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Tacrolimus trough levels after month 3 as a predictor of acute rejection following kidney transplantation: a lesson learned from DeKAF Genomics

Ajay K. Israni,[1,2] Samy M. Riad,[3] Robert Leduc,[4] William S. Oetting,[5] Weihua Guan,[4] David Schladt,[4] Arthur J. Matas,[6] Pamala A. Jacobson[5] and For the DeKAF Genomics Investigators

1 Nephrology Division, Department of Medicine, Hennepin County Medical Center, University of Minnesota, Minneapolis, MN, USA
2 Department of Epidemiology & Community Health, University of Minnesota, Minneapolis, MN, USA
3 Division of Nephrology, Department of Medicine, University of Minnesota, Minneapolis, MN, USA
4 Division of Biostatistics, University of Minnesota, Minneapolis, MN, USA
5 Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN, USA
6 Department of Surgery, University of Minnesota, Minneapolis, MN, USA

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Correspondence
Ajay K. Israni MD, MS, Department of Medicine, Hennepin County Medical Center, University of Minnesota, 701 Park Avenue, Minneapolis, MN 55415-1829, USA.
Tel.: 612 347 5871;
fax: 612 347 2003;
e-mail: isran001@umn.edu

Conflicts of interest
The authors have declared no conflicts of interest.

DeKAF Genomics Investigators:
J. Michael Cecka MD, UCLA Immunogenetics Center, Los Angeles, CA 90095, USA, e-mail: mcecka@ucla.edu; Fernando G. Cosio MD, Division of Nephrology, Mayo Clinic, Rochester, MN 55905, USA, e-mail: cosio.fernando@mayo.edu; Robert Gaston MD, Division of Nephrology, University of Alabama, Birmingham, AL 35294-0006, USA, e-mail: rgaston@uab.edu; Sita Gourishankar MD, Division of Nephrology and Immunology, University of Alberta, Edmonton, AB, Canada, e-mail: sitag@ualberta.ca; Lawrence Hunsicker MD, Nephrology Division, Iowa City, IA 52242-1082, USA, e-mail: lawrence-hunsicker@uiowa.edu; Bertram Kasiske MD, Department of Medicine, Hennepin County Medical Center and the University of Minnesota, Minneapolis, MN 55415, USA, e-mail: kasis001@umn.edu;

Summary

Most calcineurin inhibitor (CNI)-based protocols reduce blood trough goals approximately 2–3 months post-transplant in clinically stable kidney transplant recipients. The CNI target trough level to prevent rejection, after reduction, is unknown. Using a multivariate Cox proportional hazards model, we determined the association of time-varying tacrolimus (TAC) trough levels with acute rejection (AR) occurring in the first 6 months post-transplant, but specifically we assessed this association after 3 months. A total of 1930 patients received TAC-based immunosuppression prior to AR in a prospective study. Of the 151 (7.8%) who developed AR, 47 developed AR after 3 months post-transplant. In an adjusted time-varying multivariate model, each 1 ng/ml decrease in TAC trough levels was associated with a 7.2% increased risk of AR [hazards ratio (HR) = 1.07, 95% confidence interval (CI) (1.01, 1.14) $P = 0.03$] in the first 6 months. There was an additional 23% increased risk of AR with each 1 ng/ml decrease in the TAC trough levels in months 3–6 [HR = 1.23, 95% CI (1.06, 1.43) $P = 0.008$]. In conclusion, lower TAC trough levels were significantly associated with increased risk of AR in the first 6 months post-transplant with additional risk of AR between months 3 and 6 post-transplant. The timing and practice of TAC dose reduction should be personalized based on the individual's risk factors.

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Rosalyn Mannon, Division of Nephrology, University of Alabama, Birmingham, AL 35294-0006, USA, e-mail: rmannon @uab.edu; David Rush MD, Health Sciences Center, Winnipeg MB, Canada, e-mail: drush@exchange.hsc.mb.ca

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Introduction

Calcineurin inhibitors (CNIs) such as tacrolimus (TAC) are the back bone of immunosuppressant regimens and are used in greater than 80% of all kidney transplants in the United States [1]. Despite the wide use of these agents, the optimal TAC trough blood target after 3 months post-transplant to prevent rejection of transplanted allografts is unclear. Most institutions reduce TAC exposure between 2 and 3 months post-transplant in rejection-free kidney transplant recipients. According to international guidelines, the evidence behind this practice is of low quality [2]. The majority of acute rejection (AR) episodes take place in the first 6 months, a period during which this reduction takes place [3,4].

