Thrombotic Microangiopathy and Peritubular Capillary C4d Expression in Renal Allograft Biopsies

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Summary

Background and objectives This study characterizes the pathologic and clinical relationships of thrombotic microangiopathy (TMA) to antibody-mediated rejection (AMR) in renal allograft biopsies.

Design, setting, participants, & measurements Consecutive renal allograft biopsies, routinely stained for C4d over a period of 51 months ($n = 1101$), were reviewed. For comparative analysis of histology and clinical features, additional patients with TMA and peritubular capillary (PTC) C4d ($n = 5$) were combined with those identified in the 51-month period of review ($n = 6$).

Results One hundred eighty-two of 1073 adequate biopsies from 563 allografts had PTC C4d in the study period. Six of 37 biopsies with TMA had PTC C4d (five at ≤90 days and one at 213 days). Early (≤90 days) C4d+ biopsies ($n = 5$) had more frequent TMA (11.9% C4d+ *versus* 3.4% C4d−; odds ratio, 3.84; $P = 0.03$). Graft loss was significantly greater in an early C4d+TMA+ group ($n = 5$ study + 2 archival patients) than in C4d+ controls without TMA ($n = 21$) (57% *versus* 9.5%; $P = 0.02$). Early TMA+C4d+ biopsies had more severe glomerulopathy and less severe arteriolopathy than TMA+C4d− and had more frequent neutrophilic capillaritis than TMA−C4d+ biopsies.

Conclusions TMA was infrequent in this series of unselected, consecutive, renal allograft biopsies (3.4%). PTC C4d may be a significant risk factor for early TMA, and TMA is associated with glomerular thrombi and neutrophilic capillaritis. TMA in allografts with suspected AMR may portend a higher risk of graft loss.

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Introduction

Thrombotic microangiopathy (TMA) in the transplant kidney is a form of renal vascular injury that may be associated with many disorders including calcineurin inhibitor (CI) toxicity, antibody-mediated rejection (AMR), infections, malignant hypertension, and recurrent diseases like hemolytic uremic syndrome or scleroderma (1). Linear peritubular capillary (PTC) C4d staining is a sensitive and specific marker of AMR (2–4); however, diagnosis requires demonstration of donor-specific antibodies and graft injury (5). TMA has been noted in 4 to 46% of patients with AMR, with the highest frequency in the early post-transplantation period (3,6–8). Glomerular thrombi are described in AMR, and arteriolar thrombi have been described in some (9,10). Thrombi in PTC are described rarely (10). PTC mural platelet deposits may be observed at sites of severe capillary injury, with endothelial loss and interstitial hemorrhage in AMR (11).

Diagnosis of AMR requires identification of PTC C4d, graft injury, and donor-specific antibodies (5). Many patients in this series lacked serologic data, and so these allografts had immunopathologic lesions suspicious for AMR. Here we hypothesized that allografts with immunopathologic features of AMR, identified by PTC C4d and graft injury, are at greater risk for the development of TMA. To test the hypothesis, we examined the concurrence of TMA and PTC C4d in unselected consecutive renal allograft biopsies, routinely stained for C4d, over a period of 51 months. We combined the patients from the observation period with the few other examples from our files ($n = 6$) to facilitate morphologic and clinical analyses. The pathologic features in TMA+C4d+ allograft biopsies were compared with TMA+C4d− and TMA−C4d+ biopsies to elucidate differences in morphology. The effect of TMA on graft survival in suspected AMR was determined by comparing the outcome of C4d+TMA+ and a matched control group of C4d+TMA− allografts in the year after biopsy diagnosis.

Materials and Methods

Consecutive unselected renal allograft biopsies accessioned between December 1, 2004 and February 1, 2009 were included in the study. Approval for this study was obtained from the University of Chicago Institutional Review Board (protocol number 16619B). Biopsies had been obtained for allograft dysfunction

and not by protocol. Renal allograft biopsies (n = 1101) were routinely stained for C4d over this period of 51 months and had been reviewed by one of three renal pathologists. Twenty-eight biopsies were excluded for insufficiently representative tissue (n = 21) and inadequate clinical data (n = 7). TMA was defined by the following histologic features: occlusive fibrin-platelet thrombi in at least one glomerulus or one arteriole, with one or more of the following: (*1*) glomerular endothelial swelling and detachment, capillary wall thickening and double contour formation, mesangial lysis with microhemorrhage, and erythrocytolysis and/or (*2*) obliterative arteriolopathy defined as luminal occlusion with mural myxoid or fibrinoid change, thickening of the vessel wall, with or without erythrocytolysis, luminal thrombosis, and concentric spindle cell proliferation or hypercellularity.

