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ORIGINAL ARTICLE

Renal allograft biopsies in the era of C4d staining: the need for change in the Banff classification system

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Summary

C4d immunostaining in the peritubular capillaries (PTC) is a marker of antibody-mediated rejection (AMR). We evaluated the histopathologic diagnoses of 388 renal transplant biopsies since the implementation of routine C4d immunostaining at our center. Of these, 155 (40%) biopsies had evidence of acute cellular rejection (ACR), out of which 119 (77%) had pure ACR, 31 (20%) had ACR with concomitant features of AMR, and five (3%) had ACR with focal C4d staining. Sixty-four (16%) biopsies exhibited features of AMR [33 (52%) pure AMR, and 31(48%) concomitant AMR and ACR]. One hundred and fifty-five (40%) biopsies had features of interstitial fibrosis and tubular atrophy (IFTA). Of these, 20 (13%) had concomitant AMR [13 (8.5%) had pure AMR and seven (4.5%) had concomitant ACR and AMR]. Creatinine at the time of biopsy was higher in patients with mixed ACR and AMR and the clinical behavior of mixed lesions is more aggressive over time. Despite having a lower serum creatinine at the time of biopsy, patients with IFTA experienced gradual decline in graft function over time. The pathologic findings in renal allograft biopsies are often mixed and mixed lesions appear to have more aggressive clinical behavior. These findings suggest the need for change in the Banff classification system to better capture the complexity of renal allograft pathologies.

Introduction

Allograft dysfunction remains an important cause of concern in renal transplant recipients. Renal allograft biopsies provide a unique opportunity to examine and understand renal transplant pathologies. The development of C4d immunostaining provides the opportunity to understand the allograft histopathology better. C4d immunostaining in the peritubular capillaries (PTC) when present in the context of tubular injury, capillaritis, or arterial inflammation is diagnostic of antibody-mediated allograft rejection (AMR) [1–3]. However, AMR is not often present in pure form. Beyond its value in the diagnosis of AMR, positive C4d immunostaining in the peritubular capillaries (PTC) has been associated with different renal allograft pathologies including acute cellular rejection (ACR), interstitial fibrosis and tubular atrophy (IFTA), and transplant glomerulopathy (TGP). It also appears that in addition to its value as a diagnostic marker, positive C4d staining in the PTC may have prognostic implications [4,5].

As C4d staining allows better diagnostic delineation of AMR, and noting that a description of renal allograft pathologies since the introduction of this marker has not been undertaken; we sought to examine renal allograft biopsies at our center after the introduction of this marker and assess the adequacy of the current Banff diagnostic criteria in capturing the complexity of the often co-existing pathologies in renal allografts.

Methods

We reviewed the medical records and examined the kidney transplant biopsy specimens performed at Saint Louis University Hospital between May 2002 and March 2007. Data collection sheets were designed to collect pertinent information from medical records. In all the cases included, the pretransplantation screen for cytotoxic antibodies was negative. Nephropathology reports were reviewed and biopsy specimens were examined for each patient. The nephropathology examination included light microscopy, electron microscopy and immunofluorescence staining for Ig A, Ig G, Ig M, C1q, C3, albumin, fibrinogen and C4d. Banff criteria were used for the diagnosis and classification of ACR, AMR, IFTA formerly referred to as chronic allograft nephropathy and TGP [6]. ACR was defined as cortical mononuclear cell interstitial inflammation, tubulitis, and/or arteritis. AMR was diagnosed based on diffuse circumferential C4d staining of peritubular capillaries in renal cortex and evidence of tubular damage. IFTA was defined by the presence of interstitial fibrosis and tubular atrophy and, in severe cases, transplant arteriopathy. TGP was defined as the thickening of the glomerular capillary wall in at least three loops as a result of the widening of the subendothelial space by abnormal basement membrane material, and the formation of a new layer(s) of basal lamina with interposition of mesangial matrix, which gives the characteristic appearance of double contour on light microscopy [7,8]. The diagnosis of TGP was confirmed with characteristic electron microscopy features and absence of immune complexes on immunofluorescence staining. The estimated glomerular filtration rate (eGFR) was calculated according to the four variable formula used in the Modification of Diet in Renal Disease Study [9,10]. The eGFR = $186 \times (\text{plasma creatinine})^{-1.154} \times (\text{age})^{-0.203} \times$ (0.742 if female) $\times$ (1.21 if African–American). The study was approved by the Institutional Review Board at Saint Louis University.

