Histopathology and outcome of acute humoral rejection in renal allografts

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1. ABSTRACT

Our purpose was to see if histopathologic features of acute antibody-mediated rejection (AMR) in renal allografts have prognostic value; and to compare two-year graft survival with and without additional therapy with plasmapheresis and intravenous immunoglobulin (IVIG). We reviewed renal allograft biopsies taken within the first 6 months after transplant from patients with C4d positive AMR, performed between January 2000 to December 2005 (n=57). We formed two groups: Group 1: biopsied between 2003 and 2005 (n=26), when C4d staining was routinely performed and option for plasmapheresis and IVIG was available; Group 2: biopsied between 2000 and 2002 (n=31), retrospectively found to be C4d positive. Patients whose biopsies showed cortical necrosis or arterial fibrinoid necrosis had early graft loss. Other histopathologic features did not statistically correlate with graft loss. Overall, additional plasmapheresis/IVIG treatment did not show convincing improvement in graft survival or function at 2 years post-transplant, but all six patients with thrombotic microangiopathy (TMA) who received plasmapheresis/IVIG had functioning grafts at two-year follow-up.

2. INTRODUCTION

Biopsy evaluation remains the cornerstone for diagnosing acute rejection, including acute antibody mediated rejection (AMR) in renal allografts (1-13). Immunohistologic detection of the complement split product C4d in peritubular capillaries (PTC) became a powerful tool in the hands of pathologists to diagnose AMR (5-13). The following criteria for diagnosis of AMR were established 1) Clinical evidence of graft dysfunction, 2) Histologic evidence of tissue injury (inflammatory cells in PTC, fibrin thrombi, fibrinoid necrosis), 3) C4d staining in PTCs, and 4) Serologic evidence of anti-human leukocyte antigen or other anti-donor antibody at the time of the biopsy (3,12,13). Early diagnosis of AMR in presensitized patients, who received a renal allograft following a desensitization protocol, is relatively easy because these patients are usually strictly monitored from the beginning. In contrast, early diagnosis of AMR in patients with de novo donor specific antibodies (de novo AMR) is more difficult, because in most patients we do not know when the donor specific antibodies appeared and, by the time of the biopsy for graft dysfunction, the damage can already be serious.

With the decrease in the incidence of classic early T cell-mediated acute interstitial (cellular) rejection in renal allografts over the past decade, attention has now turned towards developing effective treatment protocols for AMR. There is ample evidence that patients with preexisting donor specific antibodies can be successfully transplanted after performing various desensitization protocols, which usually include plasmapheresis and IVIG (14-17). Plasmapheresis and IVIG are also successfully used in the treatment of AMR in this desensitized patient population (18, 19). AMR, secondary to de novo detected alloantibodies, tends to be resistant to conventional T-cell directed anti-rejection therapies and has a worse prognosis as compared to acute cellular rejection. Published reports indicate frequent reversal of acute de novo AMR episodes with these treatment strategies (plasmapheresis, IVIG, Rituximab) but good case controlled studies and are missing (18-21) and relevant clinicopathologic correlations in de novo AMR are scant.

The aim of our study was twofold. First, we wanted to clarify whether individual histopathologic features have prognostic value for graft outcome in non-desensitized recipients with acute AMR (with de novo detected alloantibodies) and if histologic changes can guide patient selection for treatment with plasmapheresis and IVIG. The second goal of our study was to retrospectively compare graft survival and function in these recipients with and without plasmapheresis and IVIG treatment. Although ours is a busy kidney transplant center with a relatively large number of de novo AMR cases, this was a difficult study to design. It is a single center retrospective study with historical controls, spanning a six-year period, during which treatment protocols and therapeutic approaches changed. Still, we believe our findings are of interest and raise several issues regarding de novo AMR, which could only be appropriately answered in prospective multicenter clinical trials.

3. METHODS

3.1. Biopsy selection
We reviewed renal allograft biopsies, performed for graft dysfunction within the first 6 months post-transplant, between January 2000 and December 2005. Protocol biopsies were not performed. Only biopsies with histologic features of acute AMR were included in the study. AMR was defined as diffuse (>50 percent) peritubular capillary (PTC) C4d staining, graft dysfunction and tissue injury. Because of the retrospective nature of the study, we did not use the presence of donor specific antibodies as an inclusion criterion, because in several older cases we did not find data on anti-HLA antibody studies. We found 57 patients whose biopsy findings fulfilled these three criteria. In 30 of these 57 biopsies with acute AMR, features of cellular rejection were also present.

