Positive C4d in Kidney Transplantation Biopsy: Clinical Impact

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ABSTRACT

Background. Deposition of C4d in peritubular capillaries of renal graft is normally associated with the presence of antibody-mediated rejection. The clinical impact of its presence in patients with renal transplant in Colombia is uncertain, as well as the association in acute rejection and the response to the management and survival of the graft. The aim of this study was to determine the risk of having positive C4d in biopsies of patients with episodes of acute cellular rejection.

Methods. We retrospectively reviewed 226 biopsies of kidney transplantation, all of them with acute rejection and histopathological findings classified according to Banff criteria 2009 and performed between January 2005 and December 2012 for graft dysfunction. C4d staining was performed by immunohistochemistry.

Results. C4d staining was positive in 25 of 226 biopsies. Rejection time in patients with positive C4d was 15 months in average vs 8 months with negative C4d.

Conclusions. With the use of a multivariate analysis, we found that the unique risk for C4d in our population was the positive panel reactive antibodies and elapsed time between transplant and the rejection (odds ratio: 2.12, $P = .034$) and that the other variables analyzed are not related to the expression of C4d.

THE APPEARANCE of antibody-mediated rejection has a large variability in the literature, with a rate of less than 5% for non-sensitized patients and higher levels in sensitized patients (between 40% and 90%) [1]. In the same way, the rate of graft loss varies between 27% and 63% per year, compared with only 10% of the biopsies, which does not express the C4d [2,3].

Humoral rejections can have a cellular component in up to 30% of the cases, mainly in patients with high positivity of panel reactive antibodies (PRA). The presence of C4d is associated with the increase of creatinine and steroid resistance, which increases the risk of graft failure and loss [4,5].

In 1968, Patel and Terasaki [6] described for the first time the syndrome caused by antibodies, which is actually recognized as "hyperacute rejection." In 1990, Halloran et al discovered anti-donor antibodies as the cause of severe dysfunction of the graft; in 1991, Feucht first described the pericapillar deposits of C4d in biopsies taken from highly sensitized patients, which suggests the presence of a humoral component in rejection [7–9].

Humoral rejection occurs when alloantibodies join endothelial alloantigens, activating the complement cascade and inducing the capillary injury. Most alloantibodies are directed against molecules of the major histocompatibility complex. With the development of immunopathology and the staining of C4d, a big improvement in the diagnosis of humoral rejection has been achieved [2].

C4d is a well-characterized degradation product of the classic complement pathway. After C4 activation two molecules are produced; C4d and another of bigger size estructure called C4c. The first one bounds to the endothelium and the basal membranes surfaces, by means a thioester, working as an immunological print of the complement activation, the second molecule remains soluble [4,7,10,11].

In the West, the incidence of positive C4d varies according to studies from 14% to 60% of the biopsies taken from dysfunctional grafts [4]; however, the prevalence in Asia is too low in few reported studies [12].

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Sut et al describe that the presence of positive C4d in biopsies with early acute rejection episodes less than 14 days have a better prognosis and graft survival than in later ones [4]. The higher prevalence of C4d has been observed in centers that make HLA transplants—or with ABO incompatibility reaching up to 80% of the protocol biopsies [12,13].

It is important to point out that some studies describe that C4d deposits in the absence of symptoms or changes of histological rejection can be a common discovery in grafts with ABO incompatibility [14]. Nevertheless, there are studies such as the one from Collins et al [15], in which they describe some cases of C4d deposits in patients with identical HLA that were performed before the use of CNI-based immunosuppression.

Among the reports of literature, the clinical features related to the positivity of C4d are variable. Within the described features are a previous transplant, elevated PRA, positive cross-match before the transplant, and female sex [12,16]. On the other hand, there are studies such as the one by Cheunsuchon et al [12] in which no relation with the donor's age, type (living or deceased), cold ischemia, and mismatch was found [12].