Immunofluorescence microscopy

Biopsy sections were stained with an indirect immunofluorescence technique. Four-micrometer frozen sections were cut and fixed in fresh acetone for 5 min. They were air-dried for 10 min and then added to the Dako autostainer. Sections were washed with TBS and treated with Dako avidin for 10 min, then rinsed in TBS again. Biotin was added for 10 min and then rinsed with TBS. A mouse anti-human C4d antibody (1:100 dilution) (clone 10-11, Biogenesis, Sandown, NH, USA) was added and stained for 30 min and rinsed with TBS. The secondary reagent, biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, CA, USA) antibody was added and stained for 30 min, then rinsed with TBS. The FITC-strepavidin (BioMedia, Foster City, CA, USA) was added for 30 min, then rinsed with TBS. The slides were then taken off the autostainer and auto fluorescence was quenched with Evan's blue for 7 min, then rinsed in TBS. A Dako Faramount aqueous mounting media was used for slide preparation. Biopsies from patients with membranous nephropathy as well as biopsies from patients with previously documented positive C4d in PTC served as positive controls in each biopsy specimen examined.

Detection of anti-donor antibodies

Anti-donor HLA antibody was determined only in patients with positive C4d in PTC using complement-dependant cytotoxicity assay. Both anti-human globulin-enhanced T cell and standard complement-dependent cytotoxic B cell assays were used. Donor T and B cells were isolated with immunomagnetic beads and stored at −70 °C. Serum samples were collected from patients at the time of biopsy. Patients' sera were added to the T- and B-cell trays for 30 min at 37 °C. The trays were then washed three times with 10 μl of PBS and spun in a centrifuge for 1 min at 400 × g. One microliter of AHG was then added to each T-cell tray. After 1 min, 5 μl of complement was added to each well of the trays. The B- and T-cell trays were then incubated for an hour. Ethidium bromide, acridine orange working solution and 2.5% India ink solution was added to the trays and the results were viewed under fluorescence microscopy.

Statistical analysis

Demographic as well as clinical and laboratory data were collected and entered into an electronic database. A multivariate logistic regression model was constructed to examine factors that predict positive C4d immunostaining in PTC. In the multivariate regression model, we adjusted for the following covariates: age, sex, race, hepatitis C status, cause of ESRD, source of renal allograft, and serum creatinine. For all statistical analyses, $P < 0.05$ was considered to be significant. Values are expressed as mean ± standard deviation (SD). The statistical analysis in this study was performed using SPSS 12.0 (SPSS Inc, Chicago, IL, USA).

Results

We identified a total of 388 renal transplant biopsies in 248 patients. All biopsies were performed because of deterioration in renal function and/or proteinuria. The average time of biopsy was 6.8 ± 14.2 months. The demographic and clinical characteristics of the patient population are described in Table 1.

Table 1. Demographic and clinical characteristics of the study population ($n = 248$).

	$n = 248$ (%)
Average age	46 ± 13
Sex	
Female	82 (33)
Male	166 (67)
Race	
White	168 (68)
African–American	72 (29)
Asian	8 (3)
Diabetes mellitus	136 (55)
Hypertension	119 (48)
Hepatitis C	15 (6)
Average creatinine (mg/dl) at the time of biopsy	3.8 ± 2.2
Average estimated GFR (ml/min/1.73 m^2) at the time of biopsy	27.8 ± 14.9
Source of renal allograft	
Deceased donor	164 (66)
Living related donor	79 (32)
Living unrelated donor	5 (2)
Etiology of ESRD	
Diabetic nephropathy	84 (34)
Hypertensive nephrosclerosis	60 (24)
Polycystic kidney disease	25 (10)
Focal segmental glomerulosclerosis	17 (7)
Ig A nephropathy	17 (7)
Membranoproliferative glomerulonephritis	14 (5.5)
Lupus nephritis	8 (3)
Chronic reflux nephropathy	8 (3)
Alport syndrome	7 (3)
Ureteral obstruction	4 (1.5)
Interstitial nephritis	2 (1)
Wegener's granulomatosis	2 (1)
Immunosupressive regimen	
Thymoglobulin induction, CNI, MMF, prednisone	169 (68)
Campath, CNI, MMF	67 (27)
No induction, CNI, prednisone	12 (5)

CNI, calcineurin inhibitors; MMF, mycophenolate.