3.2. Patient Groups and Subgroups
The 57 patients included in the study were divided into two groups: Group1. Patients with C4d positive acute AMR biopsied between January 01, 2003 and December 31, 2005 (n=26). C4d immunofluorescence staining had been performed as part of the routine biopsy

protocol since January 2003 and specific treatment for acute AMR (plasmapheresis, IVIG) was available for these patients during this three-year period. Group 2. Patients with C4d positive acute AMR biopsied between January 01, 2000 and December 31, 2002 (n=31). During this three-year period, C4d staining was not part of the routine biopsy workup at our institution but was performed retrospectively on all renal allograft biopsies, using immunohistochemistry on paraffin embedded tissue sections. Plasmapheresis/IVIG treatment for AMR was not in effect during this period of time. Biopsies with completely infarcted renal cortex and inconclusive C4d staining were not included in the study. Two biopsies with features of partial cortical infarction and still preserved viable surrounding cortex with interpretable positive C4d staining were included.

Not all patients in Group 1 were treated with plasmapheresis and IVIG based on C4d staining alone. The presence of one or more of the following additional criteria were used for selection of patients for plasmapheresis and IVIG treatment: TMA with *de novo* alloantibodies (high panel reactive antibody titer [PRA] at the time of biopsy), or elevated pre and post-transplant PRA, or features of partial cortical necrosis in the biopsy. Therefore Group 1 was further subdivided into: Subgroup 1A - patients that were selected for specific treatment (plasmapheresis, IVIG) for AMR, (n=12/26); and Subgroup 1B : the remaining patients with C4d positive AMR who were not selected for plasmapheresis and IVIG, but given conventional anti-rejection therapy only (n=14/26).

3.3. Biopsy avaluation
Biopsies from 2003 to 2005 were reviewed by two pathologists (TN and AS) as part of the routine sign-out. The biopsies that had been performed between 2000 and 2002 were re-reviewed by the same two pathologists for the purpose of this study. The histologic features were evaluated and semi-quantitatively scored (0 to 3) using the Banff scoring system (4, 22, 23), and the chronic allograft damage index (CADI), (24, 25). Multiple additional features that we routinely evaluate in transplant biopsies were also scored, and are described below.

Glomerular fibrin thrombi: 0: Absent, 1+: fibrin in few capillary loops of one or two glomeruli, 2+: fibrin in capillary loops of more than two but less than 50% of the glomeruli, 3+: Fibrin thrombi in more than 50% glomeruli, affecting several capillary loops.

Glomerular fragmented red blood cells (RBCs): 0: Absent, 1+: seen in one or two glomerular capillary loops, 2+: Seen in less than 25% of glomeruli, 3+ Seen in more than 25% of the glomeruli.

Glomerular hyaline: 0: Absent; 1+: mild to moderate hyaline in one to two glomeruli; 2+: Mild to moderate hyaline in less than 25% glomeruli, 3+: moderate to severe hyaline in more than 25% glomeruli.

Interstitial neutrophils, eosinophils and plasma cells: 0: 0 to 3 cells/high power field (HPF) (400x); 1+: 4 to 10 cells/HPF; 2+: 20 cells/hpf ; 3+: more than 20 cells/HPF

Interstitial edema: 0: absent; 1+: mild, 25% of the renal cortex; 2+: 25 to 50% of the renal cortex; 3+: more than 50% of the renal cortex.

Interstitial hemorrhage: 0: absent; 1+: mild patchy 1 or 2 small foci, 2+: larger foci, occupying < 25% of the renal cortex, 3+: diffuse or large bridging foci, involving >25% of the renal cortex.

Acute tubular necrosis (ATN): 0: absent; 1+: mild patchy acute tubular injury with flattening of the tubular epithelium and/or vacuolization in <25% tubules; 2+: acute tubular injury in 25 to 50% tubules with scattered tubules containing apoptotic cell debris and/or few granular casts; 3+: diffuse (>50% tubules) severe acute tubular injury with sloughed off epithelial cells and scattered granular casts in the lumina.

Tubular vacuolation: 0: absent, 1+: seen in less than 10% of tubules, 2+: seen in less than 25% of the tubules, 3+ seen in more than 25% of the tubules.

Apoptotic cell debris in tubules: 0: absent, 1+: few apoptotic cells in rare tubules, 2+: few apoptotic cells in 1 to 2 tubules/HPF; 3+: 3 to 6 or more tubules/hpf or tubules almost filled with apoptotic cell debris.

Polymorphonuclear leukocytes (PMNs) in tubules: 0: absent, 1+: few PMNs in rare tubules, 2+: few PMNs in 1 to 2 tubules/HPF, 3+: few PMNs in 3 to 6 tubules/HPF or PMNs filling up and distending more than 10% of tubules.

Tubular calcification: 0: absent; 1+: 1 to 5 small foci/5 100x fields; 2+ 6 to 10 small foci/5 100x fields; 3+: more than 10 or, fewer but larger, prominent foci/5 100x fields.

Mucoid arteriolar wall thickening: 0 absent; 1+ an occasional arteriole with mild involvement; 2+: several arterioles show mild involvement; 3+ prominent mucoid thickening and narrowing of the lumen of arterioles.

