Research Article

Tacrolimus: Influence of the Posttransplant Concentration/Dose Ratio on Kidney Graft Function in a Two-Year Follow-Up

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Keywords

Estimated glomerular filtration rate · Kidney transplantation · Outcome · Renal transplantation · Tacrolimus

Abstract

Introduction: Tacrolimus (TAC) metabolism rate has the potential to impact graft function after kidney transplantation (KTx). We aimed to analyze the relationship between the early post-KTx TAC C/D ratio (blood trough concentration normalized by total daily dose) and kidney graft function in a 2-year follow-up. **Methods:** We retrospectively analyzed data from 101 post-KTx patients at 3, 6, 12, and 24 months after KTx to identify the C/D ratio cutoff value optimal for dividing patients into fast and slow TAC metabolizers. We investigated the relationship between their TAC metabolism rate and graft function. **Results:** Patients were divided based on the TAC C/D ratio at 6 months after KTx of 1.47 ng/mL * 1 mg. Fast metabolizers (C/D ratio < 1.47 ng/mL * 1 mg) presented with significantly worse graft function throughout the whole study period ($p < 0.05$ at each timepoint) and were significantly less likely to develop good graft function (estimated glomerular filtration rate ≥ 45 mL/min/1.73 m²) than slow metabolizers. Our model based on donor and recipient age, recipient sex and slow/fast metabolism status allowed for identification of patients with compromised graft function in 2-year follow-up with 66.7% sensitivity and 94.6% specificity. **Conclusion:** Estimating TAC C/D ratio at 6 months post-KTx might help identify patients at risk of developing deteriorated graft function in a 2-year follow-up.

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Published by S. Karger AG, Basel

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Introduction

Kidney transplantation (KTx) is currently the best available treatment for end-stage renal disease. Tacrolimus (TAC), a calcineurin inhibitor (CNI), mycophenolate mofetil (MMF), and prednisolone form the immunosuppressive regimen most commonly used to prevent graft rejection [1].

Although currently used immunosuppressive protocols effectively prevent acute and chronic rejection [1], several problems arise during TAC treatment due to its variable bioavailability (8–69%), large intra- and interindividual pharmacokinetic variability, and interactions with food and drugs [2, 3], which calls for dose individualization. Therefore, no fixed dosing scheme is recommended; instead, TAC doses are tailored individually according to the guidelines regarding TAC blood trough concentrations (TAC $C_{p \text{ trough}}$) for certain periods following transplantation. TAC treatment poses a challenge, as both over- and underestimation of its dose can lead to graft damage [3–6].

Suboptimal TAC blood concentrations may result in acute or chronic graft rejection [7, 8]. On the other hand, overly high TAC concentrations may cause delayed graft function, thrombotic microangiopathy, deterioration of blood pressure control, diabetes, susceptibility to infections, and neurotoxicity [9–11]. One of side effects of TAC or cyclosporine treatment is CNI nephrotoxicity (CNIT) [12]. Acute CNIT, observed shortly after KTx and potentially reversible, is associated with vasoconstriction of the afferent arterioles, though the direct mechanism remains unclear. Chronic CNIT irreversible interstitial fibrosis and tubular atrophy (IFTA), usually progressive and contributing to poor graft function [13]. Therefore, identifying patients at risk of developing chronic graft dysfunction is crucial.

The underlying cause of TAC nephrotoxicity is yet to be discovered. Although nephrotoxicity is usually attributed to high TAC blood concentrations [12], it was suggested to be associated with the TAC metabolism rate instead, as it reportedly occurred in patients whose TAC blood concentrations fell within the therapeutic range [14].

TAC metabolism is affected by several factors including age, sex, and body mass index (BMI) [15]. It is mainly controlled by cytochrome P450 enzymes: CYP3A4 and CYP3A5 and glycoprotein P [3]. Patients with CYP3A5 * 1 are usually described as fast TAC metabolizers, while most slow metabolizers express CYP3A5 * 3 [16, 17]. However, clinical application of this observation is limited by its unconfirmed influence on graft function in long-term observation [18] and inaccessibility of routine genetic testing [19].

Recently, the relationship between TAC metabolism and kidney graft function has been increasingly appreciated; therefore, a clinically feasible indicator of TAC metabolism would be of great clinical importance. Thölking et al. [20] showed that the TAC C/D ratio (TAC blood trough concentration normalized by TAC daily dose) as an indicator of TAC metabolism rate is associated with graft function after KTx and kidney function after liver transplantation (LTx) [21].