Light microscopic diagnoses and Banff 1997 histologic indices (5) were tabulated from hematoxylin and periodic acid Schiff stained sections (n = 12 to 18 per biopsy). Polymorphonuclear neutrophilic (PMN) inflammation in capillaries was graded as follows: (*1*) glomeruli: 0, none; 0.5+, 2 or less PMN; 1+, 3 to 5 PMN; 2+, 6 to 10 PMN; 3+, more than 10 PMN in the most affected glomeruli and (*2*) peritubular capillaries: 0, none; 0.5+, 2 or less PMN in 10% or less of the PTC; 1+, >2 PMN in 11 to 25% of PTC; 2+, >2 PMN in 26 to 50% of PTC; 3+, >2 PMN in >50% PTC.

Indirect immunofluorescence staining using standard protocols was routinely performed on 4-μ frozen sections of core biopsies frozen at −20°C. The primary antibody was a monoclonal mouse anti-human C4d (clone 10-11; Biogenesis, Sandown, NH). Paraffin sections were stained in <5% of patients using previously published methods (12). C4d status, defined as the extent of involvement of PTC by linear deposition of C4d, was also recorded and correlated with histology. Focal C4d deposition was defined as 5 to 50%, and diffuse was defined as >50% mural staining of the peritubular capillary network of cortex and medulla. Kidney transplant nephrectomies were excluded.

Clinical data were obtained by retrospective chart review including age, sex, primary kidney disease, donor status, HLA mismatch, induction therapy, maintenance immunosuppressive agents, CI blood levels, clinical diagnosis at time of biopsy, treatment immediately postbiopsy, and 1-year outcomes. Serum creatinine at baseline was defined as the single lowest serum creatinine in up to 3 months before biopsy. Serum creatinine at the time of biopsy and at 1-year follow-up were also recorded. Patients with delayed graft function, defined as a requirement for hemodialysis in the first week post-transplantation, were considered to have a serum creatinine of 5 mg/dl. The CI levels included were from the time of biopsy and averaged over a 3-week period before biopsy, when available, and the results are expressed in ng/ml. All of the grafts were ABO blood group-compatible.

Induction therapy consisted of methylprednisolone plus either anti-IL-2 receptor antibody or anti-thymocyte globulin. The most common maintenance immunosuppressive regime consisted of tacrolimus, mycophenolic acid (mycophenolate mofetil or mycophenolate sodium), and prednisone. Patients who had a renal allograft nephrectomy and a creatinine of 5.0 mg/dl or more and those who required renal replacement therapy for greater than 8 weeks were defined as graft failure. At 1-year of follow-up, patients with graft failure were arbitrarily assigned a creatinine of 5 mg/dl for statistical analysis. To provide a reference end point for TMA episodes occurring at various time points in the post-transplantation period, graft failure in the first year after biopsy diagnosis of TMA was chosen as the most suitable index of graft loss.

Anti-donor antibodies were detected using flow cytometric cross-match and solid-phase assays. Three color flow cytometric cross-match analysis was performed by modification of a previously described dual-color technique (13). Donor-specific antibodies were detected by solid-phase assay. Antibody specificities were identified using the Luminex platform and the HLA phenotype and the single antigen panels as targets (14–16). The phenotype panels used were Lifematch classes I and II (Tepnel LifeCodes, Stamford, CT) and Labscreen classes I and II (One Lambda, Inc., Canoga Park, CA), and the single antigen panels were single antigen beads (One Lambda, Inc.). Donor-specific antibodies were defined as antibodies present in recipient sera with mean fluorescence intensity ≥1000 and directed against HLA antigens present on the donor cells.

