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Concurrent Acute Cellular Rejection Is An Independent Risk Factor For Renal Allograft Failure In Patients With C4d Positive Antibody-Mediated Rejection

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Abstract

Background—Identification of risk factors for renal allograft failure following an episode of acute antibody-mediated rejection (AMR) may help outcome of this difficult to treat complication.

Methods—Eight hundred thirty-three kidney graft recipients underwent 1120 clinically indicated biopsies at our center during 12/2003 to 2/2011. We reviewed these biopsies and identified 87 biopsies from 87 patients positive for C4d and acute AMR. We performed bivariate analysis using Kaplan-Meier (K-M) curve and multivariable analysis using Cox proportional hazard regression to identify risk factors for allograft failure following C4d+ acute AMR.

Results—Among the 87 patients, biopsies from 26 were diagnosed as acute AMR using Banff '09 classification schema, 29 as acute AMR and chronic active AMR, 18 as acute AMR and acute TCMR, and 14 as acute AMR, chronic active AMR and acute TCMR. K-M survival estimates showed that concurrent acute TCMR (P=0.001, Mantel-Cox log-rank test), concurrent chronic active AMR (P=0.03) and time to biopsy (P=0.04) are associated with graft survival. Cox proportional hazard regression analysis identified that concurrent acute TCMR (Hazard Ratio

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[HR] 2.59, 95 percent confidence interval 1.21–5.55, $P=0.01$) and estimated glomerular filtration rate (HR: 0.65 [0.48–0.88], $P=0.01$) are independent risk factors for allograft loss. Concurrent chronic active AMR or time to biopsy was not associated with graft failure by multivariable Cox analysis.

Conclusions—Our single center study has elucidated that concurrent acute TCMR in kidney transplant recipients with C4d+ acute AMR is an independent risk factor for graft failure. Level of allograft function at the time of biopsy diagnosis was also an independent predictor of graft loss.

Keywords

acute antibody-mediated rejection; graft survival; acute T-cell mediated rejection; renal transplantation

Introduction

Hyperacute rejection of kidney allograft is the most dramatic manifestation of antibody-mediated graft failure but donor specific humoral presensitization, unmasked with the use of ultrasensitive crossmatch protocols, leading to antibody-mediated vasculitis and early graft failure has been reported as early as in 1980 (1). The contributions of antibodies to rejection and allograft failure have reemerged forcefully with improved detection of donor specific anti-HLA antibodies (DSA) and the use of intra-graft deposition of the degradation product of complement component 4 (C4d) as a histologic feature of antibody-mediated rejection (AMR) (2–4). The current Banff diagnostic criteria for acute AMR include: (i) C4d deposits in peritubular capillaries, (ii) circulating DSA, and (iii) morphologic evidence for acute tissue injury (5). Acute allograft dysfunction distinguishes the clinical AMR from the subclinical form and the presence of chronic tissue injury (e.g., glomerular double contours) in patients with circulating DSA and intra-graft C4d are the criteria for the diagnosis of chronic active AMR. Time from transplantation to clinical manifestation of AMR has been reported to be associated with responsiveness to antirejection therapy and graft outcome (6) and histological features such as intra-graft C4d deposition, neutrophilic glomerulitis, monocyte/macrophage infiltration and immunologic characteristics such as the presence or persistence of DSA are reported to be harbingers of graft failure following an episode of AMR (7–11).

The primary objective of the current investigation was to identify risk factors for graft failure in kidney graft recipients with clinically indicated biopsies displaying both C4d and morphologic features of acute AMR. We examined whether concurrent histologic features such as acute TCMR, chronic active AMR and/ or interstitial fibrosis and tubular atrophy (IF/TA) are associated with graft survival. We also determined whether time from transplantation to biopsy and whether allograft function (estimated glomerular filtration rate, proteinuria) are related to graft survival.

Results

Characteristics of the study cohort at the time of kidney transplantation

We reviewed 1120 clinically indicated biopsies from 833 kidney allograft recipients and identified 87 biopsies from the 87 patients that were positive for C4d immunofluorescence staining and displayed morphologic evidence of acute tissue injury consistent with acute AMR. The 1120 biopsies were performed at our center between 12/2003 and 2/2011 and all were stained for C4d.

