Expanding the antibody-mediated component of plasma cell-rich acute rejection: A case series

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ABSTRACT

Renal allograft rejection is mediated by T-cells (T-cell mediated rejection) or by donor-specific antibodies (DSAs) (antibody mediated rejection, ABMR). Plasma cell-rich acute rejection (PCAR) is a unique entity due to its peculiar morphology and poor prognostic behavior. All allograft biopsies done at our center from January 2013 to October 2014 were reviewed, and seven were identified with a diagnosis of PCAR with antibody mediated rejection (ABMR). The allograft biopsies were classified as per the Banff 2007 schema. Immunohistochemistry with C4d, SV 40, CD3, CD20, CD138, kappa and lambda light chain was performed. Total 210 allograft biopsies were performed in the study period of which seven biopsies (3.3%) were diagnosed as PCAR with ABMR. All these were late ABMRs (more than 6 months) with median posttransplant duration of 17 months. The allograft biopsy showed features of PCAR along with glomerulitis, peritubular capillaritis, and positive C4d. DSA was positive in six patients. All the patients were treated with standard therapeutic measures of acute cellular rejection (ACR) and ABMR including steroids, plasma exchange, rituximab and intravenous immunoglobulins. All the patients had persistent graft dysfunction or graft loss on follow-up.

Key words: Acute rejection, allograft biopsy, antibody-mediated rejection, plasma cells

Introduction

Rejection in renal allograft is mediated by T cells (T-cell-mediated rejection) or by donor-specific antibodies (DSAs) (antibody mediated rejection, ABMR). After its introduction in Banff 1997, the criteria for ABMR have been revised and refined on the basis of morphologic tissue injury, evidence of current/recent antibody-vascular endothelial interaction, and serologic evidence of DSAs, Human leukocyte antigen's (HLA's) or other antigens.[1,2] Though not included in Banff schema, plasma cell-rich acute rejection (PCAR) is recognized as a distinct entity due to its peculiar morphology and poor prognostic behavior. Several original studies and case reports have elicited the clinicomorphologic features of PCAR along with therapeutic nuances in elaborate details.[3-9] It is known that PCAR is a combined cell-mediated and ABMR. In the last 20 months, we have observed seven allograft biopsies showing morphologic features of PCAR with positivity for C4d and DSA satisfying the Banff criteria for an additional component of ABMR.

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Access this article online	
Quick Response Code:	Website: www.indianjnephrol.org
	DOI: 10.4103/0971-4065.159300

Materials and Methods

Our centre is a tertiary care university hospital with active nephrology services routinely performing renal transplants (cadaver and live related). All biopsies were for a clinical indication biopsies and protocol biopsies were not performed. Our experience with allograft biopsies has been published earlier.[10,11] The standard triple immunosupression is followed at our centre that includes steroid based therapy, calcineurine inhibitors (Cyclosporine or Tacrolimus) along with azathioprine or mycophenolate mofetil. All biopsies from January 2013 to October 2014 were reviewed.

A total of 7 biopsies were identified with a diagnosis of PCAR with ABMR. The clinical details were noted with respect to age, type of graft, transplant duration, HLA matching, baseline serum creatinine, and creatinine at the time of biopsy, therapy given after diagnosis of acute rejection, and subsequent follow-up data.

The biopsies were classified as per the Banff 2007 schema by reading the light microscopy with the help of hematoxylin and eosin, periodic acid schiff and silver methanamine stains. Immunohistochemistry (IHC) with C4d (Biogenex, India) is performed on all allograft biopsies as a protocol by polymer horse-radish peroxidize technique. Positivity for C4d is assessed in the peritubular capillaries and scored according to Banff 2007 criteria.

The biopsies of PCAR with ABMR were subjected to additional IHC with CD20, CD3, CD 138 and kappa and lambda light chains (to detect monoclonality), and SV 40 large T Antigen (Cell Marque, USA). Nuclear staining was taken as positive for SV 40 Large T antigen. Scoring for active lesions including interstitial inflammation, tubulitis, glomerulitis, peritubular capillaritis, and arteritis was done as per Banff 2007 schema.