Clinical records were evaluated to exclude other possible causes of allograft TMA including malignant

Table 1. Summary of results of TMA and PTC C4d status

	All		Early (≤90 Days)		Late	
	n	%	*n*	%	*n*	%
C4d+	182	17	43	16.3	139	22.2
TMA+	37	3.4	14	4.6	23	3
TMA+C4d+	6	0.6	5	1.6	1	0.13
TMA+C4d−	31	2.9	9	3	22	2.87
TMA+C4d+/TMA+	6/37	16.2	5/14	35.7	1/23	4.5
Total	1073		307		766	

Table 2. Association of TMA and PTC C4d

	TMA+		TMA−		OR	*P*
	n	%	*n*	%		
For all biopsies						
C4d+	6	3.3	176	96.7	0.95	0.92
C4d−	31	3.5	860	96.5	95% CI (0.4 to 2.3)	
By time of biopsy						
early (≤90 days)						
C4d+	5	11.9	38	88.1	3.84	0.03
C4d−	9	3.4	255	96.6	95% CI (1.2 to 12.1)	
late						
C4d+	1	0.72	138	99.28	0.2	0.09
C4d−	22	3.5	605	96.5	95% CI (0.03 to 1.5)	

Table 3. Comparative histologic findings in early TMA

	C4d+	C4d−	*P*
n	7	9	
Glomeruli			
total glomeruli (mean)	24	18	
thrombi - segmental	100	33.3	0.01
thrombi - global	28.6	0	0.2
mesangiolysis	28.6	0	0.2
double contours	42.9	22.2	0.6
neutrophilic glomerulitis	100	0	0.0009
transplant glomerulitis	42.9	0	0.06
global glomerulosclerosis	71.4	88.9	0.55
segmental glomerulosclerosis	0	22.2	0.48
ischemic glomerulopathy	85.7	66	0.6
Peritubular capillaries			
peritubular capillaritis - neutrophils	100	11.1	0.001
peritubular capillaritis - mononuclear	57.1	11.1	0.1
Arterioles			
obliterative arteriolopathy	42.9	77.8	0.3
arteriolar thrombi	42.9	44.4	1
arteriolar hyalinosis	42.9	77.8	0.3
Arteries			
endothelialitis	57.1	0	0.01
arterial intimal fibrosis	42.9	44.4	1
Tubules and interstitium			
ATN	85.7	66	0.58
interstitial mononuclear cells	85.7	100	0.4
tubulitis	42.9	44.4	1
interstitial hemorrhage	42.9	0	0.06
interstitial fibrosis	42.9	55.6	0.99
tubular atrophy	28.6	55.6	0.36

The values are the percentages of biopsies with lesions in each group. ATN, acute tubular necrosis.

hypertension, hepatitis C infection, parvovirus B-19 infection, cytomegalovirus infection, graft irradiation, lupus anticoagulants, and primary or recurrent hemolytic uremic syndrome or thrombotic thrombocytopenic purpura, to the extent possible.

For comparative analysis of the effect of TMA on graft loss, five additional C4d+TMA+ archival patients were combined with the biopsies identified in the initial observational analysis ($n = 6$ of 1073 biopsies). Histologic features of early TMA+C4d+ biop-

sies ($n = 5 + 2 = 7$) were compared with TMA+C4d− biopsies from the early post-transplantation period (90 days or less) identified in the initial analysis ($n = 9$). Causes of end-stage disease in the TMA+C4d+ group included insulin-dependent diabetes mellitus ($n = 3$), hypertension ($n = 2$), scleroderma ($n = 1$), and an unknown cause ($n = 1$). Causes of end-stage kidney disease in the TMA+C4d− ($n = 9$) group included insulin-dependent diabetes ($n = 2$), hypertension ($n = 3$), membranous glomerulopathy ($n = 2$), medullary cystic disease ($n = 1$), and an unknown cause ($n = 1$).

Biopsies in the C4d+ control group were frequency-matched for time post-transplantation, recipient age, type of graft, induction, and baseline immunosuppression ($n = 35$). The effect of TMA on AMR was evaluated by comparison of clinical features of TMA+C4d+ patients and controls with PTC C4d but without TMA (TMA−C4d+, $n = 21$ early biopsies and $n = 14$ late biopsies).