Arteriolar fibrin: 0: absent, 1+: mild fibrin deposits in one or two arterioles, 2+: fibrin thrombi filling the lumen in one to two arterioles, 3+ fibrin thrombi occluding several arterioles.

Arterial and arteriolar fragmented RBCs: 0: absent, 1+: few seen in one to two arteries/arterioles, 2+: few seen in several arteries/arterioles, 3+: easily seen in more than two arteries/arterioles.

Mean of the semiquantitative scores was calculated for each variable to test for correlation with graft loss at 2 years post-transplant. Glomerular sclerosis was calculated as a percentage of sclerotic glomeruli and then semi-quantitatively scored from 0 to 3+ (24). For intimal arteritis, we tested the categories v0, v1,v2 and v3 separately since severe arteritis with arterial fibrinoid necrosis (v3), but not v1 and v2, has been shown previously to correlate with poor graft outcome in cellular rejection (26). Thrombotic microangiopathy (TMA) was treated as a categorical variable and scored as present or absent. TMA was defined as we published in a recent study (27).

3.4. Histology and C4d immunostaining

For light microscopy, tissue was fixed in 10% buffered formalin and embedded in paraffin. Paraffin sections were cut at a thickness of 3 microns and stained with hematoxylin and eosin, Periodic Acid Schiff (PAS), trichrome and methenamine silver. For biopsies performed between 2000 and 2002, C4d immunoperoxidase staining on paraffin sections was performed using a polyclonal antibody (ALPCO Diagnostic, Windham, NH), as described previously (28). For biopsies performed between 2003 and 2005, routine C4d staining on frozen section was performed by indirect immunofluorescence using the monoclonal antibody from Quidel Corporation, Santa Clara, California, as described before (28). In one of our previous studies we showed that immunofluorescence and immunoperoxidase methodologies for C4d staining in renal allograft biopsies give comparable results in our hands (28). Therefore, we did not include cases with less than 50 % PTC staining by immunoperoxidase as possible positives.

3.5. Serologic detection of anti-HLA antibodies

Sera from all kidney transplant recipient candidates underwent anti-human globulin complement dependent cytotoxic (AHG-CDC) crossmatch and T and B cell flow cytometry crossmatch (FCXM) before transplant. Recipients with positive AHG-CDC crossmatch treated with desensitization therapy before transplantation are not included in this study. Flow cytometric PRA was used for detection and quantitation of anti-HLA antibodies in the recipient serum pre-transplant as well as post-transplant (at the time of graft dysfunction coinciding with the biopsy). Although may not be donor-specific, high PRA at the time of transplant and persisting thereafter, or de novo rise in PRA after transplant, is potentially harmful to the allograft (29, 30).

3.6. Induction and maintenance immunosuppression therapy

Anti-thymocyte globulin (ATG) was used more commonly in Group 1 and Basiliximab (Simulect) in Group 2, for induction immunosuppression (Table 1). Steroid containing three drug maintenance immunosuppressive regimen was used in all but three (90%) of the patients in Group 2 but only 7% of the patients in Group 1. Transition to steroid free immunosuppressive regimen for renal allograft recipients took effect in our institute since 2003. Combination of steroid, Mycophenolate mofetil (CellCept) and Cyclosporine (microemulsion form Neoral) was used more commonly among recipients in Group 2. Two drug combination of Neoral and Sirolimus (Rapamycin) was used more commonly among recipients in Group 1. Comparison between the two Groups with regards to individual drugs (Table 1) as well as drug combinations (data not shown) by Chi-square test and was found to be significantly different. However, there were no such differences between Subgroups 1A and 1B. Therefore, Subgroup 1B (no specific AMR treatment) served as another good comparator group.

Table 1. Demographic and clinical comparisons between Groups 1 and 2 and between Subgroups 1A and 1B

	Group1 (2003 onwards)	Group 2 (before 2003)	p value	Subgroup 1A (PP, IVIG)	Subgroup 1B (conventional therapy)	p value
Age (years)	44.5+/-11.3	50.5 +/- 11.7	0.04	46.6+/-10.8	42.7+/-11.8	0.31
Gender male:female	15:12	16:15	0.64	5,7	10,4	0.12
Race C,AA,H	20,7,0	21,9,1	0.88	10,3,0	10,4,0	1
Type of transplant						
Cadaveric	17	12	0.13	7	10	0.44
Living	10	16		6	4	
Number of transplants						
1	22	28	0.45	10	12	0.64
2	5	3		3	2	
Baseline serum creatinine	1.5+/-0.3	1.7+/-0.5	0.16	1.6+/-0.3	1.4+/-0.4	0.5
Serum creatinine 2 years post Tx	1.73+/-0.7	1.84 +/-0.7	0.77	1.89 +/-0.8	1.61+/-0.5	0.4
Time from Tx to Bx (months)	1.36	1.66	0.45	1.29	1.42	0.8
De novo or elevated pre and post transplant PRA/tested recipients	14 out of 24 (58%)	8 out of 10 (80%)	0.41	10 out of 12 (82%)	4 out of 12 (33%)	0.09
Induction immunosuppression						
Thymoglobulin	25	1	<0.0001	12	13	1
Simulect	2	20		1	1	
Maintenance immunosuppression						
Steroids	2	28	<0.0001	2	0	0.2
Neoral	21	30	0.08	9	12	0.63
Rapamycin	21	3	<0.0001	10	11	1
Myfortic (CellCept)	8	26	<0.0001	4	4	1
Prograf	1	1	1	0	1	1