We aimed to analyze the relationship between the patients' TAC metabolism rate and kidney graft function and to determine an optimal cutoff C/D ratio value in order to identify patients at risk of developing deteriorated graft function in a 2-year follow-up.

Materials and Methods

The study was approved by the Bioethical Committee at the Medical University of Lodz (Lodz, Poland) on October 16, 2018, approval No. RNN/337/18/KE. Informed consent was given by all participants at the time of transplantation to record their clinical data and used in anonymized analyses.

Study Design

This retrospective study included data from 101 adult, Caucasian, primary KTx recipients from 2 Polish transplantation centers. All patients underwent KTx between 2009 and 2016 and attended the centers' outpatient posttransplant clinics for at least 12 months. We enrolled patients whose immunosuppressive regimen initially consisted of TAC: standard-release formulation (Prograf; Astellas Pharma US, Inc., Deerfield, IL, USA) administered twice daily (Tc) or extended-release formulation (Advagraf; Astellas Pharma US Inc.) and administered once daily (Tc-pr); MMF or mycophenolate sodium and prednisolone. Since all patients presented low immune risk, no induction therapy was given. TAC was administered according to the TAC $C_{p\text{ trough}}$ guidelines of the Polish Transplant Society (target TAC $C_{p\text{ trough}}$ of 12–15 ng/mL in the 1st, 8–12 ng/mL in the 2nd and 3rd and 5–8 ng/mL after the 3rd month after KTx) [22]. The immunosuppressive regimen did not change throughout the whole study period.

The initial dose of MMF was 1,000 mg twice a day, modified if adverse effects occurred (leukopenia, digestive side effects, recurrent infections) and changed to mycophenolate sodium in corresponding doses in case of digestive intolerance. The steroid dose was tapered from 250 mg of methylprednisolone on the first day, through 125 mg on days 2 and 3, to 20 mg of prednisone a day for the rest of the first month, and to the maintenance dose of 5 mg a day in the third month after KTx.

Patients who have undergone second or subsequent transplantation or required induction therapy were excluded. Other exclusion criteria were an immunosuppressive regimen originally different or converted later on, history of acute rejection in the first 12 months post-KTx, treatment with steroid doses higher than 5 mg per day after the 3rd month post-KTx, treatment with Once Daily MeltDose TAC Formulation (Envarsus® Chiesi Farmaceutici S.p.A.) or with any medication metabolized by cytochrome P450, history of hepatitis B or C, clinical symptoms of acute infection or diarrhea 7–10 days prior to laboratory assessment, history of noncompliance, and incomplete data.

Patients lost to follow-up, those after conversion of immunosuppressive regimen, and patients who died before the end of observation period were excluded in the course of the study. We gathered the patients' data using electronic and traditional files from the outpatient clinics. All data were anonymized and analyzed retrospectively.

Clinical Data, Biochemical Evaluation

The information concerning patients' medical history including time of KTx, history of acute rejection, history of diabetes mellitus (DM) or posttransplant DM (PTDM), occurrence of side effects, demographic data such as sex and age, medications (including immunosuppressive therapy), results of routine laboratory tests and graft biopsies, type of donation (living or deceased), as well as donors' sex and age were obtained from medical charts.

We analyzed clinical reports from 4 follow-up ambulatory appointments: 3 months $\pm$ 1 week, 6 months $\pm$ 1 week, 12 months $\pm$ 2 weeks, and 24 months $\pm$ 1 month after KTx. Serum creatinine concentrations as well as hematocrit were evaluated at each visit. Graft function was assessed by calculating the estimated glomerular filtration rate (eGFR) using the CKD-EPI formula [23].

The TAC $C_{p\text{ trough}}$ (12-h post-dose for Tc or 24-h post-dose for Tc-pr) was measured in whole blood using automated TAC assay (ROCHE Diagnostic, Mannheim Germany). Information about TAC doses administered prior to each appointment was also noted.

At each timepoint, we calculated the TAC C/D ratio by dividing the TAC $C_{p\text{ trough}}$ (C) by the daily dose administered the day before the measurement (D).

To ensure research objectivity, all data were collected by research team members unaware of the purpose of the analysis.