While the concept of dose reduction was introduced to limit the long-term undesirable side effect of CNIs on the renal allograft known as 'chronic CNI nephrotoxicity' as well as other CNI-related side effects [3–5], there are now data to support that most late graft loss is because of an immunologic insult [6,7]. Currently, the existence of chronic CNI nephrotoxicity is being debated by many investigators in the field [8–11]. The purpose of this study herein is to delineate the association between lower TAC trough levels and early AR within 6 months and to assess this relationship particularly after 3 months, a time most centers lower the TAC trough goal. We hypothesized that after 3 months there is an increased risk of AR in association with lower TAC trough level. Therefore, we used the prospective cohort of the Genomics of Deterioration of Kidney Allograft Function (DeKAF Genomics) study to test this hypothesis.

Materials and methods

Patients

A total of 3402 kidney or simultaneous kidney–pancreas transplant (SPK) recipients in five U.S. and two Canadian transplant centers were consented and enrolled at the time of transplantation in the DeKAF Genomics, a prospective observational, study between 2006 and 2011 [12]. The present study uses 1930 recipients from this study who received TAC within the first 6 months post-transplant. The study was approved by the Institutional Review Boards and informed consent was obtained from all participants. This trial is registered at www.clinicaltrials.gov (NCT00270712).

Immunosuppression and clinical data

Induction and maintenance immunosuppression regimens were center specific. All centers reduced TAC trough levels per their center-specific protocol (Table 1). Clinical data were collected at the time of transplantation and regularly thereafter until allograft failure and maintained in a central database. All biopsies were obtained for cause. AR was diagnosed by the treating physicians.

Table 1. Goal trough levels during the first 6 months post-transplant for all centers.

Center no.	Target tacrolimus trough level (ng/ml)	Time post-transplant (months)
1	10–12	0–2
	8–10	3–6
2	10–14	1–3
	6–10	4–6
3	8–12	0–3
	6–8	4–6
4	10–15	0–2
	5–10	3–6
5	10–12	0–1
	8–10	2–4
	6–8	5 and beyond
6	8–12	0–2
	6–10	3–6
7	6–10	0–2
	6–8	3–6

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Statistical analyses

Cox proportional hazards models were used to investigate the association between time-varying TAC trough levels between day 8 and 6 months post-transplant and time to first AR event occurring between 8 days and 6 months post-transplant. We created an interaction variable, TAC trough levels at 3 months, to assess the interaction between TAC trough levels and time after 3 months. This variable describes the effect of lower trough levels after 3 months. Individuals were considered at risk for AR beginning on the later of 8 days post-transplant or first TAC use. Censoring occurred at the earliest of permanent TAC discontinuation, 6 months post-transplant, last date of follow-up, graft failure or death. Participants who temporarily stopped TAC for reasons other than AR were excluded from the risk set until restarting TAC.

A multivariate model was created by performing backwards selection on potential clinical covariates, using a retention *P*-value of 0.10 and stratifying by transplant center. The potential clinical covariates eligible for backwards selection were TAC trough levels from day 8 to 6 months, TAC trough levels 3–6 months, recipient gender, race, age, smoking status [never, past or current], body mass index (BMI), blood type, cause of end-stage renal disease, SPK transplant, preemptive transplant, prior kidney transplant, prior nonkidney transplant, number of human leukocyte antigen (HLA) mismatches, T- or B-cell cross-match positive, panel-reactive antibody (PRA) status, dialysis prior to transplant, CMV sero-status, type of antibody induction, steroid use at day 7 post-transplant, and donor factors [age, gender, and donor status (living or deceased)].

The intra-individual variability in TAC levels was measured using the coefficient of variation (CV) for each subject. The CV was compared between subjects with no rejection in the first 6 months post-transplant, and subjects with AR before and after 3 months post-transplant using a *t*-test. Comparison of creatinine levels at 12 months post-transplant was conducted using a two-sample *t*-test. All analyses were conducted using SAS/Genetics v9.2 (The SAS Institute, Cary, NC, USA, http://www.sas.com).

Results

The characteristics of the study patients are described in Table 2. All centers undertook reduction in TAC trough levels at 2–3 months post-transplant as per their protocols described in Table 1. Figure 1 shows the decline in levels after 3 months post-transplant. In general, TAC trough levels of 8–15 ng/ml were targeted in months 0–3 post-transplant and targets of 5–10 ng/ml in months 4–6, as per the center's protocol. TAC trough levels measured prior to an oral dose, were obtained as part of clinical care, and used in this analysis. Two measurements, if available, were obtained in each of weeks 1–8 and in each of months 3, 4, 5, and 6 post-transplant, for a maximum of 24 measurements per patient. There was mean of 16.3 trough levels per patient [interquartile range (IQR) 16–22]. The median TAC trough levels during the entire 6 months post-transplant was 8.2 ng/ml (IQR 6.4–10.2 ng/ml).