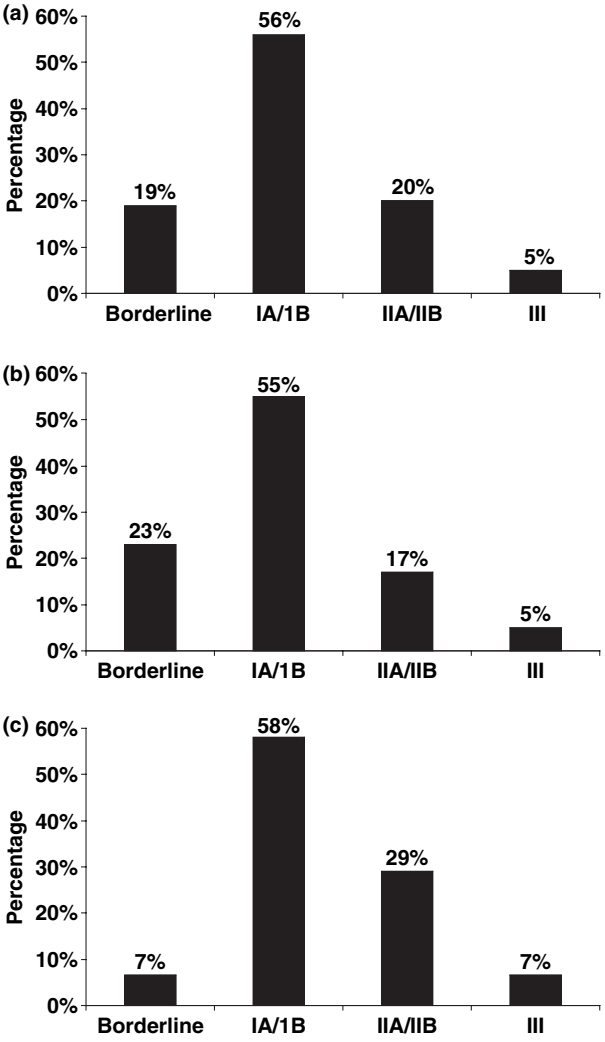

Figure 1 The Banff class distribution of biopsies with acute cellular rejection (ACR). (a) depicts all biopsies with ACR. (b) depicts biopsies with pure ACR. (c) depicts biopsies with mixed ACR and antibody-mediated rejection (AMR).

Of the 388 transplant biopsies examined, 155 (40%) biopsies had evidence of ACR; of which, 30 (19%), 86 (56%), 31(20%), and eight (5%) were borderline, IA/IB, IIA/IIB, and III, respectively (Fig. 1a). We then examined biopsies which exhibited features of pure ACR and found that 119 (77%) of all biopsies with ACR had no concomitant features of humoral rejection (Table 2a). Among biopsies with pure ACR, 27 (23%), 65 (55%), 21 (17%), and six (5%) were borderline, IA/IB, IIA/IIB, and III, respectively (Fig. 1b). 31 (20% of biopsies with ACR) biopsies had concomitant features of ACR and AMR (Table 2a); two (6.5%), 18 (58%), nine (29%), and two (6.5%) were borderline; IA/IB, IIA/IIB, and III, respectively (Fig. 1c). Compared to biopsies with tubulo-interstitial type cellular rejection (Banff borderline or IA/IB), biopsies with evidence of vascular rejection (Banff IIA/IIB, or III) had a higher percentage of C4d positivity in the PTC (of 116 biopsies with ACR (borderline and IA/IB), 20 (17%) had concomitant features of AMR; of 39 biopsies with IIA/IIB or III, 11 (28%) had concomitant C4d in PTC; $P < 0.05$). Five (3%) had ACR with focal C4d staining (Table 2a). When compared to biopsies with pure ACR, there was a lower percentage of Banff borderline and higher percentage of Banff IIA/IIB in biopsies with mixed ACR and AMR (7% vs. 23%, $P < 0.05$ and 29% vs. 17%, $P < 0.05$, respectively). The serum creatinine at the time of biopsy was not different in the patients with pure ACR and those with mixed ACR and AMR.

Sixty-four (16%) biopsies exhibited features of AMR. Thirty-three biopsies had no concomitant features of

Table 2. (a) Relative frequencies of ACR, AMR, IFTA and mixed pathologies in the study. (b) Effect of presence and grade of IFTA on the relative frequencies of AMR and ACR.