Group proportions were analyzed using the χ^2 and Fisher exact tests. Two group comparisons were made using the Mann-Whitney test for nonparametric data.

Results

Association of TMA and AMR

One thousand seventy-three biopsies from 563 allografts had adequate tissue and clinical data for inclusion in the study. One hundred eighty-two had PTC C4d expression (16.8%). C4d expression was focal in 52 and diffuse in 130. Thirty-seven of 1073 biopsies had TMA (3.4% of the total). Six biopsies (0.6% of total) had TMA and PTC C4d expression (focal in one and diffuse in five). Thirty-one biopsies had TMA without C4d expression in the PTC (3.5% of all C4d negative biopsies). Tables 1 and 2 summarize the association of TMA and C4d expression. The probability of observing TMA in C4d+ biopsies for the group as a whole ($n = 182$), given by the odds ratio (OR), is 0.95 (95% confidence interval, 0.4 to 2.3; $P = 0.92$). This observation is, however, confounded by the time after transplantation at which the biopsy

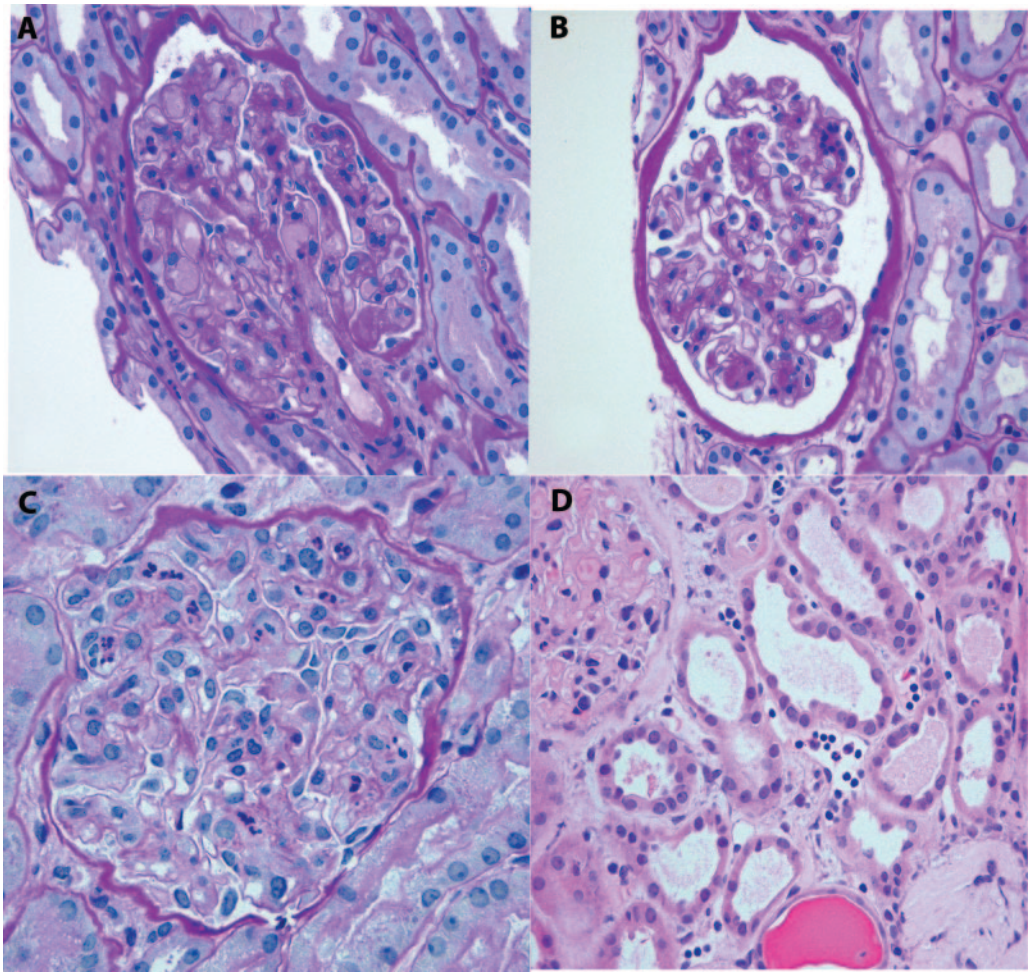

Figure 1. | (A) Glomerular capillary thrombi, segmental ischemia, and neutrophil infiltrates in a kidney allograft biopsy from post-transplant day 10 (periodic acid-Schiff [PAS]). (B) Neutrophilic glomerulitis (score 2+) in a nonthrombosed glomerulus from the same biopsy as A (PAS). (C) Neutrophilic glomerulitis (score 3+) without capillary thrombi (PAS). (D) Peritubular capillaritis with neutrophils and mononuclear cells. Tubules have acute tubular injury (hematoxylin and eosin).

was obtained. Coincidence of TMA+ and C4d+ was observed more frequently in early post-transplantation biopsies, obtained at 90 days or less after transplantation, compared with later biopsies (1.6% *versus* 0.13%; $P = 0.003$). The probability of observing TMA with PTC C4d in early biopsies (11.9%) was significantly greater than in C4d− biopsies (3.4%; OR, 3.8; 95% confidence interval, 1.2 to 12.1; $P = 0.03$). In