Results

Total 210 allograft biopsies were performed in the study period of which seven biopsies (3.3%) were diagnosed as PCAR with ABMR. Clinical data of these patients are given in Table 1. Total 45 (21.4%) biopsies showed features of acute rejection; 19 (8.0%) being cell-mediated rejections acute cellular rejection (ACR) and 26 (11.9%) of acute ABMR. The comparison of all these rejections is given in Table 2.

All patients of PCAR with ABMR had received live donor allo grafts and were on standard triple immunosuppression regimen. All these were late (more than 6 months) ABMRs with median posttransplant duration of 17 months. Three of the patients were found noncompliant to the immunosuppressive medication. Two patients were on antituberculous therapy. The cytomegalovirus and BK virus (BKV) polymerase chain reaction (PCR) were negative in all but one patient. One patient had a previous episode of ACR, which was effectively treated. DSA was positive in 6 patients. These were evaluated by Luminex fluoroanalyzer, and 41–80% positivity was taken as positive. DSA was negative in one patient.

Allograft PCR with ABMR

Plasma cells were more than 10% in all the biopsies and were seen in the cortical portion [Figure 1] separating the tubules. These were morphologically mature and were found to be polyclonal on kappa and lambda IHC. Occasional plasma cell tubulitis was also identified.

All the biopsies showed morphologic features of ACR in the form of interstitial inflammation and tubulitis as well as ABMR such as peritubular capillaritis, glomerulitis,

Table 1: Clinical and follow-up parameters

Cases	Age/ gender	Donor, age in years	HLA matching	Dialysis duration (months)	Duration of transplant (months)	Baseline creatinine	Creatinine at biopsy	Associated conditions	Previous rejection	Immunosuppression	Treatment	Posttreatment creatinine	Follow-up creatinine
1	42/ male	Wife, 36	Nil match	1	28	1.0	2.2	Pulmonary TB on ATT	0	Tacrolimus + MMF + prednisolone	Methyprednisolone, rituximab, plasmapheresis	2.0	3 months 2.8, expired
2	28/ male	Wife, 23	Haplomatch	5	17	1.2	2.4	CMV PCR positive one year back	0	Tacrolimus + MMF + prednisolone	Methyprednisolone, plasmapheresis, IVIg, bortezomib	2.0	5 months 2.8
3	46/ male	Sister, 42	Haplomatch	4	11	1.2	3.5	PTDM	0	Tacrolimus + MMF + prednisolone	Methyprednisolone, IVIg, rituximab	3.2	5 months 5.2
4	43/ male	Wife, 40	Haplomatch	1	11	0.9	1.9		0	Tacrolimus + MMF + prednisolone	Methyleprednisolone, rituximab, plasmapheresis	1.2	6 months, dialysis dependent
5	30/ male	Wife, 22	Haplomatch	3	12	1.1	4.3	Noncompliance, pulmonary TB on ATT	0	Everolimus + MMF + prednisolone	Methyprednisolone, rituximab		8 months, 4.5
6	49/ male	Wife, 40	Nilmatch	3	36	1.4	4.3	Noncompliance	0	Everolimus + MMF + prednisolone	Methylprednisolone	3.3	Lost to follow-up
7	30/ male	Mother, 47	Haplomatch	1	36	1.4	10	Noncompliance	ACR 1-year before	Tacrolimus + MMF + prednisolone	Methylprednisolone, plasmapheresis, rituximab	13	6 months Dialysis dependent

HLA: Human leukocyte antigen, MMF: Mycophenolate mofetil, ATT: Anti-tuberculosis treatment, CMV: Cytomegalovirus, PCR: Polymerase chain reaction, PTDM: Posttransplantation diabetes mellitus, TB: Tuberculosis, ACR: Acute cellular rejection, IVIg: Intravenous immunoglobulin

and intimal arteritis [Figures 2 and 3]. The features of chronicity were absent/minimal in these biopsies. The scoring for acute/active and chronic lesions is provided in Table 3. All the biopsies showed C4d positivity in peritubular capillaries (c4d3) [Figure 4].

None of the biopsies showed CD20 lymphoid aggregates. SV 40 large T antigen IHC was negative in all.

Four of the patients had follow-up biopsies and the scoring on these is given in Table 4. The morphologic features of rejection persisted in the follow-up biopsies albeit of lesser degree.