The demography, pre-transplant and transplant characteristics including donor type and induction and maintenance immunosuppression used in the 87 patients are summarized in Table 1.

Characteristics of the study cohort at the time of clinically indicated allograft biopsy

The clinical indications for performing the biopsy were acute graft dysfunction in 69 (79%) patients, delayed graft function (DGF) in 7 (8%), and significant proteinuria (defined as more than 0.5 gram per day) or chronic graft dysfunction (eGFR less than 40 ml/min/1.73m²) in 11 (13%) patients. The median time from transplantation to biopsy was 3.7 months, and the time from transplantation to biopsy was greater than one year in 35 kidney transplant recipients. At the time of diagnostic biopsy, the mean ($\pm$ SD) eGFR was 23.2 $\pm$ 15.1 ml/min/1.73m² and proteinuria was more than one gram per day in 48 of 87 patients.

All 87 biopsies showed C4d+ acute AMR and among the 87 biopsies, 26 showed acute AMR only, 43 concurrent chronic active AMR (29 acute AMR plus chronic active AMR and 14 acute AMR, chronic active AMR and acute TCMR) and 32 concurrent acute TCMR (18 acute AMR and concurrent acute TCMR, and 14 acute AMR, chronic active AMR and acute TCMR). Interstitial fibrosis and tubular atrophy (IF/TA) were present in 37 of the 87 biopsies. The histologic features of allograft biopsies, classified and graded using the Banff '09 schema, are summarized in Table 2.

Results of circulating donor specific anti-HLA antibodies (DSA) were available in 66 of 87 patients and 60 of 66 were classified as DSA positive using mean fluorescence intensity (MFI) >500 as the threshold using microbeads coated with purified single Class I or Class II HLA antigens.

Anti-Rejection treatment

Eighty-one of the 87 patients (93%) received anti-rejection therapy consisting of methylprednisolone, IVIG or PP (standard therapy) and 6 patients did not receive anti-rejection treatment due to reasons such as extensive IF/TA in their biopsies. In the 81 patients treated, the components of the anti-rejection therapy reflected evolution over time, and included the addition of antithymocyte globulin (n=39 patients), rituximab (n=25 patients) and bortezomib (n=15 patients) to standard therapy (Table 2). Reversal of rejection episode, as defined by the return of the eGFR level to within 15% of the baseline within eight weeks after initiation of anti-rejection treatment, occurred in 41 (47%) patients. The median follow up after biopsy diagnosis was 28 months at the time of preparation of this report. Thirty-seven patients (43%) developed allograft failure; defined as persistent decline in eGFR to < 15 ml/min/1.73m² or return to dialysis, during the study period and the median time from the biopsy to allograft failure was 4 months.

Kaplan-Meier graft survival analysis

The impact of concurrent biopsy findings, time from transplantation to biopsy (<12 months vs. >12 months), presence or absence of proteinuria greater than one gram/day, and the type of anti-rejection therapy were examined with the use of Kaplan-Meier curve analysis.

Figure 1 illustrates that concurrent acute TCMR (P=0.001, log-rank test), concurrent chronic active AMR (P=0.037) but not concurrent IF/TA (P=0.46) are associated with graft survival time in patients with biopsies showing C4d positive acute AMR. Figure 2 also illuminates that late rejection (biopsy>12 months) is associated with a shorter graft survival time (P=0.04) compared to early rejection (biopsy <12 months). The presence or absence of proteinuria (P=0.09) and the addition of antithymocyte globulin (P=0.27), rituximab (P=0.70) or bortezomib (P=0.96) to standard anti-rejection therapy were not associated with

allograft survival time. We did not analyze the impact of the presence or absence of DSA since 60 of 66 patients in whom DSA information was available were positive for DSA.

