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Country Data

Re-examination of Renal Allograft Biopsy Blocks for C4d Positivity: Missed Cases of Antibody Mediated Rejection

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Abstract

Introduction: C4d immunostaining of renal allograft biopsies is recommended for the diagnosis of antibody-mediated rejection (ABMR), but it was not available to us pior to June 2012. In June 2012, we were able to obtain anti-human C4d polyclonal antibody and decided to retrospectively evaluate archived kidney allograft biopsies at our center for C4d deposition.

Methods: Twenty-four paraffin blocks were available for this study. Immunostaining for C4d was performed using anti-human C4d polyclonal antibody by Immunohistochemistry. The score and pattern of C4d positivity were determined according to the Banff 2007 guidelines.

Results: All grafts were from living related donors with negative CDC cross-match. The indications for biopsy were primary, acute and chronic graft dysfunction in 29.2%, 33.3% and 37.5% of patients respectively. Two biopsies revealed extensive necrosis rendering it difficult to interpret the result of C4d staining. Among the remaining 22 biopsies, C4d staining was categorized as negative in 40.9%, minimal in 13.6%, focal in 22.7% and diffuse in 22.7%. The prevalence of C4d positivity among biopsies taken due to primary, chronic and acute graft dysfunction was 71.4%, 44.4% and 12.5% respectively.

Conclusion: C4d positivity was common in biopsies taken from this group of kidney transplant recipients and its prevalence was particularly high among biopsies taken due to primary graft non-function. This indicates that missed ABMR is an important cause for kidney allograft dysfunction in our setting.

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Introduction

Renal allograft dysfunction can be caused by a large variety of causes, most of which can only be accurately diagnosed by transplant biopsies. Kidney allograft rejection is a common cause of graft dysfunction and can be mediated by allo-reactive inflammatory cells or allo-specific antibodies. Although antibody-mediated rejection (ABMR) cannot be distinguished from cell-mediated rejection on clinical grounds, it is generally associated with a worse prognosis. It can develop years after kidney transplantation and may be triggered by a decrease in immunosuppression due to poor adherence to therapy. ABMR often doesn't respond to first line anti-rejection therapy. However, effective treatment is now available. In addition, ABMR may not impart significant long-term adverse effects on the grafts if treatment is instituted in a timely manner.

During humoral activation of the classical complement pathway, complement split products including C4d are formed. C4d has the capacity to covalently bind to target molecules on the endothelium of peritubular capillaries (PTCs) and is therefore regarded as a footprint of ABMR [1]. The Banff classification of renal allograft pathology was revised in 2003 and 2007, incorporating morphological criteria and C4d immunostaining for the diagnosis of acute antibody-mediated rejection [2, 3]. Unfortunately, the recommendation that every renal allograft biopsy should be stained for C4d [3] is not always feasible.

Table-1: C4d scoring among the 22 renal allograft biopsies in which C4d scoring was performed

C4d scoring	Number	Percentage
Negative		
Negative (0%)	9	40.9%
Minimal (1-<10%)	3	13.6 %
Positive		
Focal (10-50%)	5	22.7 %
Diffuse (>50%)	5	22.7 %

Ahmed Gasim Cardiac Surgery and Kidney Transplant Center is the largest kidney transplnat center in Khartoum, Sudan. Twenty-five renal allograft biopsies were performed in this center between September 2009 and April 2012. Unfortunately, we had no access to anti-human C4d polyclonal antibody at that time and relied on other histopathological features for the evaluation of renal allograft biopsies. In June 2012, we were able to obtain anti-human C4d polyclonal antibody and decided to retrospectively evaluate those biopsies for C4d deposition.

Methods

Twenty-four paraffin blocks were archived and available for this retrograde analysis. Immunostaining was performed on 4 µm paraffin sections using rabbit anti-human C4d polyclonal antibody (E17341;Spring Bioscience, USA). Slides were deparaffinized and pretreatment was performed as described by the manufacturer. Antigen retrieval was done by boiling tissue sections in preheated 10 mM citrate buffer at pH 6.0 for 10 minutes in water bath at 95oC followed by cooling at room temperature for 20 minutes. Primary antibody was then added to the sections on the slides and the slides were incubated for 30 minutes at room temperature. Slides were rinsed with Phosphate Buffered Saline with Tween 20 between steps. Antibody expression was detected using Anti-Rabbit HRP/DAB visualization system (Spring Bioscience, USA). Normal tonsil tissue sections were used as positive control and intrinsic glomerular staining was used as internal built in control for staining validation.

The percentage of PTC displaying positive C4d staining was estimated after excluding scar or necrotic areas. C4d scores were determined as follows: negative (positive staining of 0% of biopsy), minimal (positive staining of 1 - <10% of biopsy), focal (positive staining of 10-50% of biopsy) and diffuse (positive staining of >50% of biopsy). Negative and minimal C4d staining by immunohistochemistry (IHC) was considered negative while focal and diffuse staining was considered positive [3].