The morphologic evidence of acute or chronic tissue injury, associated to staining for C4d in peritubular capillaries and presence of donor specific antibodies (DSA) are diagnosis of antibody mediated rejection in renal allograft. The biopsy will show different levels of tissue damage such as, neutrophil infiltration, mononuclear cells in the capillaries or glomerulus, transmural arterial inflammation, fibrinoid necrosis, and capillary or glomerular thrombosis [1,6].

The presence of the humoral rejection must be suspected before the evidence of pericapillar C4d associated with the following tissue changes: acute tubular necrosis with minimal inflammation, glomerulonephritis with polymorph nuclear and monocites or macrophages in the peritubular capillaries, and transmural arterial inflammation or fibrinoid changes [17].

In reported studies, we found different techniques to detect C4d in the peritubular capillaries, among those are the use of immunofluorescence and the immunohistochemistry with monoclonal or polyclonal antibodies, by means of paraffin or frozen sections, which have a sensibility between 65% to 85% and a specificity of 90%, depending on the technique [6,18].

Among the different therapeutic options, we found the use of some methods or a combination of them such as plasmapheresis, immunoabsorption, immunoglobulin application, and profound immunosuppression with tacrolimus and mycophenolate mofetil, with or without depleted antibodies of T cells. Resistant treatments have increased the use of Rituximab [2].

Some researchers have found 30–40% of steroid therapy resistance in C4d positive biopsies [19].

C4d deposit in the first post-transplant year is a poor predictor for long-term results, but previous studies have shown different results [20].

MATERIALS AND METHODS

Two hundred twenty-six biopsies with proven acute rejection were performed between January 2008 and December 2012, and C4d was performed in all of them. We define a significant increase of creatinine as 20% over the basal. The demographic and clinical aspects were collected in a database. Based on the patient immunological risk, some patients recived Campth or Thymoglobulin anti- thymocyte-GAT (high risk) or basiliximab (low risk) as induction therapy. Steroids, CNI (Cyclosporin-Tacrolimus) and Mycophenolate was used as maintenance immunosuppressive therapy. Acute rejection was treated with steroids and thymoglobulin for steroids resistant cases. One pathologist reviewed and classified the biopsies according to the Banff score. In cases of multiple biopsies, the biopsy that expressed the C4d was considered.

Statistical Analysis

Measures of central tendency and dispersion in quantitative variables were used for individual features description of the subjects with positive and negative C4d and the variables in general. Previous confirmation of the normality in its distribution through a Shapiro-Wilk test was performed. In the case of infringement of that assumption, they were described by median and interquartile ranges. The qualitative variables were measured and analyzed by proportions. Analysis of variance was used for comparison between groups, a one-way test as reference of means when data were distributed normally, or as a default in non-parametric statistical test (Wilcoxon test). We used the Z test in qualitative variables to differentiate the proportions for the comparison between groups; in polithomic categorical variables, we used a χ^2 test of independence or a Fisher exact test when the expected values in the cells were <5.

The association between the factors studied and the presence of C4D + was estimated by odds ratio (OR) in a crude estimate and a multivariate logistic regression model unconditioned, adjusted for confounding variables and interaction. For the selection of variables was used the stepwise regression technique with a 0.15 probability of entry (15%) and output of 0.25 (25%). The reliability of each of the generated models was assessed using the deviance and the Hosmer–Lemeshov test.

A confidence level of 95% and a significance level of the 5% were used for the entire statistical test. STATA software (Version 10 SE; Stata Corporation, College Station, Texas) was used for statistical analysis.

RESULTS

Characteristics of Colombian Patients With Kidney Transplant Rejection

A total of 266 subjects were included in the study, 25 with C4d+ and 201 with C4d− for a relation case-control of 1:8.04. Table 1 reports the characteristics of each group, such as the statistical differences found among them. Figure 1 shows the distribution of the mismatch between both groups.

There were no meaningful differences between groups regarding age and sex or in transfusion and retransplant records. The time they were in dialysis before the transplant, type of donor, mismatch score, and the presence of expanded criteria and creatinine levels at the moment of acute rejection were similar between groups; however, meaningful statistical differences were demonstrated in relation to the end stage renal disease (ESRD) etiology and the presence of positive PRA.