(a)	*n* (%)
ACR	155
Pure ACR	119 (77)
Mixed ACR and AMR	31 (20)
ACR with focal C4d	5 (3)
AMR	64
Pure	33 (52)
Mixed ACR and AMR	31 (48)
IFTA	169
Pure IFTA	100 (59)
IFTA + ACR	42 (25)
IFTA + AMR	15 (9)
IFTA + ACR + AMR	9 (5)
IFTA + focal C4d	3 (2)
(b)	
Overall	
ACR	155 (71)
AMR	64 (29)
Excluding IFTA III	
ACR	148 (73)
AMR	55 (27)
Excluding IFTA II, and III	
ACR	125 (73)
AMR	47 (27)
Excluding IFTA I, II, and III	
ACR	102 (72)
AMR	40 (28)

ACR, acute cellular rejection; AMR, antibody-mediated rejection; IFTA, interstitial fibrosis and tubular atrophy.

cellular rejection and 31 biopsies had a mixed AMR and ACR (Table 2a).

One hundred and sixty-nine (44%) biopsies had features of IFTA; 46 (27%), 52 (31%), and 71(42%) were classified as grade I, II and III, respectively (Fig. 2). Of the 169 biopsies with IFTA, 100 (59%) had pure IFTA with no concomitant features of cellular or humoral rejection (Table 2a). Forty-two (25%) had IFTA with features of

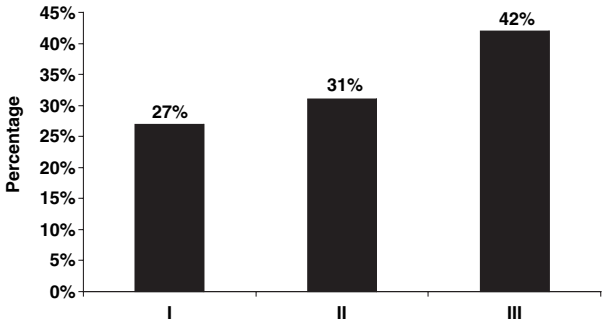

Figure 2 The Banff class distribution of biopsies with interstitial fibrosis and tubular atrophy (IFTA).

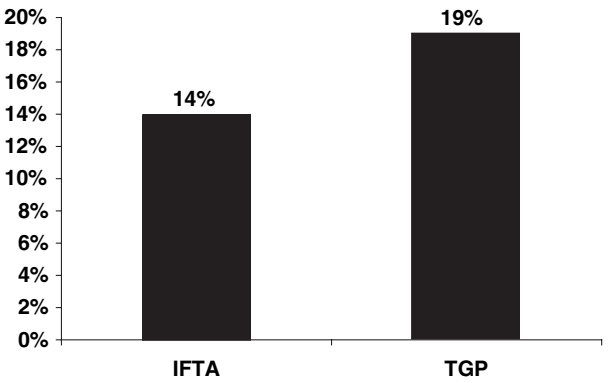

Figure 3 Percentage of C4d-positive biopsies in interstitial fibrosis and tubular atrophy (IFTA) and IFTA with additional features of transplant glomerulopathy (TGP).

ACR, 15 (9%) additional features of AMR, nine (5%) had concomitant ACR and AMR, and 3 (2%) had IFTA with focal C4 staining (Table 2a). Twenty-four (14%) had IFTA with AMR (both pure and mixed) (Fig. 3).

A total of 74 biopsies (19% of all biopsies, 44% of biopsies with IFTA) had additional features of TGP; 14 (19%) of them were associated with AMR (Fig. 3). Twenty-two biopsies had concomitant features of TGP and ACR (15 with TGP and pure ACR, and seven with TGP, ACR, and AMR).

Among biopsies with acute rejection, there were 119 (63%) with pure ACR, 33 (18%) with pure AMR, 31 (16%) with mixed ACR and AMR and five (3%) ACR with focal C4d staining. We then excluded biopsies with different degrees of IFTA and found that neither the presence nor the severity of IFTA affects the relative frequencies of ACR and AMR (Table 2b).

Donor-specific antibody

Donor-specific antibodies (DSA) were examined in patients with positive C4d. Among 64 biopsies with positive C4d, 36 had positive DSA, 18 had negative DSA and data was unavailable on 10 biopsies. Thus, among C4d-positive biopsies with available data on DSA, 67% ($n = 36$) have positive DSA. There was no difference in age, race, and gender distribution between patients with positive and negative DSA.

