level DSA (with or without a low + XM) and no specific desensitization therapy may be an acceptable option for sensitized transplant candidates. Allograft survival at approximately 5 years is similar to that for patients with undetected DSA at baseline. However, several questions remain. Long term follow-up of these patients is needed to better understand the DSA and patient characteristics associated with chronic AMR and allograft loss. We also need to study the histology and long term allograft survival of the highly sensitized transplant candidates who received kidneys as part

of the new kidney allocation system. Answers to these important questions will help with donor selection for the highly sensitized transplant candidate.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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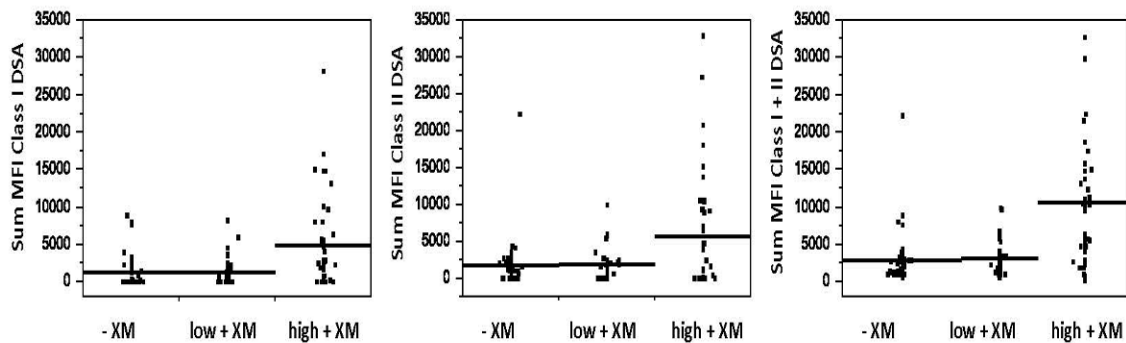
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## Abbreviations

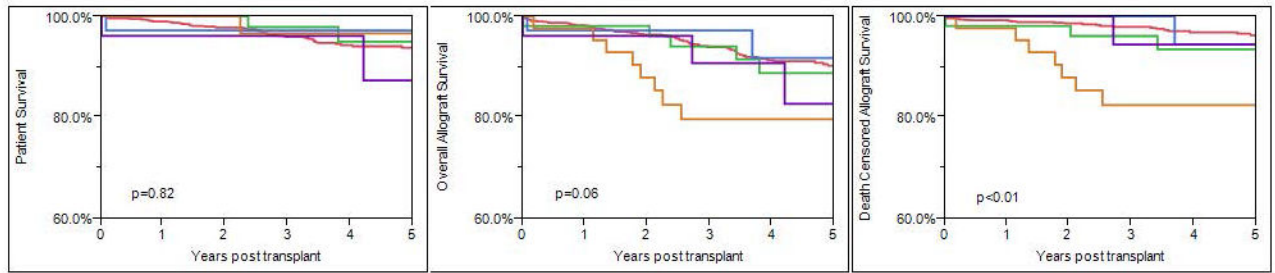
<b>AMR</b>	Antibody mediated rejection,
<b>XM</b>	B- flow cytometric crossmatch,
<b>cPRA</b>	calculated panel reactive antibody,
<b>DSA</b>	donor specific antibody,
<b>MFI</b>	mean fluorescence intensity,
<b>SAB</b>	single antigen beads



	- XM	Low + XM	High + XM	P-value
	N=53	N=36	N=43	
Anti-Class I DSA only n (%)	17(32.1)	8(22.9)	18(41.9)	P=0.17
Anti-Class II DSA only n (%)	34(64.2)	24(66.7)	13(30.2)	P<0.01
Anti-Class I + II DSA n (%)	2(3.7)	4(11.1)	12(27.9)	P<0.01
Anti-Class I DSA Sum (mean $\pm$ std)	1172.9 $\pm$ 2037.1	1267.1 $\pm$ 1905.3	4797 $\pm$ 6053.9	P<0.01
Anti-Class II DSA Sum (mean $\pm$ std)	1711.8 $\pm$ 3082.6	1861.4 $\pm$ 2145.4	5629.2 $\pm$ 7873.7	P<0.01
Anti-Class I + II DSA Sum (mean $\pm$ std)	2884.6 $\pm$ 3170.8	3128.5 $\pm$ 2421.2	10541.1 $\pm$ 9412.6	P<0.01