Acute rejection occurred in 151 of 1930 patients by 6 months. AR occurred in 104 of these patients in the first 3 months [median (IQR) of time to first AR: 20 days (15–40)] and in 47 of these patients after 3 months post-transplant [126 days (113–164)]. Most of the AR events (92.7%) were biopsy confirmed (Tables 3 and 4). The acute changes, as reflected by i and t scores greater than or equal to 2, are more common in AR biopsies after 3 months, compared to AR before 3 months post-transplant. Also, chronic changes, as reflected by ci and ct scores greater than or equal to 2, are more common in AR biopsies after 3 months, compared to AR before 3 months post-transplant (Table 3). The group with AR, had a median TAC trough level of 7.6 ng/ml (IQR 4.8–9.9) immediately proximal to the AR event with the lower quartile being below the target TAC trough goal of 5.0 ng/ml for all study sites. The lower IQR of 4.8 ng/ml means that 25% of these subjects had a level below 4.8 ng/ml. These levels were obtained at a median of 3 days prior to AR (IQR 2–5 days). The CV for subjects with AR between 3 and 6 months post-transplant was 0.31 ($\pm$0.13) and not statistically different from CV of subjects with rejection before 3 months post-transplant 0.32 ($\pm$0.15) ($P = 0.87$) and from CV of subjects with no rejection within the first 6 months post-transplant 0.33 ($\pm$0.13) ($P = 0.49$).

In a multivariate Cox proportional model stratified by transplant center, time-varying TAC trough level was an independent predictor of AR, after adjusting for HLA mismatches, positive T- or B-cell crossmatch, PRA status, donor age, gender, BMI, and steroid use at day 7 post-transplant. For each 1 ng/ml reduction in the TAC trough level there was a 7.2% increased risk of AR [hazards ratio (HR) = 1.072, 95% confidence interval (CI) (1.01, 1.14) $P = 0.03$]. Using an interaction term, we assessed that there was an additional risk of AR of 23% with each 1 ng/ml reduction in the TAC trough level after 3 months [HR = 1.23, 95% CI (1.06, 1.43) $P = 0.008$] (Table 5). These models were not adjusted for use of mycophenolate since over 99% of the patients were on this medication concomitantly. As the median TAC trough levels immediately proximal to the AR event was 7.6 ng/ml (IQR 4.8–9.9), at least 25% of patients have less than the stated goal trough level of 5 ng/ml. In a subgroup analysis excluding patients with positive T- or B-cell crossmatch, 1 ng/ml lower TAC trough level after 3 months was associated with 29% increased risk of AR [HR = 1.29, 95% CI (1.09, 1.52)

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Table 2. Characteristics of all study patients and stratified by the presence of AR in the first 6 months post-transplant.

	All (N = 1930)	Subjects AR free during first 6 months (n = 1779)	Subjects with AR during first 6 months (n = 151)
Age of recipient in years, mean (SD)	50 (13.3)	50 (13.3)	48.4 (13.7)
Male recipient	1217 (63.1%)	1111 (62.4%)	106 (70.2%)
African American recipient	353 (18.3%)	335 (18.8%)	18 (11.9%)
BMI, mean (SD)	28 (5.5)	28.2 (5.5)	27.9 (6.0)
Simultaneous kidney–pancreas transplant	157 (8.1%)	134 (7.5%)	23 (15.2%)
Diabetes pretransplant	753 (39%)	696 (39%)	57 (38%)
Immune-related factors			
HLA mismatch			
None	224 (11.6%)	218 (12.3%)	6 (3.97%)
1 or 2	297 (15.4%)	277 (15.6%)	20 (13.3%)
3 or 4	754 (39.1%)	695 (39.1%)	59 (39.1%)
5 or 6	653 (33.9%)	587 (33.0%)	66 (43.7%)
Panel-reactive antibody positive	952 (49.5%)	859 (48.4%)	93 (61.6%)
Positive T- or B-cell crossmatch	138 (7.2%)	113 (6.4%)	25 (16.7%)
Prior solid organ transplant	504 (26.1%)	444 (25.0%)	60 (39.7%)
Prior kidney transplant	302 (15.6%)	265 (14.9%)	37 (24.5%)
Donor-related factors			
Age of donor in years, mean (SD)	40.8 (14)	40.6 (14)	42.8 (13.7)
Deceased donor	816 (42.%)	754 (42.4%)	62 (41.1%)
Immunosuppression			
Induction regimen			
Monoclonal antibody	782 (40.5%)	747 (42.0%)	35 (23.2%)
Polyclonal antibody	1026 (53.2%)	928 (52.2%)	98 (64.9%)
Combination	49 (2.5%)	39 (2.2%)	10 (6.6%)
None	73 (3.8%)	65 (3.7%)	8 (5.3%)
Steroid free by 7 days post-transplant	722 (37.45%)	668 (37.6%)	54 (36.0%)
Use of mycophenolate in first 6 months	1597 (99.3%)	1463 (99.2%)	134 (100.0%)
Use of mTOR inhibitors	3 (0.2%)	2 (0.1%)	1 (0.8%)
Median TAC trough, ng/ml, immediately proximal to AR event median (IQR)	N/A	N/A	7.6 (4.8–9.9)