Predictors of antibody-mediated rejection

We then wanted to examine factors associated with AMR. We initially performed a univariate analysis to examine factors that influence the risk of AMR and found that younger age, female sex, and African–American race, serum creatinine, source of allograft, etiology of ESRD

Table 3. Risk factors associated with antibody-mediated rejection.

	Odds ratio	95% Confidence interval	*P*-value
Age (per 5 years increment)	0.976	0.956–0.966	0.018
Gender			
Male (reference group)	1.0		
Female	1.996	1.1467–3.484	0.015
Race			
White (reference group)	1.0		
African–American	6.533	1.995–21.393	0.002
Etiology of ESRD			
Hypertension (reference group)	1.00		
Diabetes mellitus	3.307	1.405–7.782	0.006
Immune complex-mediated glomerlunephritis	3.667	1.259–13.892	0.046
Creatinine (mg/dl)	0.973	0.762–1.243	0.829
Source of allograft			
Living donor (reference group)	1.0		
Deceased donor	1.265	0.516–3.104	0.608
Hepatitis C	0.554	0.171–1.796	0.325
Immunosupressive regimen			
Thymoglobulin	1.0		
Alemtuzumab	1.432	0.754–1.833	0.765

and hepatitis C seropositivity are associated with increased likelihood of having antibody-mediated allograft rejection.

We then constructed a multivariate regression model to examine independent risk factors associated with AMR and found that a 5-year increment in age is significantly associated with less risk of having antibody-mediated rejection [odds ratio (OR) = 0.976, confidence interval (CI): 0.956–0.966; $P < 0.05$ (Table 3)]. Female sex was associated with an almost a two times risk of developing AMR when compared to that male recipients (OR = 1.966, CI: 1.1467–3.484; $P < 0.05$). African–American race is significantly associated with higher risk of AMR (OR = 6.533; CI: 1.995–21.393; $P < 0.05$). Interestingly, the etiology of ESRD seems to influence the risk of developing AMR. Compared to patients whose etiology for ESRD was listed as hypertension, those patients with DM and those with immune-complex-mediated glomerulonephritis (IgA nephropathy, membranoproliferative glomerlunephritis, lupus nephritis, alport syndrome, or Wegener's granulomatosis) had a higher risk of AMR (OR = 3.307; CI = 1.405–7.782 and OR = 3.667; CI: 1.259–13.892; respectively). Serum creatinine, the source of the allograft (deceased donor or living donor), positive hepatitis C serology and the nature of the immunosuppressive regimen, did not appear to influence the risk of developing AMR significantly (Table 3).

Influence of graft pathology on graft function

Patients with pure ACR and pure AMR had lower baseline serum creatinine than patients with mixed ACR and AMR. Similarly, patients who had mixed rejection had a higher serum creatinine at 6 months and at 1 year than patients with pure form of rejection. Patients with pure IFTA or IFTA with ACR or AMR had on average a lower baseline serum creatinine than patients with pure ACR, pure AMR or mixed rejection ACR and AMR (Table 4). While patients with pure ACR, pure AMR or mixed ACR

Table 4. The effect of renal pathology on graft function.

	n	Creatinine at time of biopsy	Creatinine at 6 months	Creatinine at 1 year
Pure ACR	119	2.9 ± 1.2	1.6 ± 0.5	1.8 ± 0.4
Mixed ACR and AMR	31	3.7 ± 0.9*	2.5 ± 0.7*	2.3 ± 0.9*
Pure AMR	33	3.2 ± 0.8	1.8 ± 1.0	1.7 ± 0.9
Pure IFTA	100	1.9 ± 1.1*	2.2 ± 1.2	2.9 ± 0.9*
IFTA + ACR	42	2.4 ± 0.7*	2.5 ± 0.6	2.8 ± 0.7*
IFTA + AMR	15	2.6 ± 1.1*	2.8 ± 1.2	3.2 ± 1.4*

ACR, acute cellular rejection; AMR, antibody-mediated rejection; IFTA, interstitial fibrosis and tubular atrophy.
*$P < 0.05$.

and AMR experienced a reduction in serum creatinine at 6 months and 1 year, patients with pure IFTA or IFTA with ACR or AMR experienced a progressive increase in creatinine over time.