Table 1. Characteristics of Subjects With Kidney Transplant Rejection

Characteristic	Group C4d+ (n = 25)	Group C4d− (n = 201)	*P*
Personal characteristics			
Age (years)			
Average, SD	36.0, 15.7	41.96, 13.5	.055*
Median	43	43	
IR	31, 53	31, 53	
Sex			
% Women (n, %)	52.0%, 13	35.32%, 71	.103†
Medical history			
ESRD etiology (n, %)			
Congenital	5, 20%	12, 5.97%	.03‡
Unknown	6, 24%	65, 32.34%	
Diabetes	4, 16%	18, 8.96%	
Glomerular	2, 8%	30, 14.93%	
Hypertension	3, 12%	55, 27.36%	
SLE	4, 16%	6, 2.99%	
Polycystic	0, 0%	10, 4.98%	
HUS	1, 4%	0, 0.0%	
Urolithiasis	0, 0%	5, 2.49%	
Transfusion record			
Yes (n, %)	15, 60.0%	93, 46.27%	.194†
Retransplantation			
Yes (n, %)	1, 4.0%	7, 3.48%	.895†
Dialysis time (months)			
Average, SD	33.08, 39.33	39.03, 34.2	.193*
Median	18	32	
IR	13, 38	15, 52	
Type of donor (n, %)			
Deceased	21, 84.0%	157, 78.11%	.497†
Positive PRA (n, %)	7, 28.0%	25, 12.44%	.035†
Mismatch (n, %)			
0	1, 4.0%	1, 0.5%	.169‡
1	1, 4.0%	9, 4.48%	
2	2, 8.0%	32, 15.92%	
3	9, 36%	77, 38.31%	
4	9, 36%	37, 18.41%	
5	3, 12%	30, 14.93%	
6	0, 0%	15, 7.46%	
Expanded criteria			
Yes (n, %)	3, 12%	16, 7.96%	.492†
Creatinine at the moment of acute rejection (mg/dL)			
Average, DE	3.90, 2.32	3.81, 3.04	.278*
Median	3.0	2.49	
RI	2.3, 5.3	1.86, 4.11	

Abbreviations: SD, standard deviation; IR, interquartile range; ESRD, End Stage Renal Disease; SLE, Systemic Lupus Erythematous; HUS, Hemolitic uremic syndrome.
*Differences calculated by means of Wilcoxon test.
†Differences calculated by means of *Z* test of proportion difference.
‡Differences calculated by means of Fisher exact test.

In the group of patients with C4d+, unknown etiology was more frequent, with 24% of the cases, followed by congenital with 20%; in subjects with C4d−, although unknown etiology was found more often, this was presented in greater quantity compared with subjects with C4d+ (32.34%), and the second more frequent cause corresponds to hypertension and not to congenital causes. The distribution of the other ESRD causes is shown on Table 1.

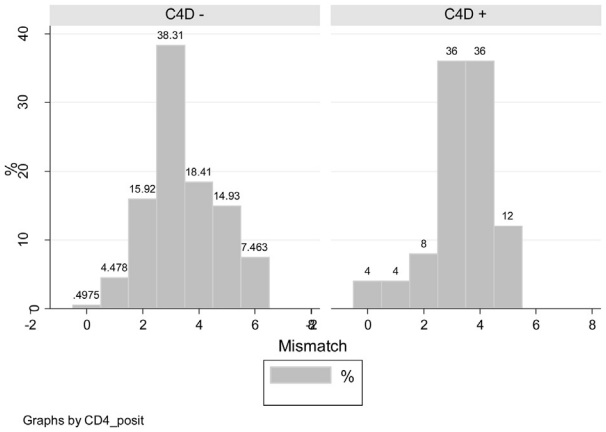

Fig 1. Mismatch distribution depending on presence of C4d.