Table 4. Clinical data in early allograft biopsy groups

	TMA+C4d+ ($n = 7$)	TMA+C4d− ($n = 9$)	P[a]	TMA−C4d+ ($n = 21$)	P[b]
Mean age (years)	46	45.3		36.4	
Gender (women:men)	3:4	2:7		11:10	
Cause of ESRD					
diabetic nephropathy	3	2	0.6	3	0.29
hypertensive nephrosclerosis	2	3	1	6	1
primary TMA	0	0	–	1	–
primary glomerular disease	0	2	0.48	6	0.28
other	2	2	1	5	1
Type of transplant					
deceased donor	6	7	1	14	0.63
living donor	1	2	1	7	0.63
kidney only	6	7	1	16	1
first transplant	6	9	0.44	11	0.19
HLA mismatches (mean)	5	5.25	0.7	4.5	0.63
Pretransplant cross-match					
positive	1	0		2	
negative	5	9	0.4	14	1
unknown	1	0		5	
Immunosuppression induction					
T cell depleting antibody	5	5	0.63	14	1
anti-IL-2 receptor antibody	0	5	0.03	5	0.37
steroids	5	7	1	14	1
unknown	0	0		3	
Baseline					
tacrolimus	7	7	0.5	14	0.29
cyclosporin	0	0		1	
sirolimus	0	0		0	
mycophenolate mofetil	7	9	1	16	1
prednisone	7	8	1	17	1
unknown	0	0		3	
Mean time of biopsy (days)	25.9	26.3	0.9	21	0.33
Serum creatinine					
baseline (median)	1.8	1.5	0.9	4.5	0.75
at biopsy (median)	4.4	2.2	0.06	2.5	0.24
at 1-year follow up (median)	5	1.9	0.1	1.5	0.05
Calcineurin inhibitor levels					
tacrolimus, at biopsy	14.8 (6)	10.5	0.57	9.5 (12)	0.14
tacrolimus, 3-week average	9.9 (5)	10.6	0.92	10.7 (12)	0.75
Treatment after biopsy					
steroid pulse	4	0	–	6	0.65
plasmapheresis	2	0	–	3	0.9
thymoglobulin	3	0	–	7	1
IvIg	4	0	–	13	0.32
rituximab	2	0	–	6	1
discontinuation of CI	0	1	–	0	–
reduction of CI dose	0	4	–	0	–
OKT3	1	0	–	0	–
unknown	1	1		5	
Graft loss	4	1		2	
Graft survival	3	8	0.1	19	0.02

[a]TMA+C4d+ *versus* TMA+C4d−.
[b]TMA+C4d+ *versus* TMA−C4d+.

contrast, TMA+C4d+ biopsies were less frequent (0.72%) than TMA+C4d− biopsies (3.5%; OR, 0.2; 95% confidence interval, 0.03 to 1.5; $P = 0.09$) for renal allograft biopsies obtained 90 days or more after transplantation. PTC C4d positivity is therefore a significant risk factor for TMA only in the early post-transplantation period.