Twenty-five grafts failed during the 12 months following the for-cause biopsy, and the one-year actual graft survival post-biopsy was 70% in the 87 patients with C4d positive biopsies. Among the 25 graft failures, 9 failed in the 55 patients without concurrent acute TCMR and the actual one-year graft survival in this subgroup was 83%. Sixteen grafts failed in the 32 patients with concurrent acute TCMR and the one-year actual graft survival in this subset was 46%.

Because concurrent acute TCMR and concurrent chronic active AMR were associated with graft survival and because time to biopsy was also associated with graft survival, we examined whether the renal allograft biopsy results were related to time from transplantation to clinically indicated biopsies. This bivariate analysis showed that the proportion of biopsies showing acute TCMR were not associated with time to biopsy ($P=0.37$, Fisher's Exact test) (Table 3). As expected, chronic active AMR ($P<0.0001$) and IF/TA ($P<0.0001$) were over-represented in late biopsies (>12 months) compared to early (<12 months) biopsies.

Cox proportional hazard multivariable analysis

We used Cox proportional hazard multivariable analysis, a statistical tool for determining the relative contributions of different causes to a single event, to identify independent predictors for graft failure. To adjust for confounding, we decided to use all of the histological and treatment variables at the time of diagnosis, *a priori*, in the Cox model, irrespective of their significance level by bivariate analyses. The six recipients who did not receive anti-rejection therapy due to clinical/histological considerations were excluded from the prediction model and the remaining 81 recipients were included in the analysis.

Multivariable analysis using Cox proportional hazard regression showed that concurrent acute TCMR (hazard ratio [HR] 2.59, 95% confidence interval [95%CI] 1.21–5.55, $P=0.01$) remained an independent predictor of graft failure (Table 4). Our analysis identified that eGFR at the time of rejection (HR: 0.65 [0.48 to 0.88], $P=0.01$) is an independent risk factor and that neither concurrent chronic active AMR nor time to biopsy is significantly associated with graft survival.

HLA-specific DSA information was available in 66 of 87 patients and 60 of 66 recipients had circulating DSA. We did not test for non-HLA antibodies in our study subjects. Of the 60 recipients who had positive circulating DSA, 55 patients had complete data such as the number donor HLA antigens recognized by DSA, the sum of MFI of all DSA detected, and the MFI of immunodominant DSA. We examined the effect of each of the three DSA characteristics in the multivariable analysis, and found that none of the DSA characteristic is independently associated with graft failure. In the analysis restricted to the same 55 patients, concurrent acute TCMR ($P=0.03$, 0.02 and 0.04 respectively) and eGFR ($P=0.002$, 0.002 and 0.002, respectively) remained significantly associated with graft failure.

Discussion

Antibody-mediated rejection generally carries a poorer prognosis compared to acute T-cell mediated rejection and it has been reported that kidney graft survival is shorter in those with C4d positive AMR compared to C4d negative AMR (7). We have identified that concurrent acute TCMR is an independent risk factor for graft failure in kidney graft recipients with biopsies positive for C4d and acute AMR.

Among the 1120 biopsies from 833 kidney transplant recipients, 87 biopsies from 87 patients were positive for C4d and acute AMR changes and the incidence of C4d+ acute AMR biopsies was 7.8% in our study. The incidence of C4d+ biopsies with acute AMR and concurrent acute TCMR among all 1120 biopsies was 2.9% and 37% of the 87 C4d+ biopsies with acute AMR features we identified displayed features of concurrent acute TCMR. Importantly the majority of graft failures, 16 of the 25 failures (64%) in the first year post biopsy, occurred in the subset with concurrent acute TCMR. An interesting biologic question is the sequence of events in patients manifesting both humoral immunity and T cell immunity, and whether acute TCMR preceded C4d positive AMR. The biology of what came first (C4d positive acute AMR vs. acute TCMR) cannot be resolved with our investigation since we did not analyze results from sequential biopsies. Because T cell help is needed for IgG antibody production, a cascade of events with T cell priming preceding a C4d+ acute AMR is biologically plausible. However, the majority (63%) of the C4d positive AMR biopsies did not display concurrent TCMR. We speculate therefore that an acute TCMR is not an essential prerequisite for the development of C4d positive AMR.