The presence of positive PRA was meaningfully higher in the group of patients with C4d+ compared with C4d−.

Regarding clinical characteristics of presented rejections (see Table 2), type of rejection was statistically different between both groups. In the group with C4d+, the more frequent rejection type corresponds to humoral IB followed by humoral IA, whereas in patients with C4d− the IA followed by the IB was more frequent. Elapsed time from transplantation to rejection was meaningfully higher in patients with C4d+, with an average difference of 5.67 months compared with subjects with C4d−. The treatment with CSTR was statistically higher in the group with C4d−, and treatment response was meaningfully better in this same group.

The use of maintenance medications was equally different between both groups. MMF-CSA was used more frequently in patients with C4d− and MMF-TACRO in patients with C4d+.

No meaningful statistical differences were found between groups regarding induction medication received and mortality. Table 3 describes cause of death recorded in patients with positive and negative C4d.

Factors Associated With the Presence of C4d+ in Colombian Patients With Rejection of Kidney Transplant

Two conditions were found associated as risk factors with the presence of C4d+ in patients with rejection of renal transplant: the presence of positive PRA and elapsed time from transplant to rejection over 3 months (see Table 4).

The presence of positive PRA was 2.73 times more frequent between the patients with C4d+ compared with those with C4d−, an association that was sustained after controlling the confusion and interaction variables (OR: 2.12, $P = .034$). Time from the transplant to the moment of rejection ≥3 months was 3.3 times more frequent in subjects with C4d+; estimation reaches 6.45 in a multi-variable model, with these results statistically significant.

No other risk factor for the expression of C4d+ was found.

Table 2. Clinical Characteristics of Presented Rejections

Characteristic	Group C4d+ (n = 25)	Group C4d− (n = 201)	P
Type of rejection (n, %)			
Humoral	2, 8.0%	0, 0.0%	.000*
IA	0, 0.0%	95, 47.26%	
Humoral IA	6, 24.0%	0, 0.0%	
IB	0, 0.0%	76, 37.81%	
Humoral IB	12, 48.0%	0, 0.0%	
IIA	0, 0.0%	11, 5.47%	
Humoral IIA	4, 16.0%	0, 0.0%	
IIB	0, 0.0	1, 0.5%	
III	0, 0.0%	4, 1.99%	
Humoral IIIB	1, 4.0%	0, 0.0%	
Borderline	0, 0.0%	14, 6.97%	
Transplant time – rejection (months)			
Average, SD	14.56–16.96	8.89–8.70	.0055*
Median	8.0	3.0	
IR	3.0–18.0	1.0–8.5	
Induction medication (n, %)			
GAT	8, 32%	38, 18.31%	.166*
Campath	17, 68%	149, 74.13%	
Basiliximab	0, 0.0%	14, 6.97%	
Received treatment (n, %)			
CSTR	21, 84.0%	195, 97.01%	.001‡
CSTR + GAT	0, 0.0%	5, 2.49%	
GAT	1, 4.0%	1, 0.5%	
Not received	3, 12.0%	0, 0.0%	
Treatment response			
Yes (n, %)	15, 60.0%	177, 88.06%	.0002†
Maintenance medication‡ (n, %)			
MMF	2, 8.0%	8, 3.98%	.024‡
MMF, CSA	7, 28%	71, 35.32%	
MMF, EVERO	0, 0.0%	1, 0.5%	
MMF, TACRO	12, 48%	49, 24.38%	
MPA	3, 12%	12, 5.97%	
MPA, CSA	1, 4.0%	48, 23.88%	
MPA, TACRO	0, 0.05	12, 5.97%	
Mortality			
Yes (n, %)	2, 8.0%	19, 9.45%	.813†

Abbreviations: SD, standard deviation; GAT, thymoglobulin anti-thymocyte; IR, interquartile range; CSTR, corticosteroid; MMR, Mycophenolate; CSA, Cyclosporine; Evero, Everolymus; Tacro, Tacrolimus.
*Differences calculated by means of Wilcoxon test.
†Differences calculated by means of *Z* test of proportion difference.
‡Measure at the moment of the rejection.