Comparative Analysis of TMA with and without PTC C4d

Histologic findings in early TMA+C4d+ and TMA+C4d− biopsies are given in Table 3. Nine TMA+C4d− biopsies were obtained ≤90 days, and 22 were obtained >90 days after transplantation. Glomerular thrombi (Figure 1A) were more frequent (100% *versus* 33.3%) and more extensive (28.3% *versus* 1.9% of glomeruli; $P = 0.0015$) in TMA+C4d+ biopsies from the early period. In contrast, acute arteriolopathic lesions (thrombi and/or obliterative arteriolopathy) were seen with higher frequency in early C4d− biopsies (78% *versus* 43%; $P = 0.3$), and these lesions tended to be more extensive in C4d− biopsies (lesions in >25% of sampled arterioles in 55.6% *versus* 0%; $P = 0.03$). The frequency and severity of arteriolar hyalinization was greater in the C4d− biopsies (55.6% *versus* 42.9%; $P = 0.9$; and Banff arteriolar hyalinosis score >1 in 44.4% *versus* 0%; $P = 0.08$). TMA in the absence of PTC C4d was attributable to CI toxicity ($n = 7$), malignant hypertension ($n = 1$), and transplanted TMA due to disseminated intravascular coagulation ($n = 1$). Diagnosis of CI toxicity was on the basis of histologic findings of hyaline or obliterative arteriolopathy ($n = 7$), with or without elevated blood levels of CI, return of serum creatinine to within 10% of baseline on reduction of the administered dose of CI, and the absence of other plausible explanations for arteriolopathy. Neutrophilic glomerulitis and peritubular capillaritis were more frequent in C4d+ biopsies ($P = 0.0009$ and 0.001, respectively). Intimal arteritis was also more frequent in the C4d+ biopsies (57% *versus* 0%; $P = 0.019$). The other histologic parameters assessed were not significantly different between these groups (Table 3). The TMA+C4d+ biopsy group had

Table 5. Histologic comparison of early C4d+ biopsies with and without TMA

	TMA+	TMA−	*P*
n	7	21	
Glomeruli			
total glomeruli (mean)	24	21	
thrombi	100	0	<0.0001
mesangiolysis	42.8	0	0.01
double contours	42.8	0	0.01
neutrophilic glomerulitis[a]	100	14.3	0.0001
acute transplant glomerulitis	57.1	47.6	0.99
global glomerulosclerosis	71.4	23.8	0.06
segmental glomerulosclerosis	0	4.8	–
ischemic glomerulopathy	85.7	23.8	0.007
Peritubular capillaries			
peritubular capillaritis - neutrophils[b]	100	23.8	0.007
peritubular capillaritis - mononuclear	57.1	52.4	1
Arterioles			
obliterative arteriolopathy	57.1	0	0.002
arteriolar thrombi	42.8	0	0.01
arteriolar hyalinosis	42.8	47.6	1
Arteries			
endothelialitis	57.1	23.8	0.16
arterial intimal fibrosis	42.8	47.6	1
Tubules and interstitium			
ATN	100	76.2	0.29
interstitial mononuclear cells	85.7	71.4	0.64
tubulitis	42.8	28.6	0.65
interstitial hemorrhage	42.8	28.6	0.65
interstitial fibrosis	57.1	14.3	0.04
tubular atrophy	11.8	14.3	0.57

The values are the percentages of biopsies with lesions in each group. ATN, acute tubular necrosis.
[a]Proportion of biopsies with more than two neutrophils in the worst affected glomeruli.
[b]Proportion of biopsies with more than two neutrophils in more than 10% of the PTC.

higher levels of serum creatinine at the time of biopsy compared with TMA+C4d− biopsies. Tacrolimus blood levels were not significantly different (14.8 *versus* 10.5 ng/ml; $P = 0.57$). The clinical details are summarized in Table 4.