AR, acute rejection; SD, standard deviation; BMI, body mass index; HLA, human leukocyte antigen; TAC, tacrolimus; IQR, interquartile range; N/A, not applicable.

P = 0.002]. In another multivariate model with the specific antibody induction agent forced into the model, the association of TAC trough levels with AR did not change in significance and direction (data not shown).

The long-term impact of the AR events after 3 months post-transplant was assessed by creatinine levels at 12 months post-transplant. Patients with AR after 3 months post-transplant had higher creatinine levels at 12 months compared to patients without AR and those with AR before 3 months post-transplant (Table 6).

Discussion

This study found that lower TAC trough levels in the first 6 months post-transplant were associated with an increased risk of AR. This is not a new finding as it is well known that TAC exposure and AR are well correlated in the first 3 months post-transplant [13–15]. The optimal timing of dose reduction or the TAC trough levels after 3 months is less clear although it is common practice that TAC is reduced at this time. The novel aspect of this study is that we showed that troughs achieved at 3 months post-transplant were equally important and lower trough levels were significantly associated with higher risk of AR. For every 1 ng/ml reduction in TAC trough levels after 3 months, the additional risk of AR increased by 23%. AR after 3 months post-transplant was associated with higher creatinine levels at 12 months post-transplant. These findings question the merits of systematic TAC exposure reduction at 3 months and/or the degree of exposure reduction. TAC trough levels remained important even after accounting for traditional risk factors such as higher number of HLA mismatches, crossmatch, and PRA status.

According to recent evidence-based guidelines, the current practice of reducing TAC trough goals is not justified by strong scientific evidence [2]. The initial trials employing TAC to prevent AR used much higher trough goals of 10–20 ng/ml in the first 3 months and 5–15 ng/ml

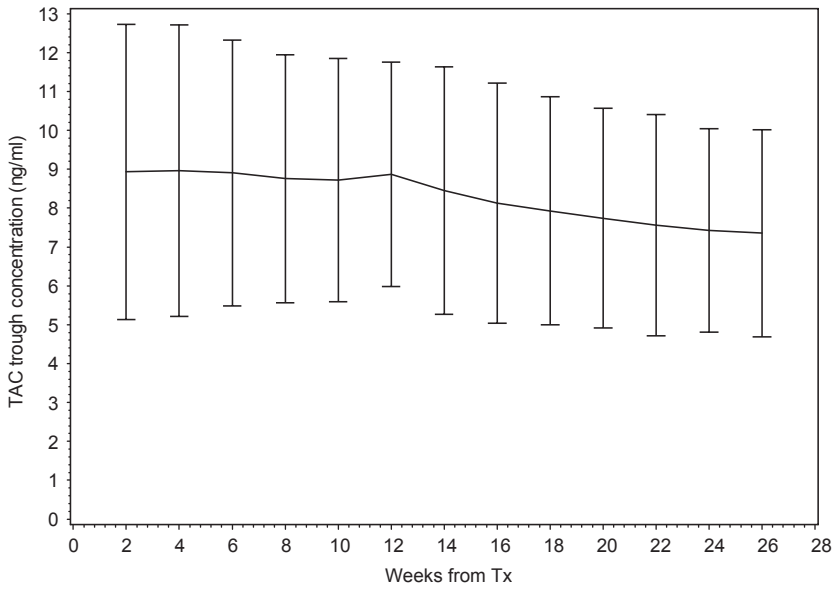

Figure 1 Mean tacrolimus (TAC) trough levels with 95% confidence intervals, during the first 6 months post-transplant in all subjects.