### Figure 1. Donor Specific Antibody Characteristics

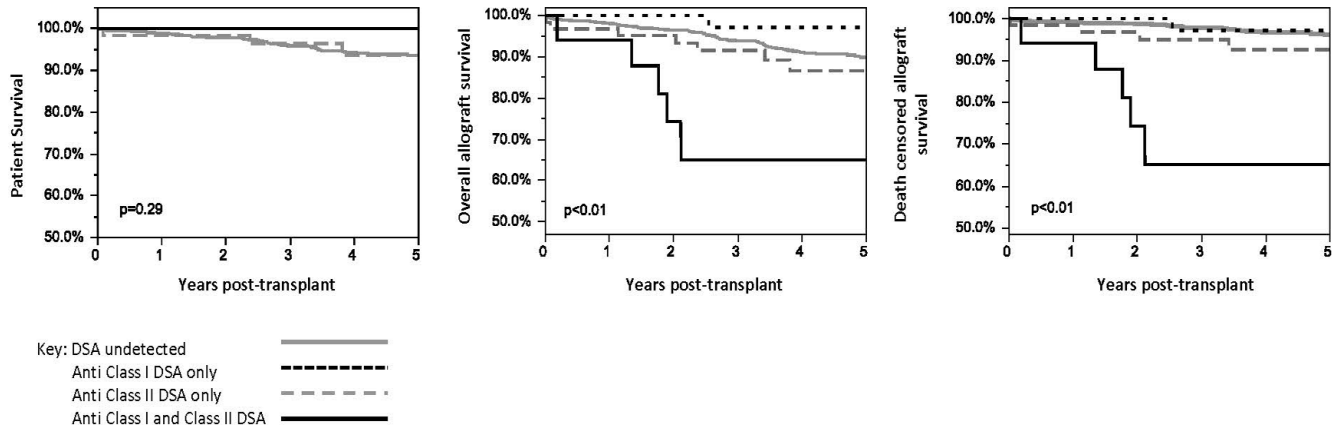
The characteristics of donor specific antibody present at baseline among the following transplant groups: +DSA/-XM (-XM), +DSA/low+XM (low+XM), and +DSA/high+XM (high+XM).



Key: -DSA/-XM  
 +DSA/-XM  
 +DSA/low + XM  
 +DSA/high + XM  
 -DSA/ +XM

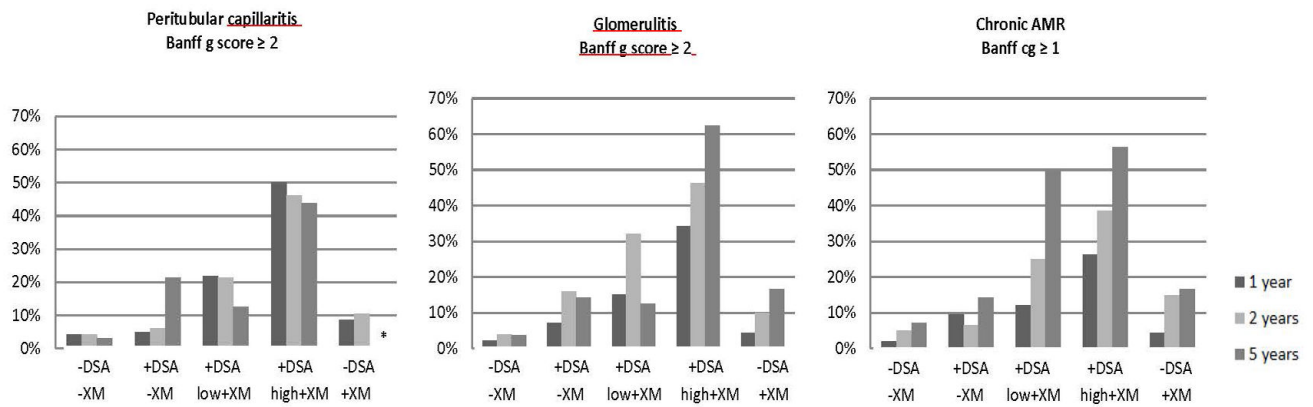
**Figure 2. Patient and Allograft Survival stratified by DSA and B-flow Crossmatch at Baseline**