Statistical Analysis

Nominal data are presented as numbers with percentages; numerical data are presented as means with SDs or as medians with the values of upper and lower quartile, depending on the normality of distribution verified with the Shapiro-Wilk test. Associations between nominal variables were tested with the chi-square test or Fisher's exact tests. To compare continuous variables between 2 groups of patients, we used unpaired *t* test for normally distributed data. To compare variables with nonnormal distribution, we used Mann-Whitney U test. Correlations between continuous variables were tested with the Pearson's test for data with normal distribution and with the Spearman's test for data with nonnormal distribution.

We aimed to assess the impact of TAC metabolism rate on kidney graft function at 24 months post-KTx. To this end, we used TAC C/D ratio measured at 6 months after KTx. Two different approaches were implemented to classify patients as slow or fast TAC metabolizers. First, as described previously by Thölking et al. [20, 21], we used the median value of TAC C/D ratio at 6 months after KTx to divide patients into 2 equally numbered groups. Then, we used receiver operating characteristic (ROC) curve analysis to evaluate the utility of C/D ratio at 6 months as a classifier identifying patients at risk for deteriorated kidney graft function in a 2-year follow-up. We calculated Youden index to select an optimal threshold value. We used this cutoff to classify patients as fast or slow TAC metabolizers. Renal function expressed as eGFR values and other clinical characteristics (age, sex, presence of diabetes, TAC formulation, and total daily dose) were compared between these 2 groups at each timepoint.

Logistic regression model with stepwise forward feature selection was fitted to create a simple model predicting decreased graft function, defined as eGFR 24 months after KTx <45 mL/min/1.73 m², that could be of use in clinical setting. The choice of the cutoff of 45 mL/min/1.73 m² was dictated by the clinical relevance, since patients with eGFR below this value have increased risk of cardiovascular events [24]. For inclusion in the model, we selected demographic data of the recipients and donors (sex and age), type of donation (living or deceased), and variables assessed at 6 months after KTx: TAC metabolism group (slow or fast), TAC dose, TAC trough concentration, TAC C/D ratio, presence of DM or PTDM.

We performed ROC curve analysis and compared both classifiers (TAC C/D ratio at 6 months post-KTx versus, the model fitted in the logistic regression). Also, in order to better describe the relationship between TAC metabolism group and eGFR in 24-month follow-up, adjusting for all potential confounders, we fitted a linear model with eGFR as a continuous response variable. In this model, we included donor age and recipient age, sex, hematocrit and C/D ratio from the 6th month posttransplantation, and the type of TAC formulation used at that time.

Two-sided tests were used in all instances, and two-sided *p* values were reported. *p* values lower than 0.05 were considered statistically significant.

All analyzes were performed with Statistica 13.1 (DELL Inc., Tulsa, USA) licensed to Medical University of Lodz.

Results

In total, 101 KTx patients, 39 F and 62 M, fulfilled the inclusion criteria and presented with good compliance with immunosuppressive regimen. Their median age was 52.3 years (40.3–58.6 years) at the time of KTx. Nanty-three patients received kidney grafts through deceased and 8 through living donation. The data were collected from 101 patients 3, 6, and 12 months after KTx and from 90 patients 24 months after KTx. Eleven patients were excluded from the study a year after KTx: 7 changed their outpatient posttransplant clinic, 2 underwent an immunosuppressive therapy conversion, and 2 died. Out of 101 patients, 8 were diagnosed with diabetes prior to KTx and 22 developed PTDM.

Fig. 1. Scatter plot of eGFR from the 24th month posttransplantation and C/D ratio (TAC trough concentration normalized by the TAC daily dose) calculated at 6 months after KTx. eGFR, estimated glomerular filtration rate.

Association of Clinical Variables with TAC Metabolism

Median C/D ratios from the 3rd, 6th, 12th, and 24th month were 0.93 (0.62–1.46) ng/mL * 1 mg, 1.51 (1.10–2.18) ng/mL * 1 mg, 1.60 (1.16–2.20) ng/mL * 1 mg, and 1.81 (1.07–2.56) ng/mL * 1 mg, respectively. TAC C/D ratios at the 6th, 12th, and 24th month after KTx were strongly correlated with each other (all $r > 0.6$; $p < 0.001$), but not with TAC C/D ratio from the 3rd month after KTx ($p = 0.629$, $p = 0.523$, and $p = 0.082$ for correlations of TAC C/D ratio at 3rd and at the 6th, 12th, and 24th month, respectively).

We observed no statistically significant differences in TAC C/D ratio between men and women (for all timepoints $p > 0.5$) or between patients receiving different TAC formulations (Tc or Tc-pr; for all timepoints $p > 0.2$). Similarly, we observed no significant associations between patients' age and TAC C/D ratio at any timepoint.