Table 3. Diagnosis and treatment of acute rejection (AR) biopsies (N = 151), as determined by local pathologist at transplant center.

	All (N = 151)	AR before 3 months post-transplant (n = 104)	AR after 3 months and before 6 months post-transplant (n = 47)
Diagnosis			
Cellular	100 (66.2)	60 (57.7)	40 (85.1)
Antibody-mediated	30 (19.9)	29 (27.9)	1 (2.1)
Both	10 (6.6)	9 (8.65)	1 (2.1)
No biopsy or indeterminate biopsy	11 (7.3)*	6 (5.77)	5 (10.6)
Drug treatment			
Steroids only	84 (55.6)	48 (46.1)	36 (76.6)
Antibodies only	4 (2.65)	4 (3.85)	0 (0.0)
Steroids and antibodies	33 (21.85)	28 (26.9)	5 (10.64)
Steroids followed by antibodies	15 (9.9)	12 (11.54)	3 (6.38)
Other	15 (9.9)	12 (11.5)	3 (6.38)

Values within parenthesis are expressed in percentage.
*Five of these subjects had no biopsies and six had indeterminate biopsies.

Table 4. Pathology scores for AR, as determined by the local pathologist at transplant center.

Banff score ≥2	All (N = 151)	AR before 3 months post-transplant (n = 104)	AR after 3 months and before 6 months post-transplant (n = 47)
i (interstitial inflammation)*	50 (42.7)	31 (34.8.0)	19 (67.9)
t (tubulitis)*	60 (50.8)	37 (41.1)	23 (82.1)
ci (interstitial fibrosis)†	7 (6)	3 (3.4)	4 (14.3)
ct (tubular atrophy)†	6 (5.1)	3 (3.4)	3 (10.7)
v (intimal artertitis)	8 (6.9)	6 (6.9)	2 (7.14)
g (glomerulitis)	1 (0.9)	1 (1.1)	0 (0.0)
cv (vascular fibrosis)	4 (3.4)	2 (2.3)	2 (7.14)
cg (glomerulopathy)	1 (0.9)	1 (1.1)	0 (0.0)
ah (arteriolar hyaline thickening)	4 (3.4)	3 (3.4)	1 (3.6)
Missing scores	34 (22.5)	15 (14.4)	19 (40.4)

Values within parenthesis are expressed in percentage. AR, acute rejection; CI, confidence interval.
*i and t-scores (range 0–3) correlated with Spearman correlation coefficient (95% CI) of 0.76 (0.67–0.86). $P < 0.0001$.
†ci and ct-scores (range 0–3) correlated with Spearman correlation coefficient (95% CI) between local ci-score and ct-score is 0.73 (0.60–0.86). $P < 0.0001$.

thereafter [3,16]. Based on this, it became standard practice to reduce TAC troughs at around 3 months post-transplant. The rationale for reducing TAC troughs even further both before and after 3 months is primarily based on the controversial concept of CNI-related nephrotoxicity and the desire to prevent long-term allograft dysfunction [17]. However, the existence of chronic CNI nephrotoxicity is debatable [8–11], whereas early rejection is a well-estab-

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Table 5. Adjusted hazards ratios of AR occurring before 6 months post-transplant.

	Hazards ratio (95% CI)	*P*-value
TAC trough levels (all troughs 0–6 months)*	1.07 (1.01, 1.14)	0.035
TAC trough levels (troughs after 3 months)†	1.23 (1.06, 1.43)	0.008
Male recipient	1.44 (0.99, 2.08)	0.051
Donor age in years	1.02 (1.00, 1.03)	0.011
Simultaneous kidney–pancreas	1.66 (0.99, 2.75)	0.051
Number of HLA mismatch		
0	Reference	
1 or 2	3.01 (1.19, 7.58)	0.02
3 or 4	3.36 (1.44, 7.87)	0.005
5 or 6	4.87 (2.08, 11.4)	0.0002
Panel-reactive antibody present	1.50 (1.05, 2.15)	0.03
T- or B-cell crossmatch positive	3.87 (2.40, 6.22)	<0.001
Steroid free at 7 days post-transplant	0.59 (0.38, 0.89)	0.012
BMI at baseline (linear)	0.99 (0.96, 1.02)	0.66
BMI at baseline (squared)	1.003 (1.0, 1.01)	0.052

AR, acute rejection; CI, confidence interval; TAC, tacrolimus; HLA, human leukocyte antigen; BMI, body mass index.
*Hazard is increased for each reduction in TAC trough level by 1 ng/ml anytime in the first 6 months post-transplant.
†Additional hazard for each reduction in TAC trough level by 1 ng/ml after 3 months. This variable assesses the interaction between TAC trough levels and time after 3 months to determine the additional effect of lower trough levels particularly after 3 months.