The mean follow-up was similar among groups and was  $4.1 \pm 1.9$  years,  $p=0.82$ . Patient and overall allograft survival were similar among groups,  $p=0.82$  and  $p=0.06$  respectively. Lower death-censored allograft survival was observed in the +DSA/high+XM group,  $p<0.01$ . Death-censored allograft survival was similar in other groups,



**Figure 3. Patient and Allograft Survival stratified by DSA Class at Baseline**  
Patients with both class I + class II DSA at baseline had inferior overall and death-censored allograft ( $p < 0.01$ ).

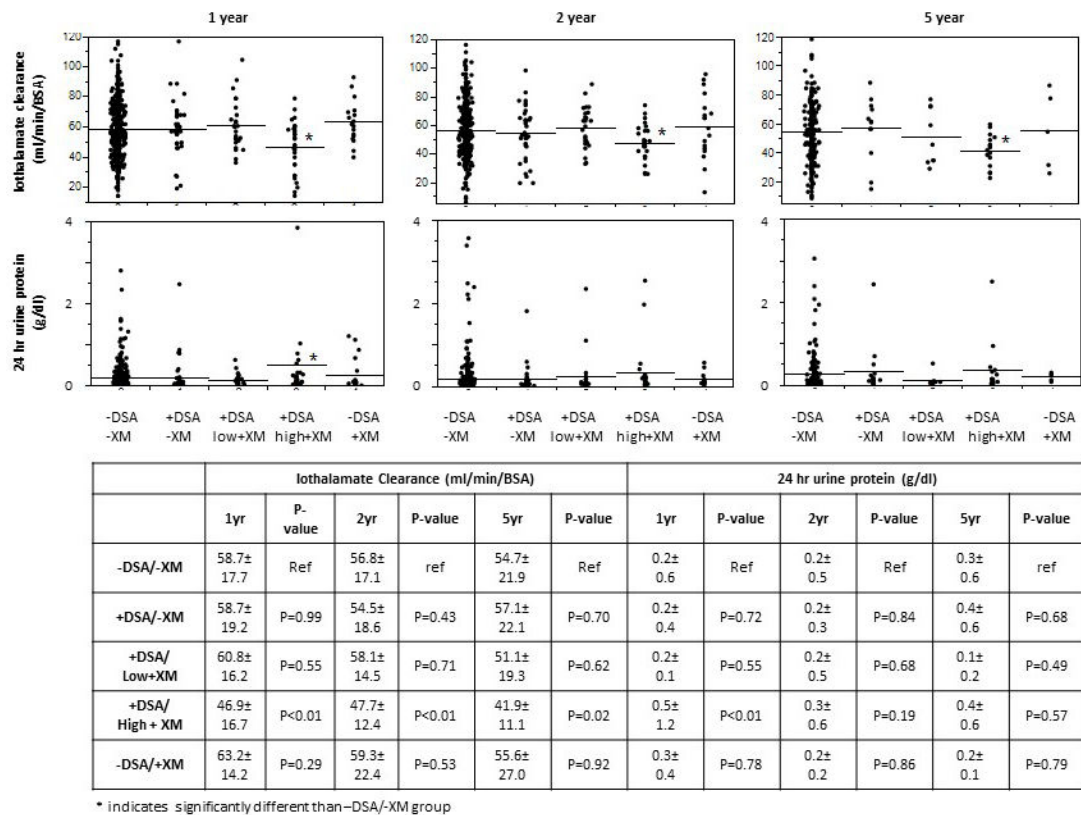




	1 year	2 year	5 years		1 year	2 year	5 years		1 year	2 year	5 years
-DSA/-XM	4.3%	4.3%	3.0%	-DSA/-XM	2.3%	3.9%	3.8%	-DSA/-XM	2.0%	5.1%	7.2%
	28/647	21/487	7/235		15/660	19/489	9/237		13/661	25/489	17/237
+DSA/-XM	4.9%	6.2%	21.4%	+DSA/-XM	7.1%	16.1%	14.3%	+DSA/-XM	9.5%	6.5%	14.3%
	2/41	2/31	3/14		3/42	5/31	2/14		4/42	2/31	2/14
+DSA/Low+XM	21.9%	21.4%	12.5%	+DSA/Low+XM	15.2%	32.1%	12.5%	+DSA/Low+XM	12.1%	25.0%	50.0%
	7/32	6/28	1/8		5/33	9/28	1/8		4/33	7/28	4/8