Follow-up

All the patients were treated with standard therapeutic measures of ACR and ABMR including methyprednisolone (1 g/day three doses), plasma exchange, rituximab and intravenous immunoglobulins (IVIg) and bortezomib.

Table 2: Comparison of all types of acute rejections

Clinical details	ACR	ABMR	PCAR with ABMR
Number of cases	19	26	7
Age (years)	32.8 (14-52)	31.2 (12-48)	38.2 (28-50)
Male: female	3.8:1	2.2:1	7:0
Duration of transplant (months)	11.6±9.1	15.3±14.2	21±10.8
Serum creatinine at presentation (mg/dL)	2.3 (1.3-4.4)	2.8 (1.6-4.5)	4.0 (1.9-10)
Banff grade			
Ia	13		2
Ib	5		5
IIa	1		
IIb			
III			
Mean follow-up period (months)	12	12	5
Graft outcome			
Functioning graft	13	19	
Graft dysfunction/loss	3	5	5
Lost to follow-up	4	2	1

ACR: Acute cellular rejection, PCAR: Plasma cell-rich acute rejection, ABMR: Antibody-mediated rejection

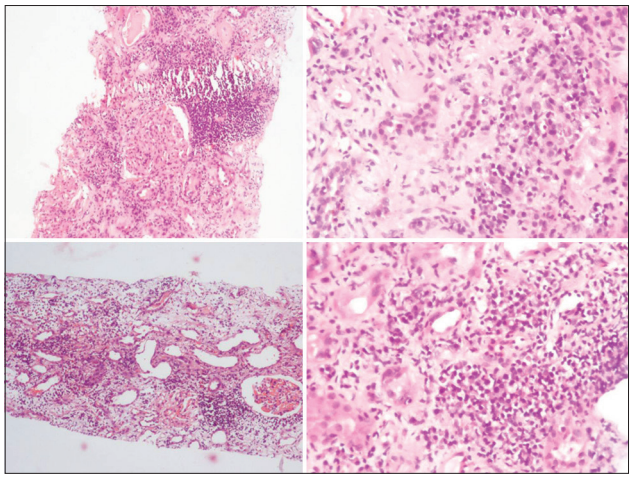

Figure 1: The allograft biopsies showing dense infiltrate of plasma cells

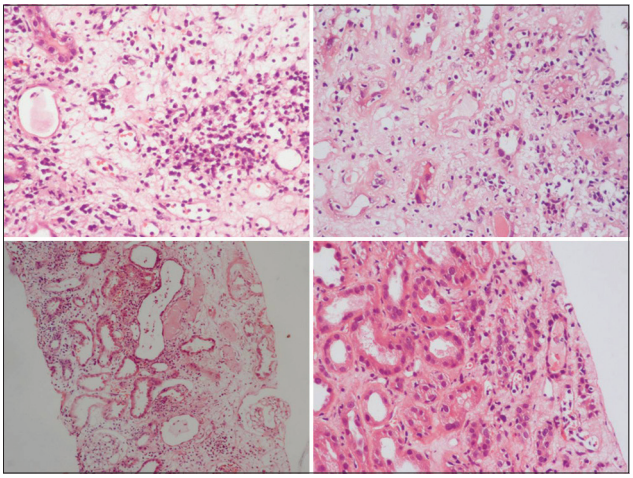

Figure 2: The allograft biopsies with interstitial edema, peritubular capillary dilaltation, and capillaritis

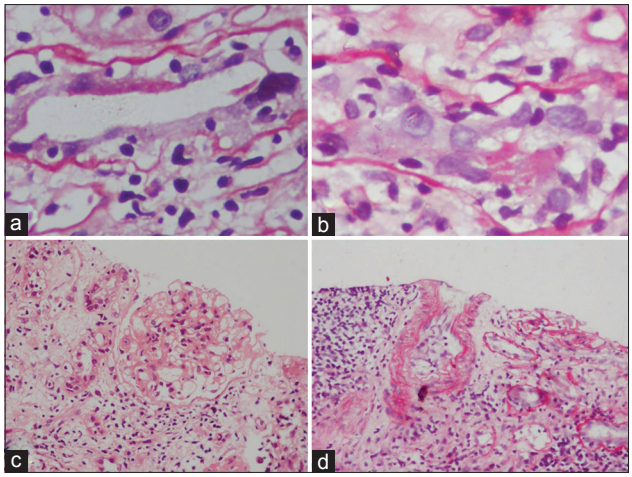

Figure 3: (a and b) The biopsies showing tubulitis, (c) glomerulitis and (d) intimal arteritis