Discussion

Feucht and collaborators first reported in 1993 on the significance of the capillary deposition of the complement degradation product C4d in renal allografts with early dysfunction [11]. C4d immunostaining of renal allograft biopsies has been increasingly used and was incorporated into the 2003 Banff classification update [12]. The presence of C4d staining in the PTC along with some evidence of tubular injury, capillaritis, or arterial inflammation is diagnostic of AMR. Positive C4d-staining in the PTC, a sine qua non of antibody-mediated response could also be present in the context of cellular rejection, chronic sclerosing nephropathy and TGP, emphasizing the possible antibody-mediated alloresponse contribution to other allograft pathology beyond AMR.

The coexistence of antibody-mediated response and cellular rejection with or without chronic sclerosing changes in a certain allograft biopsy has been described in multiple studies in the past; however, the quantitative relevance of these mixed pathologies in the light of the diagnostic marker C4d has not been examined [2,13–18]. In this study we show that ACR and IFTA are the predominant histolopathologic diagnoses. The majority of biopsies with ACR (77%) and around half of the biopsies with AMR (52%) were pure form of rejections. The remaining 23% of biopsies with ACR and the other 48% of biopsies with AMR have a mixed cellular and humoral alloresponse. Nickeleit and Andreoni recently eloquently articulated the notion that active rejection is a continuum of pathologies with pure antibody-mediated alloresponse on one end, cellular response at another end and in between a spectrum of pathologies with varying degrees of cellular and humoral rejections [19]. In this study we show that a significant percentage of biopsies with cellular rejection and antibody-mediated rejection are indeed mixed. We also show that at any certain points in this continuum, chronic sclerosing rejection is possible. IFTA previously known as chronic allograft nephropathy is present in the context of pure cellular rejection, pure humoral rejection and in mixed rejection.

The implications of the above findings are significant in terms of the effect on risk stratification, therapeutic considerations and prognosis [20–22]. A comprehensive analysis of renal allograft pathology should involve three different dimensions/axes: (i) the presence and the extent of humoral rejection, (ii) the presence and the extent of cellular alloresponse, and (iii) the presence of chronic sclerosing changes. The terms 'acute humoral rejection/antibody-mediated rejection', 'acute cellular rejection', and 'chronic humoral rejection' represent an oversimplification and are not fully adequate. Each entity is not often present in a pure form but rather in association with other pathologies and a comprehensive pathologic diagnosis should incorporate all the pathologic findings in a certain biopsy specimen. Studies have shown that 'mixed' forms of rejection require a different approach to treatment [20,23]. It is also important to note that the coexistence of ACR or AMR with IFTA significantly influence prognosis and may affect therapeutic choices [19]. As recently articulated by Nickeleit and Andreoni, classification systems and our nomenclature are 'dynamic makeshift constructs' that among other things are heavily influenced by 'experience, technology and knowledge' and that these constructs 'heavily influence therapeutic strategies at the individual patient level as well as outcome analyses of multi-center drug trials'; it is therefore important to consider incorporating a multidimensional approach in the classification of rejection to pave the way for the implementation of studies that would make the distinction between these different types of rejections and devise tailored therapeutic interventions for each type of rejection.

Our results suggest that C4d staining in PTC in patients with IFTA and TGP is uncommon [14,24]. The significance of this complement-split product in the pathobiology of IFTA and TGP is not clear. The entity called IFTA may not be one homogeneous disease. The morphologic features characteristic of IFTA could represent different mechanisms of glomerular and tubular injury. Subclassification of IFTA into two subcategories: those with negative C4d and those with positive C4d (recently referred to as chronic humoral rejection) represent a step forward to a better classification system. However, a better characterization of the natural history of these subcategories and of its pathobiology (or pathobiologies) is needed in order to further elucidate the nature of this disease and hopefully devise interventions to halt or reverse its progression [25].

In this study, a total of nine biopsies exhibited focal C4d staining. The significance of focal C4d staining is becoming increasingly clear as some investigators suggested that the histopathologic findings and clinical course in patients with focal PTC C4d staining are similar to those associated with diffuse C4d staining [26].

In this study, 67% of C4d-positive biopsies were associated with presence of DSAs. The reasons for negative DSAs in the remaining patients with positive C4d biopsies are unclear but could be probably related to subthreshold levels of DSA, the sensitivity of the assay used to detect DSA in our center, non-HLA antibodies or the

adsorption of the antibody by the allograft in some cases [20,27–32].