AA=African American, H=Hispanic, Tx=transplant, Bx= biopsy, PRA=panel reactive antibodies Wilcoxon rank sum test for age, average serum creatinines; chi-square test for gender, type of transplant; PRA. Fisher's exact test for race and transplant number, and immunosuppressants. Cut-off for p value 0.002 (using Bonferroni adjustment for multiple comparisons).

3.7. Anti-rejection therapy

Patients in Subgroup 1A received treatment with plasmapheresis (8 to 10 procedures, end-point of treatment being return of serum creatinine to baseline levels or resolution of features of acute rejection on repeat biopsy), IVIG (after each plasmapheresis or given together after all the pheresis procedures), in addition to steroids and anti-lymphocyte antibodies (ATG or OKT3). Patients in Subgroup 1B received conventional anti-rejection therapy (steroids, ATG or OKT3). In Group 2, none of the patients had received specific AMR-directed therapy.

3.8. Statistical analysis

We tested each of the histologic features for correlation with graft loss. We did not separate out the Groups here because there was no significant difference in the severity of the histologic features between the biopsies in the two groups. The mean score for each of the continuous variables was used for the analysis. Wilcoxon rank sum test with Bonferroni correction for multiple comparisons was used. Intimal arteritis and TMA were treated as categorical variables (Chi-square test was used for comparisons). Note that some of the histologic variables had a majority of patients with grades of 0. For these variables, we categorized the scores into 0, 1+ and 2 to 3+ and the association with graft loss was tested using Chi-square or Fisher-exact test. The Groups and Subgroups were matched in terms of severity of the histologic changes. We did not compare the intensity of C4d staining between Groups 1 and 2 because the methodologies used for staining were different. We did compare the degree of PTC C4d staining between Subgroups 1A and 1B.

The Wilcoxon rank sum test was used to compare recipient age, average baseline serum creatinines and average serum creatinines of functioning grafts at 2 years post-transplant. Fisher's exact test was used to compare recipient race and number of transplant (first versus second transplant). Pearson chi-square test was used to compare recipient gender, type of transplant (cadaveric versus living donor transplant), immunosuppressive drugs used, and the proportion of graft loss within two years of transplantation. Kaplan-Meier estimates of graft survival comparing the rate of graft loss as a function of time post-transplant were also produced (Figure 1) and group differences were compared using the log rank test. All analyses were performed using SAS v9.1 (SAS Institute, Inc., Cary, NC).

4. RESULTS

4.1. Histologic features and graft outcome

Two recipients whose biopsies showed arterial fibrinoid necrosis (Banff v3) lost their graft within 2 years post-transplant. Recipients whose biopsies showed partial cortical necrosis (n=2), lost their grafts within 3 to 4 weeks post-transplant (not depicted in tables), irrespective of the treatment strategy (numbers too small for statistical analysis). Biopsies from a total of eleven patients showed intimal arteritis (graded from v1 to v3 according to the Banff classification). Two of them were v3 (arterial fibrinoid necrosis). Of those eleven cases, seven were associated with graft loss (two of them with fibrinoid necrosis), (Tables 2 and 3). In Group 1 (patients biopsied between 2003 and 2005), seven patients had TMA in the biopsy; six in Subgroup 1A (who received treatment with PP and IVIG) and one in Subgroup 1B (who were not treated with PP and IVIG). Group 2 (patients biopsied between 2000 and 2002) had two patients with TMA (Tables 4 and 5). Grafts of all six patients with TMA in Subgroup 1A survived till the end of follow-up period. Out of three patients with TMA not treated with plasmapheresis and IVIG, one developed cortical necrosis and lost graft
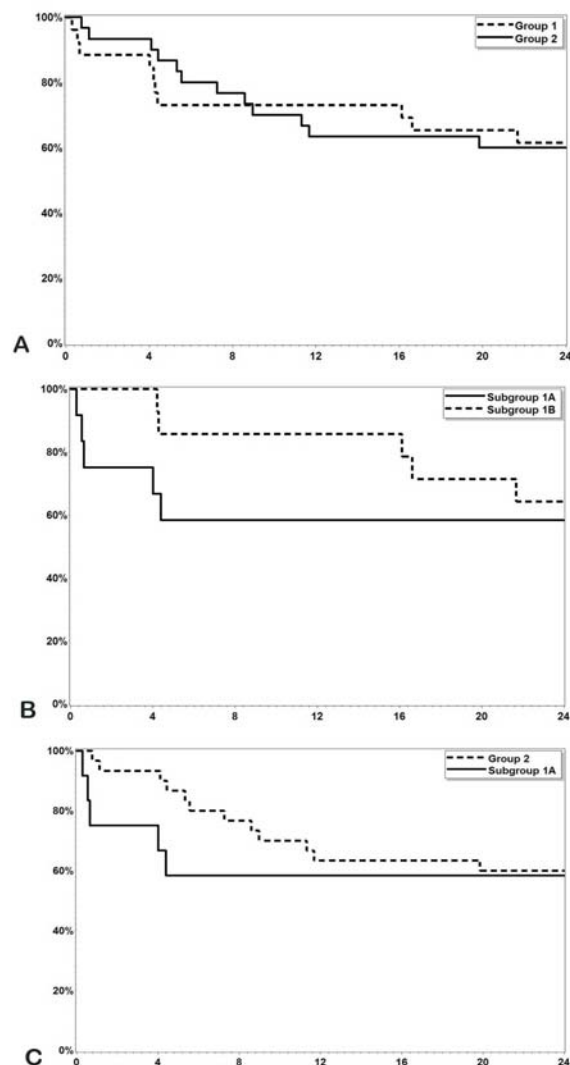
Figure 1. Kaplan-Meier curves for comparison of graft survival by Wilcoxon log rank test. Figure 1a: Group 1 versus Group 2 (p=0.9911). Since the survival curves cross, we tested for proportional hazards and did not find any severe violations (p = 0.2560). Figure 1b: Subgroup 1A versus Subgroup 1B (p=0.2143). Figure 1c: Group 2 versus Subgroup 1A (p=0.1998). Y-axis: Percent graft survival; X-axis: months post transplant.