Kidney allograft biopsy findings and graft survival time

Feucht et al. made the seminal observations that vascular deposition of complement fragments C4d and C3d is a histologic feature of kidney graft biopsies displaying cell mediated rejection, and that capillary deposition of C4d is associated with early graft dysfunction and early graft loss following deceased donor kidney transplantation (3, 7). In Feucht's study (7), the one-year graft survival was 57% to 63% in those with C4d+ biopsies and 90% in those without C4 staining of capillaries. Gaston et al. (8) also reported inferior graft survival rates in the 40 C4d+DSA+ patients compared to the 74 C4d-DSA-patients (Hazard ratio: 4.58, 95%CI: 1.75–12.00, P=0.002). Neutrophilic glomerulitis, peritubular capillary dilatation with neutrophilic infiltrates and interstitial edema at the time of first biopsy have also been reported as determinants of graft failure in AMR patients (9, 11). In our study, concurrent chronic active AMR was related to graft survival by the bivariate Kaplan-Meier curve analysis but was not significant by multivariable analysis. The presence of IF/TA was also not associated with graft survival time. The median follow up in our study however was two years and this short duration might have been insufficient to witness the adverse impact previously observed with time to biopsy or chronic changes (6, 12–13).

Potential mechanisms for the differential kidney graft outcomes in those with acute AMR vs. acute AMR and concurrent acute TCMR.

In our study, 25 grafts failed within one year of biopsy and the overall one-year actual graft survival post biopsy was 70% in the 87 patients with C4d+ biopsies that also displayed acute AMR features. The one-year actual graft survival was 83% in the 55 patients without concurrent acute TCMR and 46% in the 32 patients with concurrent acute TCMR. The plausible mechanisms for the heightened risk associated with concurrent acute TCMR include: (i) the participation of memory T cells that are relatively resistant to conventional anti-T cell therapy; (ii) the contribution of multiple cell types including macrophages/monocytes to the "cellular" component of cell mediated rejection; (iii) the antibody type (e.g., IgG subtype or complement fixing capacity) may be different with active T cell help compared to when T cells are absent; and (iv) the possible contribution of antibody dependent cell mediated cytotoxicity effector mechanism to the graft injury process (since Fc receptor bearing cells and antibodies are both present in the graft). The contribution of concurrent chronic changes to the adverse impact of concurrent acute TCMR cannot be excluded completely but appears unlikely in view of data shown in Table 4. Although the precise biologic basis for our novel observation remains unresolved, our findings advance the idea that anti-T cell therapy be an integral component of patients with biopsies displaying both humoral (AMR) and cellular (TCMR) immunity. In this regard, currently

prescribed immunosuppressive drugs such as CNI are highly active against T-cell mediated immune responses. The occurrence of concurrent TCMR in our patients may reflect under-immune suppression, due to clinical circumstances (e.g., drug toxicity) and/or non-adherence. In view of lack of robust parameters to define the immune status of organ graft recipients, we are unable to address the important issue of whether the occurrence of TCMR in our patients reflected the potentially remediable problem of under-immunosuppression.

DSA and graft outcome

The presence of DSA and persistence of DSA after treatment have both been associated with graft loss after AMR (6, 9–11, 14–17). HLA-specific DSA information was available in 66 of 87 patients and 60 of 66 recipients had circulating DSA. We did not test for non-HLA antibodies in our study subjects. The almost universal presence of DSA in our study cohort precluded an analysis regarding the significance of presence or absence of DSA on graft outcome and we restricted our analysis to investigating the impact of DSA characteristics (number of donor HLA antigens recognized, immunodominant DSA, MFI) rather than the presence or absence on graft outcome. Our focused analysis found no relationship between DSA characteristics and graft survival time. A limitation of our study is that DSA data was available only in 66 of 87 patients studied and we did not have longitudinal information. These shortcomings may have contributed to under-appreciation of the role of DSA characteristics in those with C4d+ acute AMR.