Results

The study included 24 kidney allograft biopsies taken from 24 different individuals, 16 males and 8 females, with mean age of 31 ± 13 years (range 10-53 years). All grafts were from living related donors with negative CDC cross match results. All patients received induction with methylprednisolone and were maintained on tacrolimus, azathioprine and prednisolone. Only one patient, for whom this was the second transplant, received induction with anti-thymocyte globulin. The indications for biopsy were primary graft non-function in seven cases (29.2%), acute deterioration in graft function in eight cases (33.3%) and chronic deterioration in graft function in nine cases (37.5%). All patients received empirical treatment with three pulses of methylprednisolone.

Two biopsies (8.3%) revealed extensive necrosis rendering it difficult to interpret the results of C4d staining. Among the remaining 22 biopsies, 10 biopsies (45.5%) were positive for C4d and 12 biopsies (54.5%) were negative. The details of C4d scoring are shown in Table-1.

Seven biopsies were taken because of primary graft non-function, and five of these biopsies were positive for C4d deposition indicating the likely possibility of missed ABMR (figures 1-3). The clinical and pathological features of patients who suffered from primary graft non-function are presented in Table-2.

Six biopsies were taken during the first six months post transplantation because of acute deterioration in graft function. One of the these biopsies displayed intravascular thrombosis and extensive coagulative necrosis rendering it difficult to interpret the result of C4d staining; this graft was lost. The remaining five biopsies displayed morphological features of acute cellular rejection with negative C4d staining; these five patients responded to treatment with methylprednisolone.

Another two biopsies were taken at seven and eight months post transplantation because of acute-on-chronic deterioration in graft function. Both biospies revealed interstitial fibrosis, tubular atrophy and lymphocytic infiltration with positive C4d staining. Both grafts

Table 2: Histopathological diagnoses of the studied 58 renal biopsy specimens

Num.	Clinical data	Time (days)	Key Morphological Findings	C4d staining pattern
1	10 year-old male, first transplant, donor his father	0	Glomerular inflamation and thrombosis, coagulative necrosis*	Diffuse
2	40 year-old female, first transplant, multiparous, donor her son	0	Glomerular inflamation, coagulative necrosis*	Negative
3	53 year-old male, second transplant, donor his daughter	13	Glomerular thrombosis, intravascular thrombosis, coagulative necrosis	Diffuse
4	34 year-old female, first transplant, multiparous, donor her sister	15	Glomerular thrombosis, coagulative necrosis	Diffuse
5	40 year-old female, first transplant, multiparous, donor her son	20	Acute tubular necrosis, interstitial fibrosis and lymphocytic infiltrate	Focal
6	28 year-old female, first transplant, donor her sister	30	Coagulative necrosis with small abscess formation†	-
7	44 year-old female, first transplant, multiparous, donor her son	70	Glomerular sclerosis, interstitial fibrosis and tubular atrophy	Focal

* Graft nephrectomy was performed on the same day because of extensive thrombosis

† This biopsy displayed extensive necrosis rendering it difficult to interpret the result of C4d staining

failed.Nine biopsies were taken at 1.5 to 10 years post transplantation because of chronic deterioration in graft function. All of these biopsies displayed interstitial fibrosis and tubular atrophy with various degrees of lymphocytic infiltration. All of these biopsies continued to deteriorate and eventualy failed. C4d staining was negative in two biopsies, minimal in three biopsies, focal in three biopsies and diffusely positive in one biopsy (Figure-4).

Discussions

Antibody mediated rejection (ABMR) is increasingly recognized as an important contributor to acute and chronic kidney allograft loss. The diagnosis of both acute and chronic antibody-mediated rejection requires (1) documentation of C4d positivity by immunofluoresence (IF) or immunohistochemistry (IHC), (2) detection of circulating donor specific antibodies and(3) morphologic evidence of tissue injury. In the case of acute antibody mediated rejection (ABMR), morphological changes include acute tubular necrosis-like changes with minimal inflammation, capillary or glomerular inflammation and/or thrombosis, and arteritis. In the case of chronic antibody-mediated rejection, morphological changes include glomerular double contours and/or peritubular capillary basement membrane multi-layering and/or interstitial fibrosis/tubular atrophy and/or fibrous intimal thickening in arteries [3].

The Banff-07 update recommends the routine utilization of C4d staining in all kidney allograft biopsies. Unfortunately, this practice is not always feasible. In this study, we attempted to retrospectively evaluate the prevalence of C4d positivity among archived kidney allograft biopsies in our center.

The high prevalence of C4d positivity in this series, particulary among biopsies taken because of primary graft dysfunction, was not unexpected. These patients were not tested for donor specific antibodies (DSA) and do not fulfill the diagnostic criteria of ABMR. However, the positive C4d staining pattern and consistent morphological features make it highly likely that they lost their grafts because of preformed anti-HLA antibodies in their serum. Given their significant history of sensitization, the cytotoxicity crossmatch results were probably false negatives.