function within 3 weeks but the other two survived (Chi-square test p value = 0.0095), (Table 2). Features including arterial fibrous intimal thickening, glomerular sclerosis, tubular casts, and PMNs in tubules showed a trend towards significant correlation with graft loss (p=0.02, 0.08, 0.03, 0.08 respectively), but did not hold after applying the Bonferroni correction for multiple comparisons (Table 2). The two Groups and Subgroups were matched in terms of severity of histologic features (Table 5).

4.2. Demographics

Groups 1 and 2 as well as Subgroups 1A and 1B were matched with respect to patient gender, race, type of transplant, number of transplants in each patient, duration

between transplant and biopsy and duration of followup (Table 1). Baseline serum creatinine was 1.5 mg/dl in Group 1 and 1.7 mg/dl in Group 2 (p=0.16); 1.6 mg/dl in Subgroup 1 and 1.4 mg/dl in Subgroup 2 (p=0.50). Gender and diabetes mellitus did not influence graft outcome (data not shown).

4.3. Serum creatinine at 2 years in functioning grafts

Average serum creatinine at 2 years was 1.7 and 1.8 mg/dl in Groups 1 and 2, respectively (p=0.77). The functioning grafts, in Subgroups 1A and 1B had an average serum creatinine of 1.9 and 1.6 mg/dl, respectively (p=0.40) (Table 1).

4.4. Proportion of graft loss

Graft loss was 30% in Group 1 and 42% in Group 2 (p=0.50), (Table 6). Graft loss was higher in the subgroup (Subgroup 1A) (33%), treated for AMR, compared to that in Subgroup 1B (28%) (not treated for AMR), but the difference was not statistically significant (p=0.69). Graft loss as a function of time post-transplant also did not differ significantly between Groups 1 and 2 (p=0.99, tested for proportional hazards, p=0.25), (Fig.1a); between Subgroups 1A and 1B (p=0.52) (Fig.1b), and between Subgroup 1A and Group 2 (p=0.19) (Fig.1c). Graft loss in patients with early acute cellular rejection two years post-transplant at our institution is approximately 16% (data not shown).

4.5. Post-transplant panel reactive antibody (PRA) titers

Flow PRA data at the time of the biopsy was available for 23/26 recipients in Group 1 and only 10/31 recipients in Group 2 (Table 1). Elevated de novo PRA and pre and post-transplant elevation of PRA did not correlate significantly with graft loss (Table 1); however, numbers available for statistical comparison are small, especially in Group 2. The recipients in Subgroup 1B (not treated for AMR), had only a mild elevation in PRA (>10% but <30%) and mainly involved HLA Class II, in contrast to patients in Subgroup 1A (>30%), who received plasmapheresis and IVIG treatment. Negative PRA both pretransplant and at biopsy, was seen in two patients in Subgroup 1A and eight patients in Subgroup 1B.