+DSA/High+XM	50.0%	46.2%	43.8%	+DSA/High+XM	34.2%	46.2%	62.5%	+DSA/High+XM	26.3%	38.5%	56.3%
	19/38	12/26	7/16		13/38	12/26	10/16		10/38	10/26	9/16
P-value**	P<0.01	P<0.01	P<0.01	P-value	P<0.01	P<0.01	P<0.01	P-value	P<0.01	P<0.01	P<0.01
-DSA/+XM	8.7%	10.5%	0%	-DSA/+XM	4.4%	10.0%	16.7%	-DSA/+XM	4.4%	15.0%	16.7%
	2/23	2/19	0/6		1/23	2/20	1/6		1/23	3/20	1/6
P-value†	P=0.28	P=0.21	P=1.0	P-value	P=0.43	P=0.20	P=0.07	P-value	P=0.38	P=0.09	P=0.37

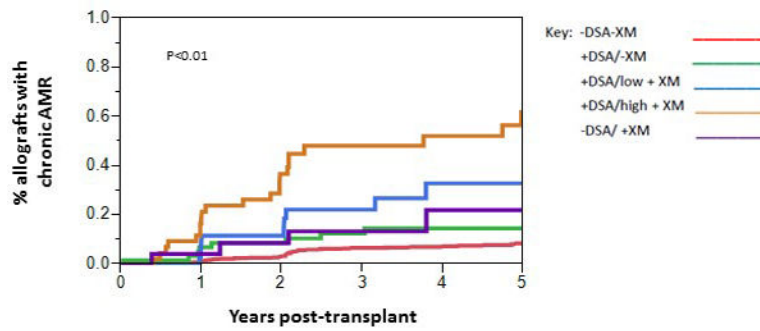
**Figure 4. Microvascular Inflammation and Chronic AMR detected on Surveillance Biopsy**

The prevalence of peritubular capillaritis, glomerulitis, and chronic AMR was higher in groups with higher DSA at all time points studied ( $p < 0.01$  \*Cochran test for trend -DSA/-XM and +DSA groups). The prevalence of peritubular capillaritis, glomerulitis, and chronic AMR was similar in patients with undetected DSA and negative or positive XM. († -DSA/-XM and -DSA/+XM groups  $p = 0.37$ ,  $p = 0.09$ , and  $p = 0.38$  respectively). \*\* No patients in the -DSA/+XM group had peritubular capillaritis at 5 years.