In late biopsies the frequency and severity of glomerular capillary thromboses were similar in C4d+ ($n = 1$ from the observation period + 3 archival patients) and C4d− biopsies ($n = 22$). All of the late TMA+C4d+ biopsies had diffuse glomerular capillary double contours, and all had diffuse C4d deposits in glomerular capillary walls and PTC. Glomerular capillary double contours were evident less frequently in late TMA−C4d+ biopsies (100% TMA+ *versus* 32% TMA−; $P = 0.4$). The frequency of acute transplant glomerulitis (75% *versus* 13.6%; $P = 0.027$), neutrophilic glomerulitis and peritubular capillaritis (50% *versus* 4.5%; $P = 0.052$), and mononuclear peritubular capillaritis (100% *versus* 18.2%; $P = 0.005$) were significantly greater in the C4d+ group. Other histologic parameters assessed were not significantly different between these groups (data not shown).

Effects of TMA on C4d-positive Rejection Episodes

Baseline demographic and clinical data for the early TMA+C4d+ and TMA−C4d+ groups are shown in Table 4. Pretransplant standard and flow cytometric cross-matches were positive in one of seven patients in the TMA+C4d+ group and negative in all of the patients in the TMA−C4d+ group. Serologic evidence of post-transplantation anti-donor alloreactivity was available in two patients from the TMA+C4d+ group; one had a positive flow cytometric cross-match, and one was donor-specific antibody (DSA)-positive. Eleven of 21 controls had serologic evaluation, nine were DSA-positive, and two had undetectable DSAs. Allograft function was not significantly different between the groups at baseline and at the time of biopsy. At 1 year of follow-up after the biopsy, the median serum creatinine was higher in C4d+ patients with TMA compared with those without TMA (5 *versus* 1.5 mg/dl; $P = 0.05$). Treatment for rejection was similar in each group. Graft failure occurred in 57.1% of TMA+C4d+ patients compared with 9.5% of the TMA−C4d+ group at follow-up 1 year after the biopsy ($P = 0.02$). In allograft biopsies obtained >3 months after transplantation, graft loss was 100% in three of three TMA+C4d+ allografts with available follow-up data, compared with 28.6% of TMA−C4d+ biopsies ($n = 14$; $P = 0.05$).

Banff 97 histologic lesions of acute transplant glomerulitis had comparable frequency in C4d+ biopsies with and without TMA (Table 5). Careful evaluation revealed at least one PMN in one glomerulus in 82% and one PMN in one PTC in 68% of the C4d+ biopsies in these two groups. Neutrophilic glomerulitis (Figure 1, B and C), with more than two neutrophils per glomerulus (score 1+ or more) was more frequent in TMA+ biopsies (100% *versus* 14.3%; $P = 0.002$). The proportion of glomeruli with any neutrophils was greater in TMA+ biopsies than in biopsies without TMA (53% (range, 29 to 87%) *versus* 20.3% (range, 3 to 45%); $P = 0.0021$). Neutrophilic peritubular capillaritis (Figure 1D) with more than two PMN in more than 10% of the PTC (score 1+ or more) was more frequent in TMA+ biopsies (100% *versus* 23.8%; $P = 0.007$). Ischemic glomerulopathy, defined as segmental or global wrinkling of the glomerular basement membrane, reduced tuft area, and periglomerular fibrosis, was more frequent in the TMA+ biopsies (85.7% *versus* 23.8%; $P = 0.007$). The frequency of type 1 (14.3% *versus* 9.6%) and 2 (57% *versus* 23.8%) T cell-mediated rejection was not significantly different between these groups. TMA+C4d+ grafts

Table 6. Odds ratios by individual studies

Study	C4d	TMA +	TMA −	OR	*P*	Time of Biopsy
Regele (17)	+	3	39	4.5 (0.45 to 45.2)	0.3	Median 14 days (1 to 532 days)
	−	1	59			
Nickeleit (3)	+	12	108	1.6 (0.7 to 3.4)	0.3	Median 38 days (7 to 5646 days)
	−	18	260			
Mauiyyedi (6)	+	4	16	incalculable	0.006	<3 months
	−	0	47			
Trpkov (9)[a]	+	11	13	5.6 (1.3 to 24.2)	0.02	<45 days
DSA data	−	3	20			
Regele (4)	+	3	70	3 (0.5 to 18)	0.34	>1 year
	−	2	138			
This study	+	5	38	3.8 (1.2 to 12.1)	0.03	≤90 days
	−	9	255			
	+	1	138	0.2 (0.03 to 1.5)	0.09	>90 days
	−	22	605			