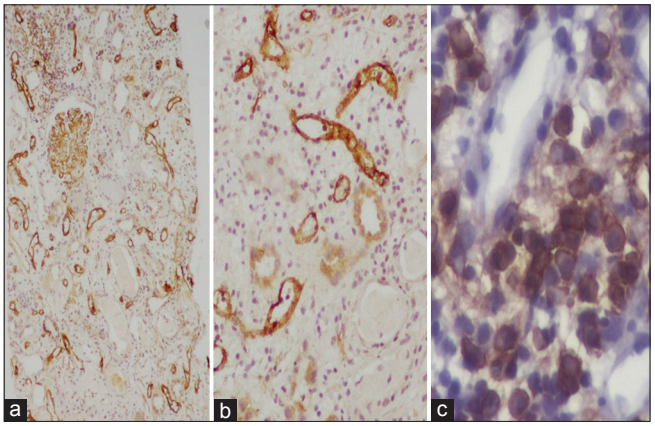

Figure 4: (a and b) Immunohistochemical positivity for C4d along the peritubular capillaries (c) positivity of plasma cells for CD 138

Table 3: Scoring of the activity and chronicity parameters

Cases	Plasma cells (%)	Tubulitis score	Interstitial inflammation score	Glomerulitis score	Peritubular capillaritis score	Arteritis score	IFTA	Vascular sclerosis
1	20	t2	i2	g0	PTC 1	V1	1	0
2	60	t2	i3	g2	PTC 2	V0	0	0
3	40	t1	i3	g0	PTC 2	V0	0	0
4	50	t1	i3	g2	PTC 3	V0	1	0
5	60	t2	i3	g2	PTC 2	V0	1	0
6	60	T2	I3	G1	PTC 2	V0	0	0
7	60	T2	I3	G2	PTC 3	V0	0	0

IFTA: Interstitial fibrosis and tubular atrophy, PTC: Peritubular capillaries

Table 4: Follow-up biopsy scoring

Histopath number	Tubulitis score	Interstitial inflammation score	Glomerulitis score	Peritubular capillaritis score	Arteritis score
Case 1	t2	i2	g0	PTC 1	V1
Case 2	t1	i2	g0	PTC 1	V0
Case 3	t0	i1	g0	PTC 1	V0
Case 4	t1	i3	g0	PTC 1	V0

PTC: Peritubular capillaries

Three patients showed minimal reduction in serum creatinine following the treatment. However, the serum creatinine showed persistent elevation in the due course in four with graft loss in two patients. The follow-up creatinine levels are given in Table 1. One patient succumbed to sepsis due to overt immunosuppression and two became dialysis dependent. The outcome in terms of graft dysfunction and loss was particularly found to be worse in the patients with PCAR with ABMR in comparison to other acute rejections.

Discussion

Plasma cells in renal allografts have generated a lot of importance and curiosity. The presence of plasma cells is associated with viral infections, drug toxicities, posttransplant lymphoproliferative disorder (PTLD) and acute rejection.[6,8]

The earliest correlation of plasma cells with graft loss was reported by Nádasdy *et al*.[12] and David-Neto *et al*.[13] that was described as acute interstitial nephritis of plasma cells. This was followed by the reports of actual PCAR was by Charney *et al*. in 1999.[3] They described 27 patients of PCAR occurring in late posttransplant duration and having poor graft survival. The series by Desvaux *et al*.[4] and Gupta *et al*.[8] further contributed to the understanding of PCAR. All these case series depict similar clinicomorphologic features of PCAR. These being relatively late onset of rejection, slight female dominance, polyclonal plasma cell infiltrate, unresponsiveness to treatment and poor prognosis. Charney *et al*.[3] and Gupta *et al*.[8] have clearly mentioned absence of tissue injury related to ABMR in their biopsies and also negativity for C4d. Desvauax *et al*.[4] however in their study demonstrated circulating anti donor antibodies (n = 12) and c4d positivity (n = 3) on the allograft biopsies. All these authors approve of an additional humoral response associated with PCAR. Charney *et al*.[3] also discuss a "Th 2 cytokine" pathway in PCAR indicating a humoral response. On similar lines, Xu *et al*. in an analysis of 40 explanted grafts, found that 57.5% of the grafts having CD138 + plasma cells and 32.5% being positive for both; CD138 + plasma cells and diffuse C4d deposits.[14] They thought that plasma cell infiltrate participate in humoral rejection through local secretion of antibodies.