At the 12th month after KTx, the hematocrit value was significantly correlated with TAC C/D ratio ($p = 0.002$). Additionally, the hematocrit values from the 3rd month and from the 12th month were significantly correlated with TAC trough levels from the respective timepoints ($r = 0.22$, $p = 0.048$ and $r = 0.31$, $p = 0.003$, respectively).

Fast and Slow TAC Metabolizers

Median TAC C/D ratio for the whole group at 6 months post-KTx was 1.51 ng/mL * 1 mg (minimum 0.38; maximum 6.27) and was correlated with eGFR from the 24th month (Spearman $r = 0.30$; $p = 0.004$; Fig. 1). The optimal C/D ratio value calculated at 6 months after KTx that allowed for identification of patients with good graft function in 2-year follow-up was 1.47 ng/mL * 1 mg (Fig. 2). Good graft function was defined as eGFR ≥ 45 mL/min/1.73 m², and the choice of the optimal cutoff value was based on Youden index calculation. This classifier provided sensitivity of 60.9% and specificity of 76.9% with the area under the ROC curve (AUC ROC) of 0.674.

The cutoff value of 1.47 ng/mL * 1 mg was therefore utilized to categorize patients as fast (FM, $n = 51$, C/D ratio < 1.47 ng/mL * 1 mg) or slow (SM, $n = 50$, C/D ratio ≥ 1.47 ng/mL * 1 mg) metabolizers (Fig. 3).

No statistically significant differences were observed between FMs and SMs in relation to patients' sex, age, TAC formulation, DM or PTDM diagnosis, and donor sex or age. To achieve

Fig. 2. ROC curve for a classifier based on C/D ratio (TAC trough concentration normalized by the TAC daily dose) calculated at 6 months after KTx that allowed for identification of patients with $\text{eGFR} \geq 45 \text{ mL/min/1.73 m}^2$ in the 24 months follow-up. Youden index is marked with red.

Fig. 3. Histogram of TAC C/D ratios (TAC trough concentration normalized by the TAC daily dose) for the whole group of patients. Cutoff value of $1.47 \text{ ng/mL} \cdot 1 \text{ mg}$ is marked, with patients classified as fast and slow TAC metabolizers.

target TAC $C_{p \text{ trough}}$, FM required approximately twice as high TAC doses as SMs (Table 1). Out of 8 patients that received kidneys through living donation, 3 were FM and 5 were SMs ($p = 0.479$).

Between the 3rd and 24th month post-KTx, a total of 11 indication graft biopsies were performed on 8 patients (5 FM, 3 SM). BK nephropathy was diagnosed in 2 patients (both FM), chronic rejection in 3 patients (2 FM, 1 SM), and recurrence of glomerulopathy in 1 patient (SM). In 2 patients (1 FM, 1 SM), biopsy revealed no pathologic findings.

During the study period, 2 patients underwent conversion of the immunosuppressive therapy, one (FM) due to trembling hands and one (SM) due to recurrence of focal segmental

Table 1. Demographics and clinical characteristics of slow and fast TAC metabolizers at all timepoints (3, 6, 12, and 24 months after KTx)