The high prevalence of C4d positivity in biopsies with chronic allograft dysfunction is consistent with the

Figure-1: Histopathology slide of a renal allograft with primary non-function displaying negative staining for C4d; this kidney was removed on the same day because of extensive thrombosis (C4d IHC. Magnification, X 80)

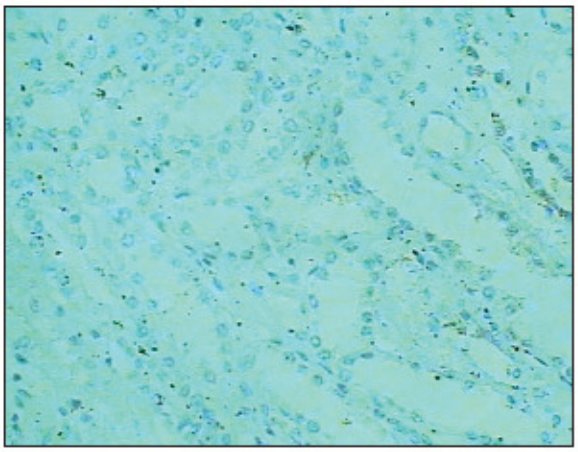
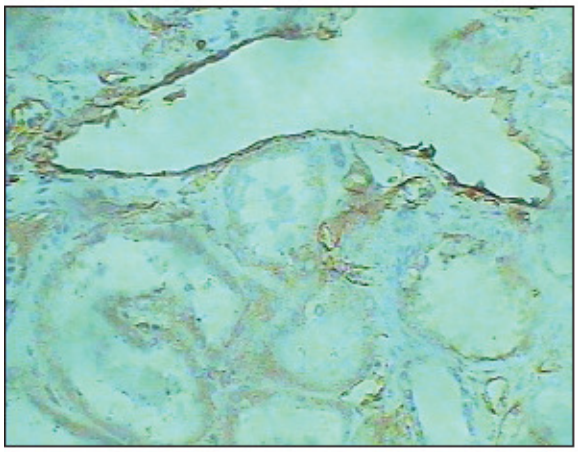


Figure-2: Histopathology slide of a renal allograft with primary non-function revealing extensive necrosis with some discernable peritubular capillaries that display positive staining for C4d (C4d IHC. Magnification, X 80)



recently identified role of humoral components in the causation of chronic allograft rejection. It may also be related to unidentified and untreated humoral component of previous episodes of acute rejection [4].

The modified Banff-07 criteria require more than 50% of PTCs involvement to label the biopsy as diffusely positive for C4d, utilizing both IHC and IF techniques. However, the significance of the focal staining is different between IHC and IF technique and IHC is less sensitive by about one grade level [3]. Focal positive C4d by IHC is possibly equivalent to diffuse positive IF and should be retested on IF when possible [5, 6]. Diffuse positive C4d by IF or IHC is highly correlated with circulating anti-donor antibody. However, for focal positive C4d by IF and for minimal C4d by IHC, the clinical significance is unknown [3]. In our setting, facilities for IF studies are not readily available and IHC is a more practical alternative.

Conclusion

C4d positivity was common in biopsies taken from this group of kidney transplant recipients and its prevalence was particularly high among biopsies taken due to primary graft non-function. This indicates that missed ABMR is an important cause for kidney allograft dysfunction in our setting.

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Figure-3: Histopathology of a renal allograft with primary non-function and severe necrosis; however, there are viable areas displaying diffuse C4d staining of peritubular capillaries (C4d IHC. Magnification, X 80)

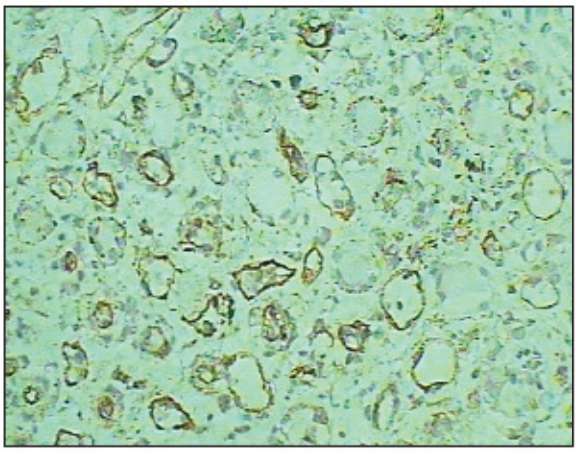
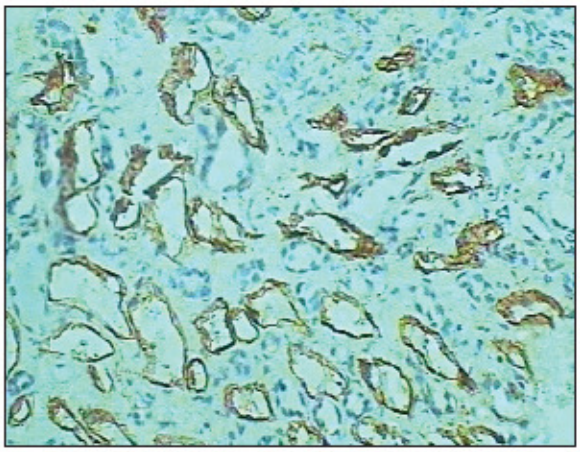


Figure-4: Histopathology of a renal allograft with chronic deterioration in graft function displaying diffuse positive staining for C4d (C4d IHC. Magnification, X 80)



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