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Absence of C4d urinary excretion in the early post-transplant period is associated with improved long-term kidney graft survival ☆

Rafał Zwiech *

Department of Kidney Transplantation, Medical University of Lodz, Poland
Dialysis Department, Norbert Barlicki Memorial Teaching Hospital No. 1, 90-153 Lodz, Kopcinskiego 22, Poland

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ABSTRACT

Introduction: C4d urinary excretion varies according to the risk of graft rejection or progression of chronic allograft nephropathy, but its influence on long-term kidney transplant (KTx) outcomes remains unclear.
The aim of the study was to determine whether the initial (1–3 months post KTx) level of C4d urinary excretion may help to predict long-term kidney allograft transplantation outcomes.
Materials and methods: The study involved 185 patients who had undergone KTx. The urinary specimens taken from the morning urine portion were assessed by ELISA test for C4d excretion. To increase the objectivity of the assessment, all measurements were divided by urinary creatinine excretion (ng/mgCr). The study population was grouped according to the C4d excretion cut-off value into low (LC4d, 109 participants) and high (HC4d, 76 participants) C4d excretion groups. Additionally a subgroup with absence of C4d in the urine (ZC4d, 26 patients) was formed.
Results: The calculated Roc curve indicated the cut off value of the urinary C4d excretion as 12.4 ng/mgCr (AUC 0.77; 95%CI 0.73–0.95). The mean C4d urinary excretions in LC4d and HC4d were 1.9 ± 3.27 and 20.6 ± 4.6 ng/mgCr, respectively, whereas after exclusion of ZC4d subgroup, the mean C4d was 14.9 ± 6.3 ng/mgCr in the remainder. Kaplan–Meier curve analysis demonstrated a slightly higher graft survival rate (GSR) in LC4d than in HC4d group ($p = 0.04$ by log-rank). The subsequent analysis showed the highest GSR in ZC4d subgroup ($p = 0.0006$ by log-rank).
Conclusion: Although lower C4d urinary excretion in the early post-transplant period seems to be associated with better long-term kidney allograft transplantation outcomes, only its absence in the urine appears to be a solid predictor of improved graft survival.

1. Introduction

Recent studies have re-introduced the complement system and its split product C4d as a potent element participating in humoral allograft rejection. At present, many centers use C4d as an immunohistochemical marker for a humoral mediated allo-response [1–9]. C4d can be detected typically early after transplantation in nearly 30% of all diagnostic kidney graft biopsies, although its presence can be occasionally seen over many months [10]. Generally, C4d positivity is associated with significant allograft dysfunction and indicates the risk of rejection episodes [10–13]. Additionally, it also may indicate a poor long-term prognosis [12,14]. In contrast, very little is known about the influence of continuous C4d deposition in stable grafts (subclinical humoral rejection) with indeterminate effects on long-term kidney graft survival [11,15,16].

In those cases, early and non-invasive diagnostic tools would be appreciated for better understanding of the clinicopathological context of chronic antibody-mediated rejection [17–19]. According to Lederer et al., measurements of C4d urinary excretion in kidney graft recipients may have the potential to detect rejection episodes [17]. However, in a study by Honger et al., urinary C4d excretion does not correlate with C4d staining but reflects a nonspecific glomerular injury [20].

On the contrary, C4d-staining in PTC is found to be a very specific marker for AMR antibody-mediated rejection (AMR and rarely positive in few other renal pathologies). Additionally, the intensity of glomerular C4d-staining mostly correlates with C4d deposition in peritubular capillaries, which is consistent with similar binding of donor-specific antibodies and subsequent complement activation within these capillary beds [21–23]. Following activation and degradation of the C4 molecule, thioester groups are exposed which allow transient, covalent binding of the degradation product C4d to endothelial cell surfaces of vascular basement membranes near the sites of C4 activation in affected glomeruli or tubules. Only about 10% of generated C4 bind to target surfaces, whereas 90% remain in the fluid phase, where it is rapidly inactivated by factor I into C4c and C4d. The unique feature of C4d is its resistance to internalization, which may facilitate its detachment and penetration

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* Department of Kidney Transplantation Medical University of Lodz, Dialysis Department, Kopcińskiego 22, 90-153 Lodz, Poland. Tel.: +48 42 6776709; fax: +48 42 6783632.
E-mail address: rzwiech@mp.pl.

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to the urine [24–27]. Irrespective of whether the presence of C4d in the urine might indicate acute/chronic humoral rejection or just be a sign of glomerular injury, if present, it may determine the therapeutic options to provide improved treatment, which might lead to a favorable KTx outcome. The aim of the study was, therefore, to determine whether the initial level of C4d urinary excretion, measured at the moment of achieving stable graft function (1–3 months pos KTx), may help to predict KTx long-term outcomes or to indicate groups of patients in whom the prognosis could potentially worsen.

2. Materials and methods

2.1. Study design

The study was performed prospectively between November 1998 and December 2006 on patients who had undergone kidney allograft transplantation (KTx) from deceased donors. The collected data have been retrospectively analyzed after the completion of observational period i.e. December 2012. The median observation period was 7.6 years (range 5–12) with minimum 60 months of post-transplant follow-up.

All patients were treated in the Outpatient Clinic of the Barlicki Memorial Teaching Hospital No 1, Department of Kidney Transplantation, Medical University of Lodz. The observation period started at the time of discharge from hospital: in most cases, on the first visit to the outpatient clinic (1–3 months post KTx).