[a]Data are for DSA status as C4d data were not available.

lost in the year of follow-up tended to have a higher frequency of transplant glomerulopathy with mesangial lysis and capillary basement membrane duplication (100% *versus* 33.3%; $P = 0.1$) than grafts surviving more than 1 year.

Discussion

The study finds a low frequency of concurrence of TMA and PTC C4d staining in renal allograft biopsy specimens obtained for graft dysfunction. Although the number of index TMA+C4d+ patients is small, the study population was derived from unselected consecutive biopsy specimens obtained over more than 4 years. Only six of 182 biopsies with PTC C4d had TMA, and 83% of these were from the early post-transplantation period (<3 months). Most patients in this series lacked donor-specific antibody data, and so these allografts had immunopathologic lesions suspicious for AMR. However, if we accept that PTC C4d staining and tissue injury are reliable diagnostic markers of AMR, then the hypothesis that allografts with immunopathologic features of AMR are at increased risk for TMA is supported by the observations of this study only in the early period after transplantation. These findings are in general agreement with the observations of others (6,9,17). The presence of TMA with AMR appears to predict a worse prognosis for graft survival in the year after diagnosis.

The reported frequency of TMA in suspected AMR varies from 4.1% (16) to as high as 49.5% in a study of 21 high-risk patients with PTC C4d (7). Such variation in frequency is difficult to explain but may reflect selectivity of subjects, variation in criteria used for definition of TMA, variation in immunosuppressive regimes, clinical course after transplantation, and primary diseases. Analysis of studies that provide numerical data on the association of PTC C4d deposition with TMA (summarized in Table 6) indicates that significant association of these lesions is observed only in biopsies from the first few months after transplantation consistent with the observations of this study. One study (9) identified a high frequency of TMA in AMR (43%), defined as graft dysfunction with circulating DSAs; however, the study predated recognition of the significance of C4d.

Lesions of TMA in TMA+C4d+ biopsies differed from the TMA+C4d− biopsies. Glomerular thrombi, mesangial lysis, and capillary double contours were more frequent and severe in C4d+ biopsies. Arteriolar lesions were more severe in the C4d− biopsies. One study of early biopsies with rejection and DSAs (9) described severe arteriolopathy resembling hemolytic uremic syndrome; however, the patients in that study had been exposed to cyclosporine immunosuppression, and hence the possible contribution of CI toxicity is hard to exclude. Observation of predominantly arteriolar lesions in TMA+C4d− biopsies in this study is consistent with patterns of injury described in CI toxicity (18,19) and malignant hypertension.

Neutrophilic glomerulitis and peritubular capillaritis were more prominent in the TMA+C4d+ biopsies compared with TMA−C4d+ biopsies. It is possible that neutrophilic inflammation predisposes to microvascular thrombosis; however, the possibility that these infiltrates may be a consequence rather than a cause of thrombosis needs to be excluded. The absence of significant intracapillary neutrophils in TMA+C4d− biopsies in our cohort suggests that microthrombi do not necessarily incite neutrophil infiltrates. One study (20) identified a strong association of TMA and PTC neutrophil margination in HLA-incompatible grafts with PTC C4d expression and a positive cross-match. Thus it is possible that an association of intravascular neutrophils and the development of thrombi may be specific to AMR (8,20).

In conclusion, the study findings suggest that AMR is a risk factor for TMA in renal allografts in the early post-transplantation period. Biopsies with suspected AMR had TMA in a minority of patients (11.9%). Prominent glomerular capillary thrombi and neutrophilic capillaritis involving the glomeruli and peritubular capillaries are characteristic histologic features. The concurrence of immunopathologic features of AMR and TMA may portend a greater likelihood of graft loss attributable to the influence of TMA.

Disclosures

None.

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