Induction Medication and Elapsed Time to Transplant Rejection

To establish the probability of not having a kidney rejection, according to the three induction medications used (Basiliximab, Campath, and GAT), time was measured in months from the moment of transplant to the rejection moment, and it was calculated by means of Kaplan-Meier estimators. Table 5 reports survival functions for each one of the three medications.

The probability of not having rejection at the month of performing the kidney transplant was 28.5% in patients treated with Basiliximab compared with 87.2% with Campath and 26% with GAT in the same elapsed time. After 15 months, these probabilities decreased to 0.0%, 20.12%, and 1.35%, respectively. The last patient to have rejection with Basiliximab had it after 12 months from transplant and after 60 with Campath and 63 with GAT. Figure 2 shows survival functions throughout the time for the three induction medications.

Patients who had induction with Campath showed better probabilities of not presenting rejection to the kidney transplant, compared with those with Basiliximab and GAT in almost every month after transplant, with a meaningful statistical difference ($P = .000$ by means of log-rank and Wilcoxon tests).

Table 3. Cause of Death in Colombian Patients With Rejection of Kidney Transplant

Characteristic	Group C4d+ (n = 25)	Group C4d− (n = 201)	P
Decease causes (n, %)			
Cryptococcosis	1, 50%	0, 0.0%	.186
AMI	1, 50%	2, 10.53%	
Meningitis	0, 0.0%	1, 5.26%	
Pneumonia	0, 0.0%	4, 21.05%	
Tuberculosis	0, 0.0%	1, 5.26%	
PTE	0, 0.0%	2, 10.53%	
CMV colitis	0, 0.0%	1, 5.26%	
GIT infection	0, 0.0%	1, 5.26%	
Unknown	0, 0.0%	7, 36.84%	

Abbreviations: AMI, acute myocardial infarction; PTE, pulmonary thromboembolism; CMV, cytomegalovirus; GIT, gastrointestinal tract.

DISCUSSION

In the analysis of the different variables studied, we found that there are no meaningful differences between both groups related to sex, transfusion records, retransplant, donor type, or expanded criteria.

In cases where we diagnose a Banff 1B rejection, we found an increased association between C4d positive biopsies and acute cellular rejection. We found that C4d-positive patients received maintenance immunosuppression with mychophenolate and tacrolimus, which corresponds to patients with higher immunological risk.

Treatment response with corticosteroids in the group of negative C4d is 88% versus 60% of those that express C4d in the biopsy. This fact has statistical significance ($P < .0002$).

There was no meaningful difference in mortality between the two groups, considering that in some cases there is no possible treatment. The leading cause of death of those patients is infection that is mainly present after the administration of steroid pulses, which generates an over-immunosuppression that can favor the infection episodes.

The only factors associated with the presence of C4d in the biopsies made were the positivity of PRA, taking as positive any value of PRA; another variable related is elapsed time after transplant >3 months.

Administration of Campath as induction therapy showed a lower risk of C4d postive rejection.

The results of this study demonstrate that in our population, we only have as a risk factor for the expression of the

Table 4. Factors Associated With the Presence of C4d+ in Colombian Patients With Rejection of Renal Transplant