Time from transplantation to biopsy diagnosis of C4d+ acute AMR

Time of onset of acute rejection has been reported to be determinant of graft outcome with late acute rejection being more recalcitrant to anti-rejection therapy compared to early onset acute rejection (6, 9, 18–19). In our study, time from transplantation to biopsy diagnosis was indeed significantly associated with graft survival by univariate analysis but lost its significance following multivariable analysis. As expected, our study did find that chronic changes are more common in late biopsies compared to early biopsies (Table 3). In this regard, the time related late features such as chronic active AMR and IF/TA were not independent predictors of graft survival time in our study whereas acute TCMR that was not related to time to biopsy heightened the risk of graft failure.

Treatment of AMR

Anti-CD20 monoclonal antibodies target B cells, plasma cells are targets of proteasome inhibitor bortezomib, and antithymocyte globulin can target multiple cell types including T cells, B cells and plasma cells. All three have been utilized for the treatment of acute AMR, particularly in refractory cases (11, 20–22), and emerging data suggest an improved treatment response with bortezomib compared to rituximab (21, 23). Contrary to our expectations, we did not find an improvement in graft survival with adjunct use of rituximab, bortezomib or antithymocyte globulin to anti-rejection therapy with corticosteroids, plasmapheresis or IVIG. Our retrospective study however was neither designed nor powered to fully evaluate the rapidly evolving therapeutic options for acute AMR.

In sum, after adjusting for potential confounders in the 87 kidney transplant recipients with biopsies that displayed C4d positive acute AMR, we show that concurrent acute TCMR and reduced eGFR at the time of biopsy are associated with inferior graft survival in this high risk population.

Methods

Kidney transplant patients and biopsies

We reviewed 1120 clinically indicated biopsies from 833 kidney allograft recipients and identified 87 biopsies from the 87 patients that were positive for C4d immunofluorescence staining and displayed morphologic evidence of acute tissue injury consistent with acute AMR. The biopsies were performed at our center between 12/2003 and 2/2011. ABO-incompatible kidney transplants were excluded.

The biopsies were classified using the Banff '09 update of Banff '97 classification (5), and the Banff-specified morphologic features and the grades are summarized in Table 2. The biopsies were read by a single pathologist (S.V.S). All biopsies were stained for C4d and C4d staining was performed on cryosections using a monoclonal anti-C4d antibody (Quidel, Santa Clara, CA). C4d was considered as positive if peritubular capillary staining involved >50% (diffuse) or 10–50% (focal) of cortical or medullary area (5).

We collected demographic, clinical, biochemical, pathology, treatment, and follow up data. DGF was defined as the need for dialysis treatment within the first week of transplant. We used the MDRD equation to estimate the GFR (eGFR) (24). Baseline eGFR was an average of the highest three measures of eGFR within three months prior to the diagnosis of acute AMR.

The Institutional Review Board of Weill Cornell Medical College approved this study.

Donor-specific antibody

Pre-transplant panel reactive antibodies (PRA) were determined using complement dependent cytotoxicity assay. Anti-HLA antibodies were detected using microparticles with individual purified HLA antigens covalently bound as targets (One Lambda Inc, Canoga Park, CA) on the Luminex[®] platform. We analyzed anti-HLA antibodies against donor HLA-A, B, DR and DQ antigens. Transplant recipients with at least one DSA with MFI > 500 were considered positive for circulating DSA. Among DSA positive recipients, immunodominant DSA was the antibody with the highest MFI. Circulating antibodies to non-HLA antigens were not tested. Information on DSA was available in 66 of 87 transplant recipients studied.

Treatment and outcome

Patients received standard anti-rejection therapy comprised of methylprednisolone, IVIG or PP. In addition to standard therapy, subsets of patients were also treated with various combinations of antithymocyte globulin, rituximab, and bortezomib. Acute AMR was classified as resistant if the eGFR did not return to within 15% of the baseline within eight weeks after initiation of antirejection treatment. The primary outcome was graft loss defined as persistent decline in eGFR to <15 ml/min/1.73m² or return to dialysis. Patients lost to follow up, died with a functioning graft or did not reach the outcome were censored at their last follow up.

Statistical analysis

We summarized continuous variables as mean (standard deviation) for variables with Gaussian distribution and median (range) for variables with non-Gaussian distribution and categorical variables as proportions.