5. DISCUSSION

There are only a few recent studies focussing on the prognostic importance of histologic features in renal allograft rejection (10,26,31). We studied a large number of histologic variables in a uniform (non-desensitized) population of renal allograft recipients with acute AMR. Our results support the previously shown finding that arterial fibrinoid necrosis (v3 of the Banff classification) in the biopsy is associated with poor graft outcome. The other histologic feature with early graft loss is partial cortical necrosis (but we could not show statistical significance because of small numbers). Unfortunately, the numerous other histologic parameters we tested did not correlate with graft loss at 2 years post-transplant. Arterial fibrointimal thickening and glomerular sclerosis are markers of chronic renal injury in the allograft. These histologic variables

Table 2. Correlation of histologic features with graft loss at 2 years. (Features that had to be treated as categorical variables)

Variable	Severity	No Graft Loss	Graft Loss	Total	Fisher's exact p-value
Intimal arteritis	0	24	12	36	0.1732
	1+(v1)	3	4	7	
	2+(v2)	1	1	2	
	3+(v3)	0	2	2	
Thrombotic microangiopathy	Present	8	1	9	0.0095
	Absent	19	29	48	
Glomerular fibrin thrombi	0	31	17	48	0.5352
	1+	2	0	2	
	2-3+	4	1	5	
Fragmented RBCs in glomerular capillaries	0	32	18	50	0.4532
	1+	2	0	2	
	2-3+	3	0	3	
Glomerular enlargement	0	30	16	46	0.8011
	1+	6	2	8	
	2-3+	1	0	1	
Glomerular wall thickening	0	33	14	47	0.3433
	1+	3	3	6	
	2-3+	1	1	2	
Mesangial expansion	0	29	14	43	1
	1+	7	4	11	
	2-3+	1	0	1	
% sclerotic glomeruli	0	35	14	49	0.0871
	1+	1	3	4	
	2-3+	1	0	1	
Arteriolar fibrin thrombi	0	34	17	51	1
	1+	1	1	2	
	2-3+	2	0	2	
Arteriolar RBCs	0	35	17	52	1
	1+	1	1	2	
	2-3+	1	0	1	
Apoptotic debris in tubules	0	19	6	25	0.4357
	1+	13	8	21	
	2-3+	5	4	9	
PMNs in tubules	0	35	14	49	0.0819
	1+	2	4	6	
Tubular calcific deposits	0	29	15	44	1
	1+	6	3	9	
	2-3+	2	0	2	
Tubular casts	0	27	8	35	0.0376
	1+	8	5	13	
	2-3+	2	5	7	
Tubular hypertrophy	0	34	16	50	1
	1+	3	2	5	
Tubular vacuolization	0	19	14	33	0.2175
	1+	12	3	15	
	2-3+	6	1	7	
Mucoid arteriolar thickening	0	22	11	33	1

Significance level p= 0.003 (Bonferroni correction for multiple comparisons)

showed low p-values,   supporting the already known fact that chronic allograft nephropathy is one of the most important factors limiting long-term renal allograft survival. The recent study by Lefaucheur *et al* (31) looked at graft outcomes after AMR in three groups of patients, namely desensitized transplant recipients, recipients with historically positive crossmatch not requiring desensitization, and other recipients without demonstrable antibodies before transplantation. Authors found that glomerular and PTC margination of inflammatory cells is associated with bad outcome in AMR. Our scoring system is comparable, but we did not find a significant correlation with graft loss.

TMA in renal allografts is an indicator of poor graft outcome. TMA is thought to be a severe form of tissue injury in AMR (27, 32); therefore, patients with TMA in the biopsy would be expected to have worse outcome. We found that all six patients with TMA in the biopsy (along with high levels of PRA) who were treated with specific therapy for AMR, recovered graft function and survived till the end of the follow-up period. Therefore, it appears that patients with AMR and TMA in the biopsy benefit from plasmapheresis/IVIG treatment and that TMA may be a useful histological variable in selecting patients with C4d positive AMR for alloantibody depleting treatment strategies. However, presence of cortical necrosis in the biopsy, which is probably best considered an extremely severe and advanced form of TMA, was associated with early graft loss irrespective of treatment. Therefore, early diagnosis and treatment for TMA are equally important for favorable outcome.

Previous reports (18-21) have described successful short-term reversal of de novo acute AMR with targeted strategies (plasmapheresis/IVIG), but long term

Table 3. Correlation of histologic features with graft loss at 2 years follow-up using mean score. Features treated as continuous variables