		Raw Model				Adjusted Model		
		IC 95%				IC 95%		
Predictor	OR*	LL	HL	*P*	OR	LL	HL	*P*
Age ≤18 years 0 ≥60 years								
No	1	Reference			1	Reference		–
Yes	2.22	0.81	6.09	.121	2.67†	0.90	7.85	.121
Sex								
Male	1	Reference		–	1	Reference		–
Female	1.98	0.85	4.57	.108	1.43‡	0.57	3.59	.445
Transfusion records								
No	1	Reference		–	1	Reference		–
Yes	1.74	0.74	4.06	.199	1.50‡	0.61	3.69	.372
Dialysis ≥60 months								
No	1	Reference		–	1	Reference		–
Yes	0.50	0.14	1.75	.280	0.427	0.111	1.645	.217
PRA								
Negatives	1	Reference		–	1	Reference		–
Positives	2.73	1.03	7.21	.041	2.12‡	1.10	12.59	.034
Retransplant records								
No	1	Reference		–	1	Reference		–
Yes	1.15	0.13	9.79	.895	0.84‡	0.07	9.42	.893
Type of donor								
Living	1	Reference		–	1	Reference		–
Deceased	1.47	0.47	4.51	.499	1.77†,*	0.55	5.68	.333
Presence of expanded criteria								
No	1	Reference		–	1	Reference		–
Yes	1.57	0.42	5.84	.496	3.55†,*	0.74	16.8	.111
Elapsed time from transplant to rejection ≥3 months								
No	1	Reference		–	1	Reference		–
Yes	3.30	1.19	9.16	.021	6.45†,*	1.84	22.58	.004
Induction medication								
GAT	1	Reference		–	1	Reference		–
Basiliximab	0.65	0.23	1.36	.19	0.259	0.06	1.11	.070
Campath	0.541	0.21	1.35	.188	0.35	0.03	1.12	.21

Abbreviations: OR, odds ratio; PRA, panel reactive antibodies; GAT, thymoglobulin anti-thymocyte; LL, Low limit; HL, High limit.
*Calculated by means of logistic regression model.
†Adjusted by sex, transfusion and retransplant background, dialysis time, donor type, positive PRA, creatinine at the moment of the transplant, and induction with Basiliximab.
‡Adjusted by age, transfusion and retransplant background, dialysis time, donor type, positive PRA, creatinine levels at the moment of the transplant, and presence of expanded criteria. In each case, it was eliminated from the model co-variable; the variable was used as predictor to avoid collinearity.

Table 5. Probability of Not Having Kidney Rejection According to Three Induction Medications

	Survival Functions*		
Time (months)	Basiliximab (n = 14)	Campath (n = 166)	GAT (n = 46)
1	0.2857	0.8720	0.2609
8	0.0714	0.3476	0.0652
15	0.0000	0.2012	0.0435
22	0.0000	0.1341	0.0217
29	0.0000	0.0732	0.0217
36	0.0000	0.0183	0.0217
43	0.0000	0.0061	0.0217
50	0.0000	0.0061	0.0217
57	0.0000	0.0061	0.0217
64	0.0000	0.0000	0.0000

Abbreviation: GAT, thymoglobulin anti-thymocyte.
*Calculated by means of the Kaplan-Meier method.

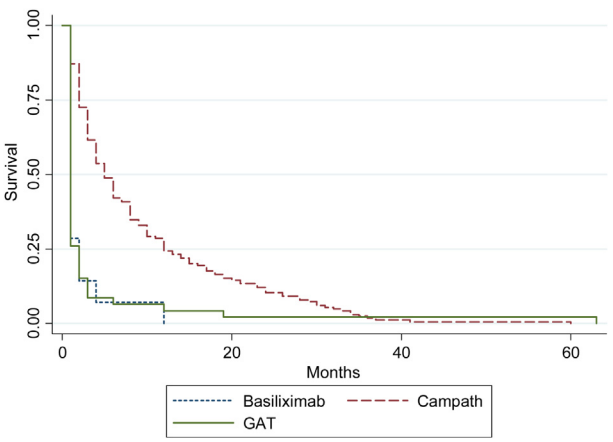

Fig 2. Curves of survival according to three types of induction medication.

C4d, the positivity of PRA and elapsed post-transplant time >3 months. These patients have an increased risk of acute cellular rejection type Banff IB. We had an optimal response to the treatment with corticoids in patients without C4d expression and a percentage of graft loss of 40%, similar to that in the literature.

In our country, unfortunately, we do not have donor-specific antibodies, to take a proper diagnoses of humoral rejection.

More long-term studies must be performed to establish the prognosis of graft survival in patients who express C4d in biopsies.