### Figure 5. Allograft Function and Proteinuria

Patients in the +DSA/high+XM group had lower GFR at 1,2 and 5 years posttransplant than the -DSA/-XM group as measured by iothalamate clearance,  $p<0.01$ . GFR was between the -DSA/-XM and +DSA/-XM, +DSA/low+XM, and -DSA/+XM groups at 1,2 and 5 years. Proteinuria was also higher in the +DSA/high+XM group as compared to the -DSA/-XM group at 1 year posttransplant,  $p<0.01$ .



**Figure 6. Incidence of Chronic AMR**

The overall incidence of chronic AMR was 11.7% and was determined by examining both surveillance and indication biopsies. The incidence correlated with increasing DSA at baseline and was the following: 8.2% -DSA/-XM, 17.0% +DSA/-XM, 30.6% +DSA/low + XM, and 51.2% in the +DSA/high+XM group,  $p < 0.01$  (Cochran Test for Trend). The incidence of chronic AMR was similar in the -DSA/-XM and -DSA/+XM groups (8.2% vs. 15.4%,  $p = 0.19$ ).

Table 1

## Patient Demographics

	-DSA-XM	+DSA-XM	+DSA Low + XM	+DSA High + XM	-DSA + XM	p-value
	N=795	N=53	N=36	N=43	N=26	
<b>Age (Mean age±std)</b>	52.7±13.9	50.4±15.1	50.6±12.1	47.0±13.4	45.6±13.9	p<0.01
<b>Female Gender n(%)</b>	297(37.4)	25(47.2)	25(69.4)	30(69.7)	12(57.7)	p<0.01
<b>Race n(%)</b>						p=0.58
White	718(90.3)	52(98.1)	34(94.4)	40(93.0)	22(84.6)	
Hispanic	18(2.3)	0(0)	1(2.9)	2(4.7)	2(7.7)	
African American	33(4.2)	0(0)	1(2.9)	0(0)	1(3.9)	
Asian	12(1.5)	1(1.9)	0(0)	1(2.3)	1(3.9)	
American Indian/Pacific Islander	14(1.8)	0(0)	0(0)	0(0)	0(0)	
<b>Donor type n(%)</b>						p<0.01
LURD	351(44.2)	35(66.0)	17(47.2)	29(67.4)	10(38.5)	
LRD	305(38.3)	14(26.4)	11(30.6)	12(27.9)	11(42.3)	
Deceased Donor	139(17.5)	4(7.6)	8(22.2)	2(4.7)	5(19.2)	
<b>Etiology of ESRD n(%)</b>						p=0.13
Glomerulonephritis	282(35.5)	21(39.6)	20(55.6)	19(44.2)	10(38.5)	
Diabetes	148(18.6)	6(11.3)	2(5.6)	1(2.3)	4(15.4)	
Hypertension	48(6.0)	1(1.9)	0(0)	2(4.7)	0(0)	
Cystic kidney disease	131(16.9)	10(18.9)	6(16.7)	6(14.0)	5(19.2)	
Other	137(17.2)	12(22.6)	4(11.4)	12(27.9)	6(23.1)	
Unknown	49(6.2)	3(5.6)	4(11.4)	3(7.0)	1(3.9)	
<b>HLA mismatch (mean +/-std)</b>	3.4±1.9	4.2±1.4	3.9±1.5	3.7±1.3	3.0±1.7	p=0.09
<b>Induction n(%)</b>						p<0.01
Anti-Thymocyte Globulin	402(50.6)	42(77.4)	34(94.4)	42(97.7)	20(76.9)	
Basiliximab	241(30.3)	5(9.4)	1(2.98)	1(2.3)	3(11.5)	
Alemtuzumab	151(19.0)	7(13.2)	1(2.8)	0(0)	3(11.5)	