Variable	Graft Loss	N	Mean	SD	p-value*
Interstitial inflammation	No	34	1.5	0.9	0.4224
	Yes	20	1.8	0.9	
Plasma cells	No	34	0.4	0.5	1
	Yes	20	0.4	0.5	
Neutrophils	No	34	0.37	0.55	0.3832
	yes	20	0.23	0.41	
Eosinophils	No	34	0.38	0.6	0.2094
	Yes	20	0.2	0.38	
Glomerulitis	No	34	0.65	0.8	0.939
	Yes	19	0.55	0.6	
Tubulitis	No	34	1.4	1.1	0.2395
	Yes	20	1.8	1.1	
Interstitial Edema	No	34	0.9	0.7	0.1615
	Yes	20	1.3	1.1	
Acute tubular necrosis	No	34	1.1	0.8	0.3338
	Yes	20	1.4	1.1	
PTC margination	No	36	1.74	0.8	0.183
	Yes	19	2.08	0.7	
Fibrointimal thickening	No	28	0.29	0.57	0.0214
	Yes	18	0.89	0.95	
Arteriolar hyalinosis	No	36	0.24	0.65	0.82
	Yes	19	0.2	0.52	
Tubular atrophy	No	34	0.38	0.55	0.6227
	Yes	20	0.35	0.65	
Interstitial fibrosis	No	34	0.37	0.54	0.4692
	Yes	20	0.33	0.65	
CADI	No	34	2.6	1.7	0.3541

Histologic variables semiquantitatively scored (0 to 3+) and treated as continuous variables. Wilcoxon rank sum test. Cut-off for p-value= 0.006 (Bonferroni correction). PTC=peritubular capillary; CADI=chronic allograft dysfunction index.

Table 4. Patients from Group 1 and group 2 with thrombotic microangiopathy (TMA) in the biopsy

	Tx to Bx (days)	time to loss	Baseline creatinine (mg/dl)	Creatinine at 2 years (mg/dl)	Other morphologic features	Pre Tx PRA	Post Tx PRA	de novo rise	Treatment for rejection	Maintenance immunosuppression
1	14	functioning	1.2	1.2	ATN	Neg	Neg	Absent	PP,Simulect, Pred	Myfortic, Neoral, Pred
2	12	functioning	1.3	1.4	ATN	Neg	Pos	Present	PP,IVIG,Pred	Myfortic, Neoral, Pred
3	13	functioning	1.4	1.4	moderate PTC margination, moderate ATN	Neg	Pos	Present	PP,IVIG,ATG,Pred	Myfortic, Rapa, Pred
4	12	functioning	1.6	1.4	Mild PTC margination, moderate ATN	Neg	Pos	Present	PP,IVIG,Rit, ATG	Myfortic, Neoral, Pred
5	73	functioning	2.3	3.5	mild PTC margination	Pos	Pos	Absent	PP, Pred	Neoral, Rapa, Pred
6	28	functioning	2.2	1.5	ATN	Pos	Pos	Absent	PP, IVIG, Pred	Prograf, Myfortic, Pred
7	5	functioning	1.2	1.3	prominent PTC margination	Neg	not done	not done	Conventional	Myfortic, Neoral, Pred
8	9	23 days	4	lost	prominent PTC margination, arteritis	Pos	Pos	Absent	Conventional	Myfortic, Neoral, Pred
9	7	functioning	1.2	1	moderate PTC margination	Neg	not done	not done	Conventional	Myfortic, Rapa, Pred

Tx=transplant, Bx=biopsy, ATN=acute tubular necrosis, PRA=panel reactive antibodies, PP=plasmapheresis, IVIG=intravenous immunoglobulin, Pred=Prednisone, Rapa=Rapamycin, Rit=rituximab, Pos=positive, Neg=negative.

outcomes in de novo AMR are lacking. We have compared 2 year graft outcomes in patients before and after 2003, as well as patients diagnosed with AMR since 2003, but those who received and those who did not receive plasmapheresis and IVIG treatment. This is a more meaningful approach than comparing patients with AMR to patients with acute cellular rejection after conventional T-cell directed anti-rejection treatments as done in some older reports. One study (19) does mention better 2-year graft survival after AMR treated with plasmapheresis and IVIG compared to

"historical controls", but detailed description of these "historical controls" is not given.

We did not find statistically significant improvement in graft survival, or level of graft function 2 years post-transplant in patients who received targeted treatment for AMR (Subgroup 1A), relative to patients with AMR who received only conventional anti-rejection treatment (Group 2 and Subgroup 1B). One could argue that the treated patients in Subgroup 1A had more severe

Table 5. Comparison of histologic features semi-quantitatively scored using Banff criteria[1]