REFERENCES

[1] Fior F, Nacchia F, Minicozzi A, et al. Focal C4d staining in peritubular capillaries and kidney graft survival: results of a retrospective study. Transplant Proc 2010;42:1095–7.

[2] Gomes A, Pedroso S, Martins L, et al. Diagnosis and treatment of acute humoral kidney allograft rejection. Transplant Proc 2009;41:855–8.

[3] Matignon M, Muthukumar T, Seshan S, et al. Concurrent acute cellular rejection is an independent risk factor for renal allograft failure in patients with C4d-positive antibody-mediated rejection. Transplantation 2012;94(6):603–11.

[4] Demirci C, Sen S, Sezak M, et al. Incidence and Importance of C4d deposition in renal allograft dysfunction. Transplant Proc 2008;40:174–7.

[5] David-Neto E, Prado E, Beutel A, et al. C4d-positive chronic rejection: a frequent entity with a poor outcome. Transplantation 2007;84(11):1391–8.

[6] Banasik M, Boratynska M, Nowakowska B, et al. C4D deposition and positive posttransplant crossmatch are not necessarily markers of antibody-mediated rejection in renal allograft recipients. Transplant Proc 2007;39:2718–20.

[7] Neves M, Cotovio P, Machado S, et al. C4d deposits in acute "cell-mediated" rejection: a marker for renal prognosis? Transplant Proc 2012;44:2360–5.

[8] Loupy A, Hill G, Suberbielle C, et al. Significance of C4d Banff scores in early protocol biopsies of kidney transplant recipients with preformed donor-specific antibodies (DSA). Am J Transplant 2011;11:56–65.

[9] Mengel M, Bogers J, Bosmans J, et al. Incidence of C4d stain in protocol biopsies from renal allografts: results from a multicenter trial. Am J Transplant 2005;5:1050–6.

[10] Xu H, He X, Sun J, et al. The expression of B-cell activating factor belonging to tumor necrosis factor superfamily (BAFF) significantly correlated with C4D in kidney allograft rejection. Transplant Proc 2009;41:112–6.

[11] Moll S, Pascual M. Detection of alloantibody deposition in allografts: capillary C4d deposition as a marker of humoral rejection. Curr Opin Organ Transplant 2004;9:29–35.

[12] Cheunsuchon B, Vongwiwatana A, Premasathian N, et al. The prevalence of C4d-positive renal allografts in 134 consecutive biopsies in Thai patients. Transplant Proc 2009;41:3697–700.

[13] Hayde N, Bao Y, Pullman J, et al. The clinical and molecular significance of C4d staining patterns in renal allografts. Transplantation 2013;95(4):580–8.

[14] Haas M. The significance of C4d staining with minimal histologic abnormalities. Curr Opin Organ Transplant 2010;15:21–7.

[15] Collins A, Chicano S, Cornell L, et al. Putative antibody-mediated rejection with C4d deposition in HLA-identical, ABO-compatible renal allografts. Transplant Proc 2006;38:3427–9.

[16] Lorenz M, Regele H, Schillinger M, et al. Risk factors for capillary C4d deposition in kidney allografts: evaluation of a large study cohort. Transplantation 2004;78(3):447–52.

[17] Valente M, Furian L, Marino S, et al. Histological markers of humoral rejection in renal transplant patients. Transplant Proc 2007;39:1827–9.

[18] Ludovico-Martins H, Silva C, Teodoro W, et al. Analysis of different staining techniques for C4d detection in renal allograft biopsies. Transplant Proc 2009;41:862–5.

[19] Crary G, Raissian Y, Gaston R, et al. Optimal cutoff point for immunoperoxidase detection of C4d in the renal allograft: results from a multicenter study. Transplantation 2010;90(10):1099–105.

[20] Perkowska-Ptasinska A, Ciszek M, Chmura A, et al. Transplant glomerulopathy: clinical and pathological correlations. Transplant Proc 2009;41:141–9.