	Slow metabolizers (n = 50)	Fast metabolizers (n = 51)	p value
Age at baseline, years	49.0±12.2	48.3±13.3	0.765
Gender, male/female	31 (60.8)/20 (39.2)	31 (62.0)/19 (38.0)	0.900
Donor age, years	47.6±13.0	44.6±12.4	0.253
Donor gender, male/female	32 (62.7)/19 (37.3)	28 (56.0)/22 (44.0)	0.490
Type 2 DM	6 (11.8)	2 (4.0)	0.154
New onset DM	12 (23.5)	10 (20.0)	0.627
3 months post KTx			
TAC daily dose, mg	6.8±4.0	10.6±4.7	<0.001*
TAC C _{p trough} , ng/mL	10.83±3.68	9.71±3.12	0.105
C/D ratio, ng/mL * 1 mg	1.28±0.96	1.06±0.54	0.172
Creatinine concentration, mg/dL	1.55±0.48	2.02±1.12	0.006*
eGFR, mL/min/1.73 m ²	47.91 (36.73–63.82)	40.05 (30.16–51.40)	0.015*
6 months post KTx			
TAC daily dose, mg	3.6±1.4	7.7±4.3	<0.001*
TAC C _{p trough} , ng/mL	8.30±2.38	6.70±2.40	0.001*
C/D ratio, ng/mL * 1 mg	2.52±1.03	1.00±0.33	<0.001*
Creatinine concentration, mg/dL	1.39±0.42	1.72±0.61	0.002*
eGFR, mL/min/1.73 m ²	58.46 (42.33–72.71)	49.45 (32.97–57.77)	0.002*
12 months post KTx			
TAC daily dose, mg	3.1±1.2	5.9±2.8	<0.001*
TAC C _{p trough} , ng/mL	6.25±1.98	6.61±2.61	0.434
C/D ratio, ng/mL * 1 mg	2.32±1.18	1.39±1.18	<0.001*
Creatinine concentration, mg/dL	1.31±0.38	1.62±0.58	0.002*
eGFR, mL/min/1.73 m ²	61.21 (48.42–73.65)	51.82 (36.95–63.36)	0.002*
24 months post KTx			
TAC daily dose, mg	2.7±1.0	5.1±2.5	<0.001*
TAC C _{p trough} , ng/mL	5.76±1.69	6.08±1.79	0.379
C/D ratio, ng/mL * 1 mg	2.56±1.44	1.46±0.80	<0.001*
Creatinine concentration, mg/dL	1.27±0.38	1.54±0.49	0.005*
eGFR, mL/min/1.73 m ²	64.83 (53.04–77.32)	48.77 (39.71–61.44)	0.006*

Nominal variables are presented as absolute and relative numbers and continuous data are presented as means with SDs or as medians with the values of upper and lower quartile. Data for the 3rd, 6th, and 12th month after KTx are presented for 101 patients (slow metabolizers: n = 51, fast metabolizers: n = 50), data for the 24th month are presented for 90 patients (slow metabolizers: n = 45, fast metabolizers: n = 45).

KTx, kidney transplantation; DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; TAC, tacrolimus; TAC C_{p trough}, TAC trough concentration.

glomerulosclerosis in the transplanted kidney. Two patients died (both FMs), one due to cervical cancer and one due to cardiovascular complications.

Kidney Graft Function: Associations with Time after KTx, FM/SM Status, Sex, Age, and TAC Formulation

For the whole group, graft function improved notably during the analyzed period, with median eGFR 45.34 (33.66–61.31) mL/min/1.73 m² after 3rd, 52.63 (39.23–62.17) mL/min/1.73 m² after 6th, 55.35 (42.82–68.34) mL/min/1.73 m² after 12th, and 56.01 (43.93–71.36) mL/min/1.73 m² after the 24th month post-KTx (p < 0.001 for comparison between the first and last timepoint). Twenty-four months post-KTx, 64 patients presented eGFR ≥45 mL/min/1.73 m² and 26 eGFR <45 mL/min/1.73 m².

Fig. 4. eGFR values in consecutive time points (from the 3rd, 6th, 12th, and 24th month after KT_x) for patients categorized as slow and fast TAC metabolizers. Horizontal lines represent medians, boxes represent the values of upper and lower quartile, and the error bars represent nonoutlier range. eGFR, estimated glomerular filtration rate.

Table 2. Parameters in the model used to identify patients with eGFR <45 mL/min/1.73 m² in 24 months follow-up

Parameter	β	SE	OR (95% CI)	p value
Intercept	-11.52	2.88		<0.001
TAC metabolism (fast vs. slow)	2.67	0.77	14.452 (3.22–64.92)	<0.001
Donor age, years	0.10	0.03	1.109 (1.04–1.18)	0.001
Recipient age, years	0.07	0.03	1.075 (1.01–1.14)	0.017
Recipient gender, female vs. male	1.27	0.64	3.56 (1.01–12.54)	0.048

After 24 months, 26 patients had eGFR <45 mL/min/1.73 m² and 63 patients had eGFR ≥45 mL/min/1.73 m². Fast TAC metabolism rate was coded as 1 and slow metabolism rate was coded as 0.

TAC, tacrolimus; β , regression coefficient; eGFR, estimated glomerular filtration rate.

At each timepoint, FM_s presented significantly worse graft function compared with SM_s: 40.05 (30.16–51.40) versus 47.91 (36.73–63.82) mL/min/1.73 m² ($p = 0.019$) after 3rd, 49.45 (32.97–57.77) versus 58.46 (42.33–72.71) mL/min/1.73 m² ($p = 0.008$) after 6th, 51.82 (36.95–63.36) versus 61.21 (48.42–73.65) mL/min/1.73 m² ($p = 0.002$) after 12th, and 48.77 (39.71–61.44) versus 64.83 (53.04–77.32) mL/min/1.73 m² ($p = 0.002$) after the 24th month post-KTx (Fig. 4).