We generated Kaplan-Meier survival curves to estimate the time to allograft loss. Survival curves were compared by Mantel-Cox log-rank test. We used the Cox proportional hazard

regression to test for the independent contribution of each of the variable at the time of diagnosis of C4d positive acute AMR in predicting subsequent graft loss. To adjust for confounding, we decided to use the following variables at the time of diagnosis of rejection, *a priori*, in the Cox model, irrespective of their significance level by univariate analyses: (i) early versus late rejection, (ii) eGFR, (iii) proteinuria, (iv) presence of the following concurrent features on biopsy: acute TCMR, chronic active AMR or IF/TA, and (v) use of antithymocyte globulin, rituximab or bortezomib in addition to the standard treatment of steroids, IVIG or PP.

We expressed the results of Cox regression as relative hazard with 95 percent confidence intervals. We verified the proportionality assumption of the model by the residuals analyses. We used Stata/IC 10.1 for Windows (StataCorp, College Station, TX) and GraphPad Prism 5.02 for Windows, (GraphPad Software, La Jolla, CA) for all statistical analyses. A two-sided P value of <0.05 was considered statistically significant.

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Abbreviations

AMR	antibody-mediated rejection
CNI	calcineurin inhibitors
DGF	delayed graft function
DSA	anti-HLA donor specific antibody
eGFR	glomerular filtration rate estimated using the 4-parameter Modification of Diet in Renal Disease (MDRD) formula
IF/TA	interstitial fibrosis/tubular atrophy
IVIG	intravenous immunoglobulin
MDRD	Modification of Diet in Renal Disease
MFI	mean fluorescence intensity
MMF	mycophenolate mofetil
TCMR	T-cell mediated rejection

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Figure 1. Kaplan-Meier survival estimated probability of survival following clinically indicated allograft biopsy

All 87 biopsies from the 87 patients showed C4d+ acute AMR and Kaplan-Meier estimated probability of survival is shown in the absence (green) or presence (red) of concurrent acute T cell mediated rejection (TCMR) (panel A), absence (green) or presence (red) of concurrent chronic active antibody mediated rejection (AMR) (panel B), or absence (green) or presence (red) of concurrent interstitial fibrosis/tubular atrophy (IF/TA) (panel C). The survival estimates based on time from transplant to biopsy <12 months (green) or >12 months (red) (panel D) and absence of proteinuria at the time of biopsy <1 gram/day (green) or presence of >1 gram/day (red) (panel E) are also shown. Eighty-one of 87 patients received standard anti-rejection therapy consisting of steroids, intravenous immunoglobulins or plasmapheresis and survival analysis based on additional therapy without (green) or with (red) of antithymocyte globulin (panel F), without (green) or with (red) of rituximab (panel G), or without (green) or with (red) of bortezomib (panel H) are illustrated. For each variable the difference in survival between the two groups was analyzed by Mantel-Cox log-rank test. Biopsies were categorized using Banff '09 classification by a single pathologist (S.V.S) blinded to the clinical outcomes.

Table 1

Characteristics of Patients at the Time of Kidney Transplantation

Characteristics	N (%) 87 Patients (100)
Demography	
Recipient age, years, <i>mean</i> ±SD	45±14
Gender, <i>female</i>	38 (44)
Race, <i>African-American</i>	28 (32)
Native kidney disease	
Hypertension	20 (23)
Glomerular disease	15 (17)
Diabetes	11 (13)
Others	25 (29)
Unknown	16 (18)
Donor age, years, <i>mean</i> ±SD	41±17
Type of donor, <i>deceased donor</i>	47 (54)
Donor race, <i>African-American</i>	18 (21)
Pretransplant characteristics	
Transfusion before transplant	42 (48)
Repeat transplant	24 (28)
Panel Reactive antibodies (PRA) ^a	
Available	74 (85)
Peak PRA, <i>median (range)</i>	12% (0–100)
Pre-transplant PRA, <i>median (range)</i>	0% (0–80)
Donor specific anti-HLA Antibodies (DSA) ^b	
Available	63 (72)
Positive DSA (MFI ^c > 500), Recipients, N	26 (41)
Immunodominant DSA	
Class I	13 (50)
Class II	13 (50)
MFI, <i>median (range)</i>	5612 (742–20680)
Sum of MFI, <i>median (range)</i>	10493 (742–102081)
Number of donor HLA recognized by DSA, <i>median (range)</i>	3 (1–8)
Class I, <i>median (range)</i>	1 (0–4)
Class II, <i>median (range)</i>	1.5 (0–5)
Transplant characteristics	
Pre-transplant desensitization therapy	20 (23)
Cold ischemia time, hours, deceased donor, <i>mean</i> ±SD	21.5±8.5
Delayed graft function, deceased donor	18 (38)
Induction immunosuppression	76 (87)
Antithymocyte globulin	63 (72)
Interleukin receptor-2 blockers	15 (17)