	-DSA-XM	+DSA-XM	+DSA Low + XM	+DSA High + XM	-DSA + XM	p-value
None	1(0.1)	0(0)	0(0)	0(0)	0(0.0)	
<b>Prior solid organ transplant n(%)</b>	133(16.7)	11(20.8)	18(50.0)	23(53.5)	6(23.1)	p<0.01
<b>Dialysis prior to transplantation n(%)</b>						
Yes	424(53.3)	26(49.1)	24(66.7)	34(79.1)	12(46.2)	P=0.04
No	288(36.2)	21(39.6)	11(30.6)	7(16.3)	12(46.2)	
Unknown	83(10.4)	6(11.3)	1(2.8)	2(4.6)	2(7.8)	
<b>Time on Dialysis* (mean +/-std)</b>	2.2±0.1	1.8±0.5	2.5±0.5	3.5±0.4	2.9±0.7	P=0.03
<b>Percent cPRA** (mean +/-std)</b>	12.3±26.9	42.2±36.3	54.5±38.2	79.8±26.3	16.2±29.2	p<0.01
<b>cPRA 80% n(%)**</b>	51(6.6)	12(23.1)	15(41.6)	28(68.3)	2(8.0)	p<0.01
<b>B flow XM (mean channel shift ±std)</b>	NA	NA	147.9±37.1	283.3±67.7	163.1± 56.2	p<0.01
<b>Auto-B flow XM positivity n/N (%)</b>	NA	NA	1/7 (14.3)	1/5 (20.0)	2/8(25.0)	P=0.87
<b>Desensitization n(%)</b>						
Plasmapheresis/IVIG(alone)	0(0%)	0(0%)	0(0%)	3(7.0%)	0(0%)	
Eculizumab <sup>†</sup>	0(0%)	0(0%)	2(5.6%)	35(81.0%)	0(0%)	p<0.01
Bortezomib	2(0.25%)	1(1.9%)	0(0%)	7(14.3%)	0(0%)	
<b>Follow-up(Mean years±std)</b>	4.1±1.9	4.3±1.7	3.8±1.8	4.1±2.1	3.7±1.9	p=0.49

\* Patients who received a preemptive transplant were excluded.

\*\* Calculated based on MFI 2000.

<sup>†</sup> All patients who received eculizumab also received plasmapheresis and IVIG.

## Causes of Allograft loss

Table 2

	-DSA-/XM N=30	+ DSA-/XM N=8	+DSA/low+XM N=1	+DSA/high+XM N=8	-DSA/+XM N=1	p-value
N=number of allograft failures						
Chronic antibody mediated rejection n(%)	5 (16.7)	3(37.5)	0(0)	6(75.0)	1/(100.0)*	P=0.22
Documented nonadherence: acute rejection n(%)	6(20.0)	2(25.0)	0(0)	0(0)	0(0)	P=0.24
Recurrent/De novo Glomerular Disease n(%)	6(20.0)	1(12.5)	1(100.0)	1(12.5)	0(0)	P=0.29
Allograft thrombosis n(%)	3(10.0)	0(0)	0(0)	0(0)	0(0)	P=0.27
IFTA <sup>‡</sup> n(%)	3(10.0)	0(0)	0(0)	0(0)	0(0)	P=0.27
Systemic Illness n(%)	6(20.0)	2(25.0)	0(0)	1(12.5)	0(0)	P=0.24
Unknown n(%)	1(3.3)	0(0)	0(0)	0(0)	0(0)	P=0.29

\* In setting of de novo DSA,

<sup>‡</sup> IFTA=interstitial fibrosis and tubular atrophy

**Table 3**Early Acute *Clinical/AMR* based on Histologic Criteria

	-DSA/- XM n=795	+ DSA/- XM n=53	+ DSA/Low +XM n=36	+ DSA/High +XM n=43	-DSA/+XM n=26
Early acute clinical AMR n (%)	7(0.9%)	0(0%)	2 (5.5%)	7 (16.3%)	1 (3.9%)
p-value	reference	p=1.0	p=0.05	p<0.01	p=0.22