We observed no statistically significant differences in eGFR values between men and women at any timepoint (all $p > 0.4$). Patient age was inversely correlated with eGFR at the 3rd ($r = -0.32$; $p = 0.001$), 6th ($r = -0.23$, $p = 0.019$) and 12th month post-KTx ($r = -0.22$, $p = 0.026$), meaning that older patients presented significantly worse graft function.

Most patients entered the study while being treated with immediate-release TAC and were gradually switched to a prolonged-release formulation, to achieve better compliance: 80 patients received Tc and 21 Tc-pr after the 3rd; 70 vs. 31 after the 6th, 65 vs. 36 after the 12th and 55 vs. 35 after the 24th month post-KTx. Three months after KTx patients receiving Tc-pr presented significantly better graft function (eGFR: 62.6 ± 33.6 vs. 42.9 ± 16.4 mL/min/1.73 m², $p = 0.031$). No such relationship was evidenced at consecutive timepoints ($p = 0.219$ for comparison at 6 months, $p = 0.16$ at 12 months, and $p = 0.17$ at 24 months).

Fig. 5. Comparison of models allowing for identification of patients with eGFR ≥ 45 mL/min/1.73 m² in the 24 months follow-up: based only on C/D ratio (TAC trough concentration normalized by the TAC daily dose) from the 6th month (red) and based on slow/fast metabolizer status, donor age, recipient age, and recipient sex (blue).

Table 3. Parameters in the linear regression model with eGFR in 24 months follow-up as a continuous response variable, adjusted for the donor age and recipient hematocrit, TAC formulation, age, and gender

Parameter	β (95% CI)	SE	p value
Intercept	0.16 (−0.228 to 1.595)	0.107	0.007
Hematocrit, %	0.277 (1.494 to 9.264)	0.100	0.139
C/D ratio from the 6th month	−0.156 (−0.565 to 0.077)	0.103	0.007
Recipient age, years	−0.362 (−0.889 to 0.248)	0.102	0.135
Donor age, years	−0.033 (−4.882 to 3.542)	0.105	0.001
Gender(reference class: female)	−0.011 (−5.357 to 4.788)	0.102	0.752
TAC formulation (reference class: advagraf)	0.16 (−0.228 to 1.595)	0.107	0.911

eGFR, estimated glomerular filtration rate; TAC, tacrolimus; β , regression coefficient.

Model Identifying Patients with Compromised Graft Function in Two-Year Follow-Up

Multiple logistic regression analysis with forward stepwise variable selection showed that SM/FM status (for fast vs. slow metabolizers, OR 14.45, 95% CI 3.22–64.92, $p < 0.001$), donor age (OR 1.11, 95% CI 1.04–1.18, $p = 0.001$), recipient age (OR 1.07, 95% CI 1.01–1.14, $p = 0.017$), and recipient sex (for female vs. male, OR 3.56, 95% CI 1.01–12.54, $p = 0.048$) were factors significantly associated with decreased graft function (eGFR < 45 mL/min/1.73 m²) at 2 years post-KTx. All model parameters and regression coefficients are presented in Table 2. The number of observations used to fit this model was 90, because eGFR values were available for 90 patients at 24 months posttransplant.

The model had AUC ROC of 0.875 and allowed for identification of patients with compromised graft function with 66.7% sensitivity and 94.6% specificity. This model was also significantly better than a classifier based solely on Tac C/D values from the 6th month (AUC ROC difference 0.201 ± 0.097 , $p = 0.038$; Fig. 5).

To better describe the relationship between TAC metabolism and eGFR in 24-month follow-up, adjusting for all potential confounders, we performed linear regression analysis adjusting for donor age and recipient age, sex, hematocrit, and C/D value from the 6th month after transplantation and the TAC formulation type he was utilizing at the time. However, due to missing data, this model was built only using 79 observations. After adjusting for these confounders, C/D ratio remained significantly associated with the outcome ($p = 0.007$). Parameters for this model are presented in Table 3.

Discussion

Our results suggest that KTx recipients with lower TAC C/D ratio present notably worse kidney graft function.