Characteristics	N (%) 87 Patients (100)
Alemtuzumab	1 (1)
Maintenance immunosuppression	
Calcineurin-inhibitor based	81 (93)
Early corticosteroid withdrawal	44 (51)
Baseline eGFR ^d , ml/min/1.73m ² , <i>mean± standard deviation</i>	44.4±21.9

^a panel reactive antibodies detected with the use of complement dependant cytotoxicity assay and using a 40–50 member peripheral blood mononuclear cell panel.

^b donor specific anti-HLA antibody (DSA) detected using microparticles with individual purified HLA covalently bound as targets (One Lambda Inc, Canoga Park, CA) on the Luminex platform.

^c mean fluorescence intensity, transplant recipients with at least one DSA with MFI > 500 was considered positive for circulating DSA.

^d Modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate.

Baseline eGFR was an average of the highest three measures of eGFR within three months prior to the diagnosis of acute AMR.

Table 2

Characteristics of Patients at the Time of Clinically Indicated Kidney Allograft Biopsy

Characteristics	N (%) 87 Patients (100)
<i>Renal Allograft Biopsy Results^a</i>	
C4d ^b positive Acute AMR	87 (100)
C4d diffusely positive	76 (87)
Acute tubular necrosis- like minimal inflammation	9 (10)
Peritubular capillary inflammation- <i>ptc</i>	65 (75)
Grade 1/2/3	26/14/25 (40/22/38)
Glomerular inflammation- <i>g</i>	54 (62)
Grade 1/2/3	24/22/8 (44/41/15)
Concurrent chronic active AMR	43 (49)
Glomerular double contours	22 (51)
Peritubular capillaries multilayering	18 (42)
Fibrous intimal thickening in arteries	28 (65)
Concurrent acute TCMR	32 (37)
Grade I/II/III	18/14/0 (56/44/0)
Concurrent IF/TA	37 (43)
Grade I/II/III	23/9/5 (62/24/14)
<i>Donor specific Anti-HLA antibodies (DSA)^c</i>	
Performed, N (%)	66 (76)
Positive DSA (MFI ^d > 500), Recipients	60 (91)
Immunodominant DSA	
Class I	13 (22)
Class II	47 (78)
MFI, <i>median (range)</i>	9935 (1054–22680)
Sum of MFI, <i>median (range)</i>	19514 (1768–71283)
Number of donor HLA recognized by DSA, <i>median (range)</i>	3 (1–9)
Class I, <i>median (range)</i>	1 (0–4)
Class II, <i>median (range)</i>	2 (0–6)
Time to rejection, months, <i>median (range)</i>	3.7 (0–261)
eGFR ^e , ml/min/1.73m ² , <i>mean ± SD</i>	23.2 ± 15.1
Proteinuria, > 1g/day	48 (55)
<i>Treatment</i>	
Standard therapy ^f	81 (93)
and Antithymocyte globulin	39 (48)
and Rituximab	25 (31)
and Bortezomib	15 (19)

^aClinically indicated kidney allograft biopsy specimens were read by a single pathologist and categorized using the Banff '09 update of Banff '97 classification (5).

^b C4d staining was performed on cryosections using a monoclonal anti-C4d antibody (Quidel, Santa Clara, CA). C4d was considered as positive if peritubular capillary staining involved >50% (diffuse) or 10–50% (focal) of cortical or medullary area.