Table 6. Creatinine levels at 12 months post-transplant for patients based on AR status during the first 6 months post-transplant.

AR status during first 6 months post-transplant	*N**	MSC ± SD	*P*-value†
No rejection	1410	1.42 ± 0.66	<0.0001
Rejection before 3 months post-transplant	95	1.67 ± 0.64	0.16
Rejection between 3 and 6 months post-transplant	37	2.02 ± 2.19	–

AR, acute rejection; MSC, mean serum creatinine; SD, standard deviation.
*The *n* is smaller than stated in Tables 1 and 3 as not all subjects had a creatinine level around 12 months post-transplant.
†*P*-value compares creatinine between two groups. The comparator group is the patients with rejection between 3 and 6 months post-transplant.

lished risk factor for allograft loss [18]. TAC trough levels that are too low or reduced too early may negate any potential gains made by reduction in chronic CNI-related nephrotoxicity. A growing body of evidence now shows that the most late graft loss is caused by immunologic insults, i.e. late rejection and not necessarily related chronic CNI nephrotoxicity. For example, Gaston *et al.* showed that 35% of 171 patients had biopsies for cause diagnosed as CNI-related chronic nephrotoxicity [6]. However, this chronic CNI nephrotoxicity did not impact late all-cause allograft failure in their study [6].

Our findings are consistent with that of previously published studies. Initial randomized trials published in 1996–1997 of three different doses of TAC [13,14] showed that a therapeutic trough range of 5–15 ng/ml during the first year post-transplant had the maximum benefit and least side effects, compared to higher trough levels. Using the same trial data, Kershner and Fitzsimmons [15] showed that the higher the TAC trough levels (range 26–40 ng/ml) the lower the incidence of rejection post-transplant at the expense of elevated creatinine and nonrenal CNI side effects. In contrast, currently TAC trough goals are 8–15 ng/ml in months 0–3 post-transplant are generally well tolerated. Our study shows that further dose reduction at 3 months post-transplant may not be the optimal time for all patients because of the increased risk of AR. Naesens *et al.* also noted that low mean TAC levels between 3 and 12 months post-transplant were independently associated with higher increase in chronicity scores on protocol biopsies at 12 months post-transplant [19]. In our study, the rejections that occurred between 3 and 6 months post-transplant had higher chronicity scores compared to rejections before 3 months post-transplant (Table 4).

Mycophenolate is a key component of CNI-based regimens today. Previous studies using TAC and mycophenolate reduced the TAC trough levels around 3 months post-transplant with higher [20,21] or similar rates of AR [22–25]. However, none of these trials was large enough to study the association of TAC trough level reduction after 3 months post-transplant on risk of AR.

The finding of the ELITE Symphony trial may not be generalizable to the present study population. In the ELITE Symphony trial [26], low TAC trough levels were targeted (3–7 ng/ml) during the first year post-transplant; however, the study patients achieved higher average trough level of 6–8 ng/ml. Moreover, the TAC trough levels did not vary significantly through the duration of the study because levels were not lowered at 3 months, and 30–40% of patients remained above the target [27]. This may partially explain why the TAC group in the ELITE trial was not associated with increased risk of AR which suggested that medium TAC trough goal (7 ng/ml), without reduction by 3 months, was acceptable. Also, the ELITE Symphony trial did not include any U.S. transplants and excluded moderate- to high-risk patients such as those with high PRA and positive crossmatch. Therefore, the ELITE Symphony trial may not be applicable to the moderate-risk patients

enrolled in the present study [28]. In the ELITE Symphony trial, all subjects were required to have at least nine trough levels checked during their 12 month study [28]. In contrast, the present study was an observational study that did not require subjects to have trough levels checked at specific time-points and had an average of 17 trough levels over the first 6 months post-transplant [29]. Therefore, the more frequent trough level monitoring suggests more closer follow-up in the present study consistent with the moderate-risk patient population enrolled in the present study than in the ELITE Symphony trial.