We also show that among patients undergoing a renal allograft biopsy younger age, African–American race, and female gender are associated with increased risk of humoral rejection. Furthermore, we show that the etiology of ESRD is important in risk stratification with regard to acute humoral rejection and that those patients with diabetes mellitus or immune-complex-mediated glomerulonephritis have a higher risk of acute humoral rejection. Interestingly hepatitis C seropositivity, which has been shown in some studies to be a risk factor for AMR, was not associated with increased risk of AMR in the current studies [33]. This is most probably on account of the small number of patients with hepatitis C in the current studies.

We also show in this study that baseline creatinine is higher in patients who have mixed ACR and AMR and that the clinical behavior of mixed lesions is more aggressive over time. We also show that despite having a lower baseline serum creatinine, patients with IFTA experience gradual decline in graft function over time. These findings highlight the need for a multidimensional classification system that allows better diagnostic delineations of the different facets or renal allograft pathologies, and for specifically tailored therapeutic interventions to ameliorate outcomes in this setting.

Finally, our study is mostly descriptive and attempts to shed light on the landscape of allograft nephropathology in the era of C4d staining in the hope that it partially elucidates the interplay between antibody-mediated, cellular and chronic sclerosing type of rejections and provide the underpinning for a multidimensional classification system that takes into account the multiple facets of renal allograft pathologies.

Authorship

ZA-A: Designed the study, analyzed the data and wrote the manuscript. VR: Collected data. AM: Collected data. GB: collected data. CMC: Analyzed the biopsies. LS-M: Analyzed the biopsies. BB: Conceived the idea, and designed the study.

References

1. Racusen LC, Colvin RB, Solez K, *et al.* Antibody-mediated rejection criteria – an addition to the Banff 97 classification of renal allograft rejection. *Am J Transplant* 2003; **3**: 708.
2. Takemoto SK, Zeevi A, Feng S, *et al.* National conference to assess antibody-mediated rejection in solid organ transplantation. *Am J Transplant* 2004; **4**: 1033.
3. Montgomery RA, Hardy MA, Jordan SC, *et al.* Consensus opinion from the antibody working group on the diagnosis, reporting, and risk assessment for antibody-mediated rejection and desensitization protocols. *Transplantation* 2004; **78**: 181.
4. Jianghua C, Wenqing X, Huiping W, Juan J, Jianyong W, Qiang H. C4d as a significant predictor for humoral rejection in renal allografts. *Clin Transplant* 2005; **19**: 785.
5. Kawamura N, Tomita M, Hasegawa M, *et al.* Complement C4d deposition in transplanted kidneys: preliminary report on long-term graft survival. *Clin Transplant* 2005; **19**(Suppl. 14): 27.
6. Solez K, Colvin RB, Racusen LC, *et al.* Banff '05 Meeting Report: differential diagnosis of chronic allograft injury and elimination of chronic allograft nephropathy ('CAN'). *Am J Transplant* 2007; **7**: 518.
7. Ivanyi B, Fahmy H, Brown H, Szenohradszky P, Halloran PF, Solez K. Peritubular capillaries in chronic renal allograft rejection: a quantitative ultrastructural study. *Hum Pathol* 2000; **31**: 1129.
8. Ivanyi B. Transplant capillaropathy and transplant glomerulopathy: ultrastructural markers of chronic renal allograft rejection. *Nephrol Dial Transplant* 2003; **18**: 655.
9. Lin J, Knight EL, Hogan ML, Singh AK. A comparison of prediction equations for estimating glomerular filtration rate in adults without kidney disease. *J Am Soc Nephrol* 2003; **14**: 2573.
10. Levey AS, Kusek JW, Beck GJ, MDRD Study Group. A simplified equation to predict glomerular filtration rate from serum creatinine. *J Am Soc Nephrol* 2000; **11**: 155A.
11. Feucht HE, Schneeberger H, Hillebrand G, *et al.* Capillary deposition of C4d complement fragment and early renal graft loss. *Kidney Int* 1993; **43**: 1333.
12. Racusen LC, Halloran PF, Solez K. Banff 2003 meeting report: new diagnostic insights and standards. *Am J Transplant* 2004; **4**: 1562.
13. Herzenberg AM, Gill JS, Djurdjev O, Magil AB. C4d deposition in acute rejection: an independent long-term prognostic factor. *J Am Soc Nephrol* 2002; **13**: 234.
14. Mauiyyedi S, Pelle PD, Saidman S, *et al.* Chronic humoral rejection: identification of antibody-mediated chronic renal allograft rejection by C4d deposits in peritubular capillaries. *J Am Soc Nephrol* 2001; **12**: 574.
15. Al Aly Z, Yalamanchili P, Cortese C, Salinas-Madrigal L, Bastani B. C4d peritubular capillary staining in chronic allograft nephropathy and transplant glomerulopathy: an uncommon finding. *Transpl Int* 2005; **18**: 800.
16. Nickeleit V, Mihatsch MJ. Kidney transplants, antibodies and rejection: is C4d a magic marker? *Nephrol Dial Transplant* 2003; **18**: 2232.
17. Nickeleit V, Zeiler M, Gudat F, Thiel G, Mihatsch MJ. Detection of the complement degradation product C4d in renal allografts: diagnostic and therapeutic implications. *J Am Soc Nephrol* 2002; **13**: 242.