^c donor specific anti-HLA antibody (DSA) detected using microparticles with individual purified HLA covalently bound as targets (One Lambda Inc, Canoga Park, CA) on the Luminex platform.

^d mean fluorescence intensity (MFI), transplant recipients with at least one DSA with MFI > 500 were considered positive for circulating DSA.

^e modification of Diet in Renal Disease (MDRD) estimated glomerular filtration rate.

^f standard anti-rejection treatment consisted of steroids, intravenous immunoglobulins or plasmapheresis.

Table 3

Concurrent kidney graft biopsy findings in 87 kidney transplant recipients with C4d positive acute AMR, analysis by time to biopsy from transplantation^a

Renal Allograft Biopsy Findings in patients with C4d+ Acute AMR ^b	Time from Kidney Transplant to Allograft Biopsy		
	<12 months	>12 months	P Value ^c
	N=52 Patients	N=35 patients	
<i>without</i> Acute T-cell mediated rejection	35	20	0.37
<i>with</i> Acute T-cell mediated rejection	17	15	
<i>without</i> Chronic active antibody mediated rejection	43	8	<0.0001
<i>with</i> Chronic active antibody mediated rejection	9	27	
<i>without</i> Interstitial Fibrosis/Tubular Atrophy	40	10	<0.0001
<i>with</i> Interstitial Fibrosis/Tubular Atrophy	12	25	

^aClinically indicated kidney allograft biopsy specimens were reviewed by a single pathologist and categorized using the Banff '09 update of Banff '97 classification (5).

^bC4d staining was performed on cryosections using a monoclonal anti-C4d antibody (Quidel, Santa Clara, CA). C4d was considered as positive if peritubular capillary staining involved >50% (diffuse) or 10–50% (focal) of cortical or medullary area.

^cP-value calculated using Fisher's exact test.

Table 4
Multivariable analysis of risk factors for graft failure by Cox proportional hazards regression

Characteristics	N (%) 81 Patients (100)	Coefficient	Standard error	Hazard ratio	95% CI	P Value ^a
Renal Allograft Biopsy Results ^b	81 (100)			1		
C4d Positive acute antibody mediated rejection						
Presence of						
Concurrent acute T-cell mediated rejection	30 (37)	0.982	0.395	2.67	1.23–5.79	0.01
Concurrent chronic active antibody mediated rejection ^c	32 (40)	0.569	0.579	1.77	0.57–5.49	0.33
Concurrent interstitial fibrosis/tubular atrophy	33 (41)	–0.223	0.482	0.79	0.31–2.05	0.63
Time from transplant to biopsy						
< 12 months	51 (63)			1		
> 12 months	30 (37)	0.936	0.568	2.55	0.83–7.75	0.10
eGFR at biopsy ^d	81 (100)	–0.409	0.157	0.66 ^d	0.49–0.90	0.01
Proteinuria						
<1 gram/day	37 (46)			1		
>1 gram/day	44 (54)	0.263	0.388	1.30	0.61–2.78	0.50
Treatment ^e						
Standard therapy	81 (100)			1		
and Antithymocyte globulin	39 (48)	0.319	0.388	1.38	0.64–2.94	0.41
and Rituximab	25 (31)	–0.083	0.384	0.92	0.43–1.95	0.83
and Bortezomib	15 (19)	0.175	0.573	1.19	0.39–3.66	0.76

^a Hazard ratio (relative hazard) and 95 percent confidence interval and P value derived using Cox proportional hazard regression analysis.

^b Clinically indicated kidney allograft biopsy specimens were reviewed by a single pathologist and categorized using the Banff '09 update of Banff '97 classification (5).

^c Defined as glomerular double contours or peritubular capillary basement membrane multilayering and/or fibrous intimal thickening in arteries.

^d For every 10 ml change in eGFR.

^e Standard anti-rejection treatment consisted of steroids, intravenous immunoglobulins or plasmapheresis, and additional therapy consisted of antithymocyte globulin, rituximab or bortezomib.