Our study found that conventional risk factors, such as more HLA mismatches, positive T- or B-cell crossmatch, PRA positive status, and older donor age, were associated with increased risk of AR. Being steroid free by 7 days post-transplant varied by center, but was associated with a lower risk of AR in the multivariate model. It is likely that lower immunologic risk patients were selected to be steroid free by 7 days post-transplant. Only after adjusting for other risk factors of AR, being off steroids appeared to be associated with a lower risk of AR. This is consistent with the finding that in the unadjusted analysis, steroid free by 7 days, was not associated with a reduced risk of AR [HR = 0.93, 95% CI (0.67–1.30), $P = 0.07$].

The present study has several limitations. It is an observational study and not a randomized controlled trial with prespecified trough levels. Several different induction regimens were used in this study, and steroid withdrawal was transplant center dependent. Therefore, the impact of selection bias and unmeasured confounders, such as doses of steroids and mycophenolate, may not be accounted for in our study. The present study did not collect detailed information on hypertension, lipid profiles; therefore, the impact of TAC reduction on these outcomes could not be described. The present study only collected information about the use of steroid and mycophenolate, but not the exact doses of these immunosuppressants. The TAC troughs were not measured in a single central laboratory. However, the majority (97.1%) of TAC whole blood concentrations were obtained from centers using liquid chromatography–mass spectroscopy to measure trough concentrations. All troughs were measured using CLIA-certified assays or CLIA quality assays [29]. Another limitation is that we did not collect information on adherence to immunosuppressive medications, therefore we cannot rule out the role of poor adherence on AR after dose reduction.

We conclude that low TAC trough levels are significantly associated with AR with an additional risk of AR with a reduction in trough levels at 3 months. These AR events after 3 months post-transplant have a detrimental impact on kidney function at 12 months post-transplant. This study questions the practice at most transplant centers of reducing TAC trough goal at 2–3 months post-transplant for fear of chronic nephrotoxicity and other side effects. Given the ongoing debate about the existence of chronic nephrotoxicity, transplant centers may be reducing TAC at the expense of AR and potentially poor long-term allograft survival. Based on the results of this study, a personalized approach is needed to determine the ideal timing and degree of TAC dose reduction after assessing the risks and benefits for each patient, particularly high-risk patients.

Authorship

AKI, RL, WSO, WG, DS, AJM, PAJ: participated in research design, performance of research, data analysis, and writing of article. SMR: participated in performance of research, data analysis, and writing of article.

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References

1. Organ Procurement and Transplantation Network (OPTN) and Scientific Registry of Transplant Recipients (SRTR). *OPTN/SRTR 2010 Annual Data Report*. Rockville, MD: Department of Health and Human Services, Health Resources and Services Administration, Healthcare Systems Bureau, Division of Transplantation, 2010: Figure KI 7.1.
2. Kidney Disease Improving Global Outcomes (KDIGO). KDIGO clinical practice guideline for the care of kidney transplant recipients. *Am J Transplant* 2009; **9**: S14.
3. Pirsch JD, Miller J, Deierhoi MH, Vincenti F, Filo RS. A comparison of tacrolimus (FK506) and cyclosporine for immunosuppression after cadaveric renal transplantation. FK506 Kidney Transplant Study Group. *Transplantation* 1997; **63**: 977.