Thölking et al. [20] used this parameter to express TAC metabolism rate and showed that patients with faster TAC metabolism presented worse graft function and higher mortality rate in a 24-month follow-up, compared with intermediate and slow metabolizers. The same researchers reported that LTx recipients classified as FMs presented worse kidney function in a 3-year observation [21]. The authors expressed the need to further investigate the applicability of C/D ratio as a practical indicator of TAC metabolism.

They used the mean of C/D ratios from 3 timepoints – the 1st, 3rd, and 6th month after KTx, to divide patients into 3 equally numbered groups: fast (C/D ratio $<1.05 \text{ ng/mL} \cdot 1 \text{ mg}$), intermediate (C/D ratio $1.05\text{--}1.54 \text{ ng/mL} \cdot 1 \text{ mg}$), and slow (C/D ratio $>1.55 \text{ ng/mL} \cdot 1 \text{ mg}$) TAC metabolizers [20]. In LTx patients they used the median C/D ratio from 6 months after surgery of $1.09 \text{ ng/mL} \cdot 1 \text{ mg}$ in order to classify patients as FMs or SMs [21]. In our retrospective analysis, we established the C/D ratio of $1.47 \text{ ng/mL} \cdot 1 \text{ mg}$ assessed 6 months after KTx as the cutoff value for dividing patients into FMs ($<1.47 \text{ ng/mL} \cdot 1 \text{ mg}$) and SMs ($\geq 1.47 \text{ ng/mL} \cdot 1 \text{ mg}$) by using ROC curve analysis and calculating the Youden index to identify the cutoff value with the highest sensitivity and specificity for identifying patients with $\text{eGFR} < 45 \text{ mL/min/1.73 m}^2$ in the 24th month after KTx.

To build a simple classifier, we included other clinical and laboratory variables in a model that could be used by clinicians to identify patients at risk of impaired graft function. This model, based on TAC metabolism rate, age and sex of the recipient and age of the donor, significantly outperformed the classifier based solely on TAC C/D ratio, yielding 66.7% sensitivity and 94.6% specificity for detection of patients with $\text{eGFR} < 45 \text{ mL/min/1.73 m}^2$ in the 24th month after KTx. This is in concordance with previous findings and indicates the need to include TAC metabolism rate in early risk assessment alongside well-established factors such as donor and recipient age. Interestingly, the adjusted OR of developing $\text{eGFR} < 45 \text{ mL/min/1.73 m}^2$ was 14 times higher for fast metabolizers compared with slow metabolizers, highlighting the importance of considering this factor in patient management after KTx. However, in order to widely and efficiently incorporate this parameter in clinical practice, we ought to study a far larger population and establish a common cutoff value.

As the relation between TAC C/D ratio and impaired graft function might have been confounded because of the stepwise algorithm used to build the model, we estimated this relation by linear regression model adjusting for the donor age and recipient age, sex, hematocrit, and type of TAC formulation. The highly significant relationship between C/D ratio and outcome variable after adjustment for these additional confounders ($p = 0.007$) further validates our observation that TAC metabolism rate is an important factor, associated with the kidney graft function.

Several factors influence TAC absorption and metabolism in the early period after KTx, including the induction of TAC metabolism by steroids [15, 18, 25]. Therefore, to exclude the impact of early immunosuppressive regimen on TAC metabolism rate, we chose to assess TAC C/D ratio no sooner than 3 months post-KTx, when all patients receive a stabilized prednisolone dose (5 mg/day) and have completed a routine 90-day opportunistic infections preventive treatment with valganciclovir, trimethoprim-sulfamethoxazole, and nystatin. TAC C/D ratios from the 6th, 12th, and 24th month after KTx were strongly correlated with each other, but not with the TAC C/D ratio at the 3rd month after KTx, supporting the hypothesis that the C/D ratio at very early periods after KTx should not be utilized to predict long-term graft function. Hence, we chose the C/D ratio from the 6th month after KTx, a timepoint when immunosuppressive regimen is established, and most patients receive a fixed, specific treatment for comorbidities, as, in our opinion, an optimal timepoint for early prediction of kidney graft function in a long-term follow-up.

Egeland et al. [26] reported that high TAC clearance (calculated by dividing the total TAC daily dose by TAC $C_{p\text{ trough}}$) was associated with an increased risk of acute rejection during the first 3 months after KTx. Recently, they showed that high TAC clearance was associated with the development of IFTA from 7 weeks to 1 year after KTx, compared in paired protocol biopsies. They noted that patients who required 7.5 mg TAC per day to achieve a proper trough concentration were twice as likely to develop IFTA as patients taking 2.5 mg per day [6]. In our study group, FM patients required notably higher TAC doses than SM patients to achieve the same, recommended TAC $C_{p\text{ trough}}$. However, our patients did not undergo protocol biopsies, so we could not confirm whether FM patients were also more likely to develop IFTA.