18. Kayler LK, Farber JL, Colombe B, LaCava D, Friedewald JJ, Ratner LE. Characterization of rejection episodes in patients following positive crossmatch and ABO-incompatible live donor renal transplantation. *Transpl Int* 2006; **19**: 128.
19. Nickeleit V, Andreoni K. The classification and treatment of antibody-mediated renal allograft injury: where do we stand? *Kidney Int* 2007; **71**: 7.
20. Sun Q, Liu ZH, Cheng Z, *et al.* Treatment of early mixed cellular and humoral renal allograft rejection with tacrolimus and mycophenolate mofetil. *Kidney Int* 2007; **71**: 24.
21. Sureshkumar KK, Hussain SM, Carpenter BJ, Sandroni SE, Marcus RJ. Antibody-mediated rejection following renal transplantation. *Expert Opin Pharmacother* 2007; **8**: 913.
22. Magee CC. Transplantation across previously incompatible immunological barriers. *Transpl Int* 2006; **19**: 87.
23. Becker YT, Samaniego-Picota M, Sollinger HW. The emerging role of rituximab in organ transplantation. *Transpl Int* 2006; **19**: 621.
24. Racusen LC, Solez K, Colvin R. Fibrosis and atrophy in the renal allograft: interim report and new directions. *Am J Transplant* 2002; **2**: 203.
25. Worthington JE, McEwen A, McWilliam LJ, Picton ML, Martin S. Association between C4d staining in renal transplant biopsies, production of donor-specific HLA antibodies, and graft outcome. *Transplantation* 2007; **83**: 398.
26. Magil AB, Tinckam KJ. Focal peritubular capillary C4d deposition in acute rejection. *Nephrol Dial Transplant* 2006; **21**: 1382.
27. Bohmig GA, Exner M, Watschinger B, *et al.* C4d deposits in renal allografts are associated with inferior graft outcome. *Transplant Proc* 2001; **33**: 1151.
28. Karpinski M, Rush D, Jeffery J, *et al.* Flow cytometric crossmatching in primary renal transplant recipients with a negative anti-human globulin enhanced cytotoxicity crossmatch. *J Am Soc Nephrol* 2001; **12**: 2807.
29. Mauiyyedi S, Crespo M, Collins AB, *et al.* Acute humoral rejection in kidney transplantation: II. Morphology, immunopathology, and pathologic classification. *J Am Soc Nephrol* 2002; **13**: 779.
30. Martin L, Guignier F, Mousson C, Rageot D, Justrabo E, Rifle G. Detection of donor-specific anti-HLA antibodies with flow cytometry in eluates and sera from renal transplant recipients with chronic allograft nephropathy. *Transplantation* 2003; **76**: 395.
31. Thiel S, Vorup-Jensen T, Stover CM, *et al.* A second serine protease associated with mannan-binding lectin that activates complement. *Nature* 1997; **386**: 506.
32. Dragun D, Muller DN, Brasen JH, *et al.* Angiotensin II type 1-receptor activating antibodies in renal-allograft rejection. *N Engl J Med* 2005; **352**: 558.
33. Forman JP, Tolkoff-Rubin N, Pascual M, Lin J. Hepatitis C, acute humoral rejection, and renal allograft survival. *J Am Soc Nephrol* 2004; **15**: 3249.