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4. Spencer CM, Goa KL, Gillis JC. Tacrolimus. An update of its pharmacology and clinical efficacy in the management of organ transplantation. *Drugs* 1997; **54**: 925.
5. Pallardo LM, Oppenheimer F, Guirado L, *et al.* Calcineurin inhibitor reduction based on maintenance immunosuppression with mycophenolate mofetil in renal transplant patients: POP study. *Transplant Proc* 2007; **39**: 2187.
6. Gaston RS, Cecka JM, Kasiske BL, *et al.* Evidence for antibody-mediated injury as a major determinant of late kidney allograft failure. *Transplantation* 2010; **90**: 68.
7. Sellarés J, de Freitas DG, Mengel M, *et al.* Understanding the causes of kidney transplant failure: the dominant role of antibody-mediated rejection and nonadherence. *Am J Transplant* 2012; **12**: 388.
8. Kandaswamy R, Humar A, Casingal V, Gillingham KJ, Ibrahim H, Matas AJ. Stable kidney function in the second decade after kidney transplantation while on cyclosporine-based immunosuppression. *Transplantation* 2007; **83**: 722.
9. Matas AJ. Chronic progressive calcineurin nephrotoxicity: an overstated concept. *Am J Transplant* 2011; **11**: 687.
10. Matas AJ, Leduc R, Rush D, *et al.* Histopathologic clusters differentiate subgroups within the nonspecific diagnoses of CAN or CR: preliminary data from the DeKAF study. *Am J Transplant* 2010; **10**: 315.
11. Snanoudj R, Royal V, Elie C, *et al.* Specificity of histological markers of long-term CNI nephrotoxicity in kidney-transplant recipients under low-dose cyclosporine therapy. *Am J Transplant* 2011; **11**: 2635.
12. Israni A, Leduc R, Holmes J, *et al.* Single nucleotide polymorphisms, acute rejection and severity of tubulitis in kidney transplantation, accounting for center-to-center variation. *Transplantation* 2010; **90**: 1401.
13. DA Laskow VF, Neylan JF, Mendez R, Matas AJ. An open-label, concentration-ranging trial of FK506 in primary kidney transplantation: a report of the United States Multicenter FK506 Kidney Transplant Group. *Transplantation* 1996; **62**: 900.
14. Vincenti F, Laskow DA, Neylan JF, Mendez R, Matas AJ. One-year follow-up of an open-label trial of FK506 for primary kidney transplantation. A report of the U.S. Multicenter FK506 Kidney Transplant Group. *Transplantation* 1996; **61**: 1576.
15. Kershner RP, Fitzsimmons WE. Relationship of FK506 whole blood concentrations and efficacy and toxicity after liver and kidney transplantation. *Transplantation* 1996; **62**: 920.
16. Mayer AD, Dmitrewski J, Squifflet JP, *et al.* Multicenter randomized trial comparing tacrolimus (FK506) and cyclosporine in the prevention of renal allograft rejection: a report of the European Tacrolimus Multicenter Renal Study Group. *Transplantation* 1997; **64**: 436.
17. Golshayan D, Pascual M. Minimization of calcineurin inhibitors to improve long-term outcomes in kidney transplantation. *Transpl Immunol* 2008; **20**: 21.
18. Kidney Disease Improving Global Outcomes (KDIGO). KDIGO clinical practice guideline for the care of kidney transplant recipients: treatment of acute rejection. *Am J Transplant* 2009; **9**: S21.
19. Naesens M, Lerut E, Damme BV, Vanrenterghem Y, Kuypers DRJ. Tacrolimus exposure and evolution of renal allograft histology in the first year after transplantation. *Am J Transplant* 2007; **7**: 2114.
20. Ciancio G, Burke G, Gaynor JJ, *et al.* A randomized trial of thymoglobulin vs. alemtuzumab (with lower dose maintenance immunosuppression) vs. daclizumab in renal transplantation at 24 months of follow-up. *Clin Transplant* 2008; **22**: 200.
21. Filler G, Webb NJ, Milford DV, *et al.* Four-year data after pediatric renal transplantation: a randomized trial of tacrolimus vs. cyclosporin microemulsion. *Pediatr Transplant* 2005; **9**: 498.
22. Ciancio G, Burke GW, Gaynor JJ, *et al.* A randomized trial of three renal transplant induction antibodies: early comparison of tacrolimus, mycophenolate mofetil, and steroid dosing, and newer immune-monitoring. *Transplantation* 2005; **80**: 457.
23. Grenda R, Watson A, Vondrak K, *et al.* A prospective, randomized, multicenter trial of tacrolimus-based therapy with or without basiliximab in pediatric renal transplantation. *Am J Transplant* 2006; **6**: 1666.
24. Heilman RL, Chakkera HMazur M, *et al.* Outcomes of simultaneous kidney-pancreas transplantation with positive cross-match. *Transplant Proc* 2009; **41**: 303.
25. Gaston RS, Kaplan B, Shah T, *et al.* Fixed- or controlled-dose mycophenolate mofetil with standard- or reduced-dose calcineurin inhibitors: the Opticept trial. *Am J Transplant* 2009; **9**: 1607.
26. Ekberg H, Tedesco-Silva H, Demirbas A, *et al.* Reduced exposure to calcineurin inhibitors in renal transplantation. *N Engl J Med* 2007; **357**: 2562.
27. Ekberg H, Mamelok RD, Pearson TC, Vincenti F, Tedesco-Silva H, Daloze P. The challenge of achieving target drug concentrations in clinical trials: experience from the Symphony Study. *Transplantation* 2009; **87**: 1360.
28. Leichtman AB. Balancing efficacy and toxicity in kidney-transplant immunosuppression. *N Engl J Med* 2007; **357**: 2625.
29. Jacobson P, Oetting WS, Brearley AM, *et al.*; for DeKAF investigators. Novel polymorphisms associated with tacrolimus trough concentrations: results from a multicenter kidney transplant consortium. *Transplantation* 2011; **91**: 300.

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