To the best of our knowledge, we are the first to analyze the TAC metabolism rate (C/D ratio) and its association with graft function both in patients who received standard Tc formulation and patients treated with Tc-pr. In our study, numerous patients were switched from Tc to Tc-pr, mainly to improve compliance. No significant differences in TAC C/D ratio and graft function at any timepoint (except for better graft function in Tc-pr patients 3 months post-KTx) were evidenced between Tc and Tc-pr recipients. This is hardly surprising, given the similarities in their absorption site, distribution, metabolism and secretion. Tc-pr exhibits a slightly lower maximum serum concentration (C_{max}), requires more time to achieve it (T_{max}) [14], and appears to have more stable blood concentrations compared with Tc [27].

In our study, 11 indication graft biopsies were performed, 3–24 months after KTx.

BK nephropathy was diagnosed only in FM patients (2 cases). This happens to be in concordance with the observations of Thölking et al. [20], who reported significantly more histologically proven cases of BK nephropathy and CNIT among fast TAC metabolizers. There were no cases of biopsy-proven CNIT in our patients.

We would like to emphasize that our findings do not determine the cause of deteriorated graft function in fast TAC metabolizers; it might result from the occasional occurrence of either too low concentrations, and therefore immunosuppression deficiency and chronic graft rejection, or too high concentrations exerting toxic effect.

We observed no impact of age or sex on individual TAC metabolism rate in our patients. Some authors suggest that females require higher TAC doses [28] but others disagree [20, 29]. Likewise, some found no relationship between age and TAC metabolism [30, 31], while others report that younger patients metabolize TAC faster [20, 32, 33].

An ethnically homogenous group analyzed in our study can be viewed as an advantage, limiting the confounding impact of ethnic differences in CYP3A variants. African Americans require higher TAC doses than Caucasians or Asians [15, 34], and this has been attributed mainly to differences in CYP3A or glycoprotein P variants [35]. Since our study group consisted

solely of Caucasians, our results should not be extrapolated to other populations. Furthermore, CYP3A genotypes are unknown in our patients. Although genetic polymorphisms have been shown to significantly influence TAC metabolism, genetic testing of CYP3A is unavailable in routine clinical practice; furthermore, no relationship between CYP3A genotype and serum creatinine was evidenced in 5-year follow-up after KTx [18, 36].

Our study is retrospective and based solely on documentation analysis. For maximum reliability, only complete records were included in the analysis. The influence of TAC metabolism on glycemia and proteinuria was not assessed because these parameters had not been measured at all timepoints.

As the BMI records were not available for all patients at all timepoints, we were unable to include this factor in the analysis. A previous study shows a link between higher BMI and slow TAC metabolism [15]. However, Thölking et al. [20, 21] did not find any statistically significant association between patients' BMI and TAC metabolism rate or kidney function.

Conclusions

Faster TAC metabolism is associated with worse kidney graft function. Estimating the C/D ratio, a simple indicator of TAC metabolism rate, at 6 months post-KTx may be useful in clinical practice for early identification of patients at risk of deteriorated graft function in a long-term follow-up.

Acknowledgement

None.

Statement of Ethics

The study was approved by the Bioethical Committee at the Medical University of Lodz (Lodz, Poland) on October 16, 2018, approval no. RNN/337/18/KE. Informed consent was given by all participants at the time of transplantation to record their clinical data and use in anonymized analyzes.

Disclosure Statement

The authors declare no conflicts of interest.

Funding sources

None.

Data Accessibility

The data that support the findings of this study are available upon request from the corresponding author ilona.kurnatowska@umed.lodz.pl.

Author Contributions

M.N.: study design, data acquisition, data interpretation, literature search, manuscript preparation, manuscript revision. M.G.: study design, data acquisition, data interpretation, manuscript preparation, manuscript revision. Z.N.: statistical analysis, data interpretation, manuscript preparation, manuscript revision. K.E., P.E., S.W., A.Z.-B., J.S., and J.M.: data acquisition, manuscript revision. I.K.: study design, data interpretation, literature search, manuscript preparation, manuscript revision.

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