**Table 4**

## Acute Cellular Rejection Detected on Surveillance Biopsy

	1 year	p - value	2 years	p-value	5 years	p-value
-DSA/-XM	4.1% 27/661	Ref	5.4% 28/518	Ref	2.1% 5/238	Ref
+DSA/-XM	4.8% 2/42	P=0.68	3.2% 1/31	P=1.0	21.4% 3/14	P<0.01
+DSA/Low+XM	6.1% 2/33	P=0.64	0% 0/28	P=0.39	0% 0/8	P=1.0
+DSA/High +XM	2.6% 1/36	P=1.0	7.6% 2/26	P=0.68	0% 0/16	P=1.0
-DSA+XM	4.3% 1/23	P=1.0	0% 0/20	P=0.62	16.7% 1/6	P=0.14



**Table 5**

Cox Regression Models of Chronic Antibody Mediated Rejection in Different Patients Groups

<b>All Patients</b>				
<b>Variables</b>	Univariate		Multivariate	
	HR (CI)	p-value	HR (CI)	p-value
<b>Early Acute clinical AMR</b>	4.1(1.6-8.6)	p<0.01	1.3(0.5-3.0)	p=0.6
<b>Acute cellular rejection</b>	3.0(2.1-4.4)	P<0.01	2.3(1.5-3.4)	P<0.01
<b>cPRA 80%</b>	3.8 (2.5-5.7)	p<0.01	1.6(0.9-2.7)	p=0.12
<b>Class I DSA only</b>	2.6(1.3-4.5)	p<0.01	0.9(0.4-2.3)	p=0.98
<b>Class II DSA only</b>	3.5(2.2-5.6)	p<0.01	1.5(0.7-3.5)	p=0.29
<b>Class I + II DSA</b>	6.4(2.9-12.3)	p<0.01	1.5(0.4-4.4)	p=0.50
<b>Sum all DSA*</b>	1.8(1.6-2.1)	p<0.01	1.1 (0.8-1.5)	p=0.43
<b>+DSA/-XM</b>	1.4 (0.7-2.6)	p=0.35		
<b>+DSA/low+XM</b>	3.7 (1.8-6.5)	p<0.01	2.3(0.9-5.7)	p=0.07
<b>+DSA/high+XM</b>	7.3 (4.5-11.5)	p<0.01	2.8(1.1-7.4)	p=0.03
<b>-DSA/+XM</b>	1.5 (0.5-3.6)	p=0.44		
			Full model	p<0.01
<b>Patients with DSA detected at the time of transplant (+DSA/-XM, +DSA/low+XM, +DSA/high+XM)</b>				
<b>Variables</b>	Univariate		Multivariate	
	HR (CI)	p-value	HR (CI)	p-value
<b>Early Acute clinical AMR</b>	1.7(0.4-4.8)	p=0.40		
<b>Acute cellular rejection</b>	1.3(0.7-2.5)	P=0.40		
<b>cPRA 80%</b>	1.7(0.9-3.2)	p=0.09		
<b>Class I DSA only</b>	0.7(0.4-1.4)	p=0.33		
<b>Class II DSA only</b>	1.0(0.6-1.9)	p=0.99		
<b>Class I+II DSA only</b>	2.2(0.9-4.4)	p=0.07		
<b>Sum MFI all DSA</b>	1.5(1.2-1.8)	p<0.001		
<b>Patients with no DSA detected at time of transplant (-DSA/-XM and -DSA/+XM)</b>				
<b>Variables</b>	Univariate		Multivariate	
	HR (CI)	p-value	HR (CI)	p-value
<b>Early Acute clinical AMR</b>	4.8(1.2-12.9)	p=0.03	2.1(0.5-6.0)	p=0.26
<b>Acute cellular rejection</b>	3.9(2.4-6.3)	P<0.01	3.6(2.2-5.8)	P<0.01
<b>Number of HLA mismatches</b>	1.2(1.0-1.4)	P=0.01	1.3(1.1-1.5)	P<0.01
<b>cPRA 80%</b>	3.1(1.5-5.7)	p<0.01	4.5(2.1-8.7)	p<0.01
			Full model	p<0.01