The evidence of ABMR was proved without doubt in our biopsies on the basis of light microscopic features as well as positivity for C4d and DSA. Peculiarly all our patients of PCAR were male unlike that reported by Charney *et al*.[3] and Gupta *et al*.[8] to have slight female predominance.

Furuya *et al*.[9] have most recently described a patient of PCAR with ABMR occurring 1-year posttransplant who responded to antirejection medication. The biopsy features described by them are most similar to that observed in our biopsies.

Late ABMR is being thought as a distinct form of rejection away from chronic ABMR. Chronic ABMR is characterized by transplant glomerulopathy, peritubular capillary basement membrane multilayering, interstitial fibrosis/tubular atrophy, fibrous intimal thickening with C4d deposition, and positive DSA. The term "chronic" is not related to posttransplant duration and thus late AMR can have a phenotype of acute or chronic AMR. Late AMRs are associated with reduction in

immunosuppression/noncompliance, unresponsiveness to treatment and graft loss.[15,16] All our biopsies of PCAR with ABMR are actually late ABMRs. Though not reported earlier with PCAR, three of our patients were found to be noncompliant to the immunosuppression therapy.

DSAs were negative in one patient. The diagnosis of ABMR requires the presence of morphologic features, c4d positivity and also positive DSA.[17] However, it is known that there is no absolute correlation between DSA and AMR or C4d positivity.[18] DSA is detected in only 63% to 90% of cases with C4d positivity.[18] In a recent publication by Larpparisuth *et al*., it was shown that DSA was detected in 25 of the 34 patients with Late Acute ABMR.[19]

The presence of plasma cells in allografts has also been studied with respect to chronic graft dysfunction. Martin *et al*. have reported the presence of plasma cells, diffuse C4d staining of PTC and DSA on serial allograft biopsies of recipients with chronic dysfunction as compared to a control group with normal renal function.[20]

BK virus nephropathy and PTLDs are important considerations with allograft plasma cells. Distinguishing BK nephropathy from acute rejection is of utmost importance due to diverse line of management. PCAR biopsies can show tubular epithelial atypia that can be mistaken for viral cytopathic effects. In addition, BK nephropathy can show overlapping features of rejection like tubulitis and peritubular capillaritis.[21] This can create diagnostic issues. The absence of staining with SV 40 antigen and negative BKV PCR rules out the presence of BKV nephropathy in our biopsies.

Polymorphic PTLDs can show plasma cell infiltrate with expression of Epstein-Barr virus (EBV) RNA.[22] Plasma cells in our biopsies were not atypical, but the presence of EBV RNA could not be established in our biopsies.

The treatment options for ABMR include steroids, plasmapheresis, rituximab, bortezomib and IVIg.[23] ABMRs need to be tackled aggressively using many of these options. PCARs have shown a distinct treatment failure to all these lines of treatment with a poor prognosis in most of the published literature. The same was observed in our series. The other cases of nonplasma cell ACR and ABMR showed better therapeutic response and good graft outcome with similar lines of treatment.

The study has limitations since protocol biopsies are not followed at our center. In most of these patients, earlier allograft biopsies were not done which could have picked up possible subclinical rejections or early appearance of plasma cell infiltrates and other morphologic features.

We report seven patients of PCAR with ABMR diagnosed in late posttransplant period with resistance to the standard antirejection therapy and persistent graft dysfunction with graft loss in two patients.

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How to cite this article: Uppin MS, Gudithi S, Taduri G, Prayaga AK, Raju SB. Expanding the antibody-mediated component of plasma cell-rich acute rejection: A case series. Indian J Nephrol 2016;26:176-81.

Source of Support: Nil, **Conflict of Interest:** None declared.