After informed consent had been obtained, the patients' records were evaluated and participants were asked in detail of their previous medical history. Only patients who had been treated with maintenance immunosuppression (IS) using one of three protocols, i.e. prednisone + cyclosporine + azathioprine (PCA), prednisone + cyclosporine + mycophenolate mophetil (PCM) or prednisone + tacrolimus + mycophenolate mophetil (PTM), were included in this study. The dosing of calcineurin inhibitors (CNI) was based on the whole blood level 2 h after a dose (C2) or not the trough level (C0). The choice of the method of CNI levels monitoring as well as their therapeutic ranges were based on the then guidelines.

Any deviation or conversion in the IS scheme during this early period, as well as any surgical complications, any signs of infection at baseline or history of severe infection (viral or bacterial) in the follow-ups were the exclusion criteria. Induction therapy was administered in none of the participants. Within 10 years of the conducted trial, 49 patients were excluded from the 234 involved in the primary analysis, and thus 185 participants were included in the final assessment. The main reasons of exclusion were: surgical complications – 19 patients, infections or IS conversion in the early post-transplant period – 11 and 8 patients, severe infections during observational period – 7 patients, missing data – 4 patients.

2.2. Patients and outcomes

The present analysis included 185 Caucasian patients, with a mean age of 45.4 ± 11.0 years at the moment of KTx, who had been recipients of cadaveric renal allografts. The collected data have been retrospectively analyzed. The most frequent cause of end-stage renal disease was glomerulonephritis (38%) and diabetes mellitus (18%). The majority of participants prior to kidney allograft transplantation were treated with hemodialysis (87%).

To adjust for patient comorbidities, the major components of the comorbidity score proposed by Davies et al. [28] were assessed, the results being given in Table 1. During the post-transplant period, the early and late complication incidences considered as covariates, which were believed to present a potential source of confounding, were monitored — Table 2.

The primary outcome was graft survival defined by the status of the patient: a living recipient with a functioning renal transplant, not on dialysis, at the study completion date (the first visit in outpatient clinic between November 2011 and December 2012).

2.3. Urine samples and C4d assessments

The morning urine samples from patients on three different maintenance IS regiments were collected on the first visit to the outpatient clinic, generally within 30 days from discharge. The time period between KTx and obtaining the urine samples was 36 days (median), range 21–82 days. After determination of urinary creatinine and proteinuria, the urine was centrifuged for 10 min at 2000 rpm, and stored at −70 °C until analysis. The urinary excretion of C4d was measured by enzyme-linked immunosorbent assay (ELISA) in undiluted urine, using commercial immunoassays, according to the manufacturer's instructions (Quidel, San Diego, Calif., USA). The lower limit of quantification for C4d was 0.022 μg/mL.

Table 1
Baseline characteristics of patients divided into low and high C4d excretion groups (the cut off value being 12.4 ng/mg urinary creatinine). Continuous variables are presented as mean ± SD. Continuous variables in the groups were compared using the Mann–Whitney test, categorical variables were compared by the chi^2 test with Yates correction.

	Low C4d	High C4d	P values
N	109	76	NS
Male	68	43	NS
Age at the moment of KTx (years)	45.7 ± 12.3	45.3 ± 10.5	NS
BMI	24.6 ± 4.3	24.2 ± 5.5	NS
ESRD causes			
Diabetes mellitus	21	13	NS
Glomerulonephritis	40	31	NS
Polycystic kidney disease	7	3	NS
Hypertension nephropathy	6	6	NS
Tubulo-interstitial diseases	11	7	NS
Vasculitis	8	4	NS
Unknown	16	12	NS
Dialysis modality			
Hemodialysis	95	66	NS
Peritoneal dialysis	14	10	NS
Total duration of pretransplantation ESRD (months)	18.1 ± 20.8	17.4 ± 23.6	NS
Number of transplant			
One	97	70	NS
Two or more	12	6	NS
Cold ischemia time (hours)	15.9 ± 8.6	16.8 ± 6.6	NS
Number of HLA matched antigens	17.3 ± 4.0	17.0 ± 3.3	NS
PRA level (%)	6.0 ± 16.2	6.2 ± 14.7	NS
Maintenance immunosuppression			
Cyclosporine	66	53	NS
Tacrolimus	43	23	NS
Azathioprine	31	42	NS
Mycophenolate mophetil	78	34	NS
C0 to C2 measurements ratio	0.68	0.65	NS
Proteinuria (mg/mg of urine creatinine)	1.84 ± 1.77	1.81 ± 1.72	NS
GFR (mL/min)	59.1 ± 22.6	57.6 ± 20.8	NS
Smokers	14	4	NS
Comorbidities			
Ischemic heart disease	13	8	NS
Diabetes mellitus	36	18	NS
Treated hyperlipidemia	31	16	NS
Statins	36	18	NS
Fibrates	4	2	NS
Treated hypertension	80	46	NS
Beta-blockers	27	21	NS
Calcium blockers	29	23	NS
ACEi or ARB	75	32	NS
Alpha-blockers	16	13	NS

KTx — Kidney transplantation, BMI — body mass index, ESRD — end stage renal disease, HLA — human leukocyte antigens, PRA — panel reactive antibodies, GFR glomerular filtration rate, ACEi — angiotensin II inhibitors, ARB — angiotensin II receptor blockers. NS — not significant.