Histologic variable	Group 1 (2003 onwards)	Group 2 (before 2003)	p-value*	Subgroup 1A (PP, IVIG)	Subgroup 1B (conventional therapy)	p-value*
Interstitial inflammation (i)	1.62 +/-1.0	1.61 +/- 0.7	0.97	1.6 +/- 1.1	1.64 +/- 1	0.92
Tubulitis (t)	1.41 +/-1.2	1.63 +/- 0.97	0.56	1.4 +/-1.3	1.43 +/- 1.2	0.92
PTC margination	1.85 +/- 0.81	1.95 +/- 0.91	0.69	1.75 +/- 0.7	1.93 +/- 0.8	0.6
Glomerulitis	0.58 +/- 0.5	0.71 +/- 0.9	0.69	2.95 +/- 0.6	3.04 +/- 0.5	0.54
CADI	3 +/- 2.2	2.84 +/- 1.7	0.77	2.9 +/- 1.9	3 +/- 2.5	0.74
Intimal thickening	0.31 +/- 0.90	0.35 +/- 0.65	0.13	0.67 +/- 0.66	0.73 +/- 1.05	0.68
Arteriolar hyalinosis	0.46 +/- 0.83	0.3 +/- 0.54	0.61	0.64+/-1.03	0.32+/-0.61	0.56
Atrophy	0.29 +/- 0.51	0.43 +/- 0.64	0.54	0.2 +/- 0.35	0.36 +/- 0.6	0.73
Fibrosis	0.27 +/- 0.51	0.42 +/- 0.63	0.45	0.3 +/- 0.42	0.29 +/- 0.58	0.97
TMA (n)	7	2	0.03^	6	1	0.02^
Arteritis (n)	6	4	0.48^	2	4	1.0^
Arterial fibrinoid necrosis (n)	2	0	not done	0	2	not done
Cortical Necrosis (n)	1	1	not done	1	0	not done
PTC C4d intensity			not done	2+/-0.8	1.9+/-0.4	0.78

[1]Average scores indicated here. *Wilcoxon rank sum test. ^Fisher's exact p-value. n=number of biopsies. Cut off p-value = 0.002 (Bonferroni), PTC=peritubular capillary

Table 6. Proportion of graft loss 2 years post-transplant

	Group 1 (2003 onwards)	Group 2 (before 2003)	Subgroup 1A (PP, IVIG)	Subgroup 1B (conventional therapy)
Graft loss	8(30%)	13 (42%)	4 (33%)	4 (28%)
No graft loss	18 (69%)	18 (58%)	8 (62%)	10 (71%)

Chi-square p=0.50 for Groups 1 and 2; Chi-square p=0.69 for Subgroups 1A and 1B

AMR; however, this would not explain the lack of difference between Groups 1 and 2 and between Subgroup 1A and 2, because no patient before 2003 (Group 2) was treated for AMR, regardless of the severity of rejection. The induction immunosuppression protocols in our two major groups (Group 1 and 2) did differ. There was also a change in the maintenance regimen from steroid containing to steroid free two drug regimen with increased use of Sirolimus in Group 1. This was inevitable since immunosuppression protocols are subject to change over the years as newer and newer drugs are discovered. However, there was no significant difference in the rate of graft loss between Groups 1 and 2 or between Subgroups 1A and 1B (these subgroups were on similar induction and maintenance protocols). The two-year death-censored graft survival for all transplant patients at our institution was 92.3% between 1/1/2000 and 12/31/2002 (the three-year period from which patients for Group 2 were selected) and 93.8% between 1/1/2003 and 12/31/2005 (the three year period from which patients for Group 1 were selected). Thus, changes in treatment regimens did not alter two-year graft survival within the study periods. None of the immunosuppressive drug regimens have been convincingly shown to affect or improve long-term outcome in antibody-mediated rejection. Therefore the possibility that the different immunosuppression protocols have greatly affected this study is unlikely.

The disadvantage of our study is that it is retrospective. Prospective case-controlled studies are needed, but they are difficult to conduct because of the current consensus that acute AMR has to be treated with plasmapheresis and/or IVIG. The lack of beneficial effect of specific treatment for de novo AMR in our study may be the result of late diagnosis. Early intervention may improve outcome of AMR secondary to de novo donor specific antibodies. This could only be achieved with superb, rigorous post-transplant immune monitoring.

In summary, our retrospective study shows that, specific additional treatment for AMR (plasmapheresis and IVIG) as compared to conventional anti-rejection therapy in non-desensitized patients with C4d positive AMR during the first six months post-transplant, did not improve graft survival and level of graft function at our institution. Treatment of C4d positive AMR with plasmapheresis/IVIG however, appears to benefit patients with TMA associated with high levels of anti-HLA antibodies, provided it is diagnosed and treated before the development of severe graft injury such as cortical necrosis. Cortical necrosis and fibrinoid arterial necrosis in the biopsy most likely portend poor outcome. Unfortunately, other histologic parameters appear to have little if any predictive value and did not prove helpful in guiding the selection of patients for plasmapheresis and IVIG treatment.

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Abbreviations: AHG-CDC: Anti-Human Globulin Complement Dependent Cytotoxicity Crossmatch; ATG: Anti-Thymocyte Globulin; ATN: Acute Tubular Necrosis; AMR: Antibody Medicated Rejection; CADI: Chronic Allograft Damage Index; FCXM: Flow Cytometric Crossmatch; HLA: Human Leukocyte Antigen; HPF: High Power Field; IVIG: Intravenous Immunoglobin; PAS: Periodic Acid-Schiff; PMNs: Polymorphonuclear Leukocytes; PRA: Panel Reactive Antibodies; PTC: Peritubular Capillary; RBCs: Real Blood Cells; TMA: Thrombotic Microangiopathy.

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