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ORIGINAL RESEARCH

Impact of High Tacrolimus Exposure on Graft Function and Development of a Prediction Model in Kidney Transplant Recipients: A Single-Center Retrospective Cohort Study

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Purpose: To evaluate the association between early supratherapeutic tacrolimus exposure (trough concentration >20 ng/mL within the first postoperative week) and graft outcomes in kidney transplant recipients, and to develop a prediction model for this early high exposure.

Patients and Methods: This single-center retrospective cohort study included 210 kidney transplant recipients (105 exposed, 105 non-exposed) after propensity score matching. Renal function (eGFR, cystatin C) and infection rates over the first year were compared using linear mixed-effects models with multiple imputation. A Cox proportional hazards model with LASSO variable selection was developed to predict the risk of high exposure.

Results: The exposed group had significantly lower eGFR at 7 days, 1, and 3 months (adjusted mean differences: −11.32, −10.20, −10.75 mL/min/1.73 m^2) and higher cystatin C (0.83, 0.36, 0.36 mg/L). At 1 year, eGFR remained lower (−7.54 mL/min/1.73 m^2, P=0.038) and cystatin C higher (0.40 mg/L, P=0.043) in the exposed group. The 1-year infection rate was higher in the exposed group (80.0% vs 52.3%; adjusted OR=4.27, P<0.001). The prediction model identified *CYP3A5 *3/*3* genotype (HR=2.19), elevated C-reactive protein on day 1 (HR=1.27), and higher weight-adjusted tacrolimus dose (HR=1.58) as key predictors (C-index=0.716; optimism-corrected C-index=0.728).

Conclusion: Early high tacrolimus exposure is associated with impaired renal function and increased infection risk in the first year after transplantation. A prediction model incorporating genetic, inflammatory, and dosing factors could aid in early risk stratification.

Keywords: therapeutic drug monitoring, pharmacogenomics, predictive model, early exposure, risk prediction

Introduction

In kidney transplantation, calcineurin inhibitors form the cornerstone of standard immunosuppressive therapy, with tacrolimus (TAC) and mycophenolate serving as the core agents.[1] According to the 2021 US Annual Data Report on kidney transplantation,[2] over 90% of patients receive a TAC and mycophenolate-based regimen. Although the management of TAC concentrations is debated, most studies[1,3–5] support maintaining higher levels, and current guidelines[6] recommend elevated trough concentrations (C0) early after transplantation to enhance immunosuppressive efficacy. This recommendation is further substantiated and refined by recent international consensus and reviews.[7,8] However,

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significant interindividual variability in TAC metabolism often leads to C0 exceeding the recommended upper limit in the early post-transplant period.[9]

A TAC C0 of 15 ng/mL is considered a toxicity threshold, while levels exceeding 20 ng/mL signify high nephrotoxicity risk.[10] Notably, international consensus acknowledges a target C0 range extending up to 20 ng/mL in the initial months post-transplant.[7] Chinese clinical guidelines[11,12] also recommend maintaining TAC C0 within 8–15 ng/mL early postoperatively, frequently following a "higher is safer" principle to minimize rejection risk. Based on long-term observations at our center showing a marked increase in adverse events when C0 surpasses 20 ng/mL, this study defines >20 ng/mL as the critical "high-risk exposure" threshold. Real-world studies in kidney transplantation[13–17] confirm that a subset of patients present with TAC C0 approaching or even exceeding 20 ng/mL shortly after transplant. This phenomenon contrasts with conventional early therapeutic targets (eg, 8–12 ng/mL for months 0–3).[18] These findings highlight the clinical tension between effective immunosuppression and the nephrotoxic potential of high TAC exposure. The precise impact of such early high-level exposure on graft function remains unclear: does it facilitate faster recovery due to potent immunosuppression, or does it cause impairment due to direct nephrotoxicity? This critical question warrants investigation.

This single-center retrospective cohort study aims to evaluate the impact of early postoperative high TAC exposure (C0 > 20 ng/mL) on graft outcomes, hematological and metabolic parameters, and infection risk. Furthermore, we seek to develop a prediction model for early high-exposure risk. We anticipate that our findings will inform more precise TAC dosing strategies to optimize therapy, improve safety, and enhance long-term graft prognosis.

Materials and Methods

Study Design

This single-center retrospective cohort study compared kidney transplant recipients with early high tacrolimus exposure (≥1 trough concentration >20 ng/mL within 7 days post-transplant) to a non-exposed group, defined as having all trough concentrations <20 ng/mL during the same 7-day window. Patients who underwent transplantation between January 2019 and June 2023 were retrospectively enrolled. The study protocol was approved by the Ethics Committee of the First Clinical Medical College of Southern Medical University (NFEC-2024-390). The ethics committee granted a waiver of informed consent because this retrospective analysis used only existing medical records, posed no more than minimal risk to the subjects, the waiver would not adversely affect their rights and welfare, and all subject privacy and personal information were protected. All patient data were de-identified and kept strictly confidential throughout the analysis. All kidneys were obtained from voluntary deceased donors, with written informed consent procured for donation, in full compliance with national regulations and the Declaration of Istanbul. To minimize confounding from baseline imbalances, a propensity score-matched (PSM) cohort was constructed for the primary analysis. The flow of participant screening, inclusion, and matching is illustrated in Figure 1. After matching, 105 patients were included in each group (exposed vs non-exposed), resulting in a total analytical sample of 210 patients.

Patient Selection

Data were extracted from the electronic medical record system. Inclusion criteria were: first-time kidney transplant recipients who received a tacrolimus-based maintenance immunosuppressive regimen (tacrolimus, mycophenolate mofetil [MMF] or mycophenolate sodium [MPS], and corticosteroids) throughout the first post-transplant year. Exclusion criteria comprised: immediate postoperative conversion to cyclosporine or sirolimus; death within the first year due to acute rejection or other unknown causes; and lack of complete medical records or key laboratory follow-up data.

Cohort Construction

From an initial screen of patients transplanted between January 2019 and June 2023, 286 recipients (144 exposed, 142 non-exposed) met the selection criteria and constituted the pre-matching cohort for propensity score analysis.

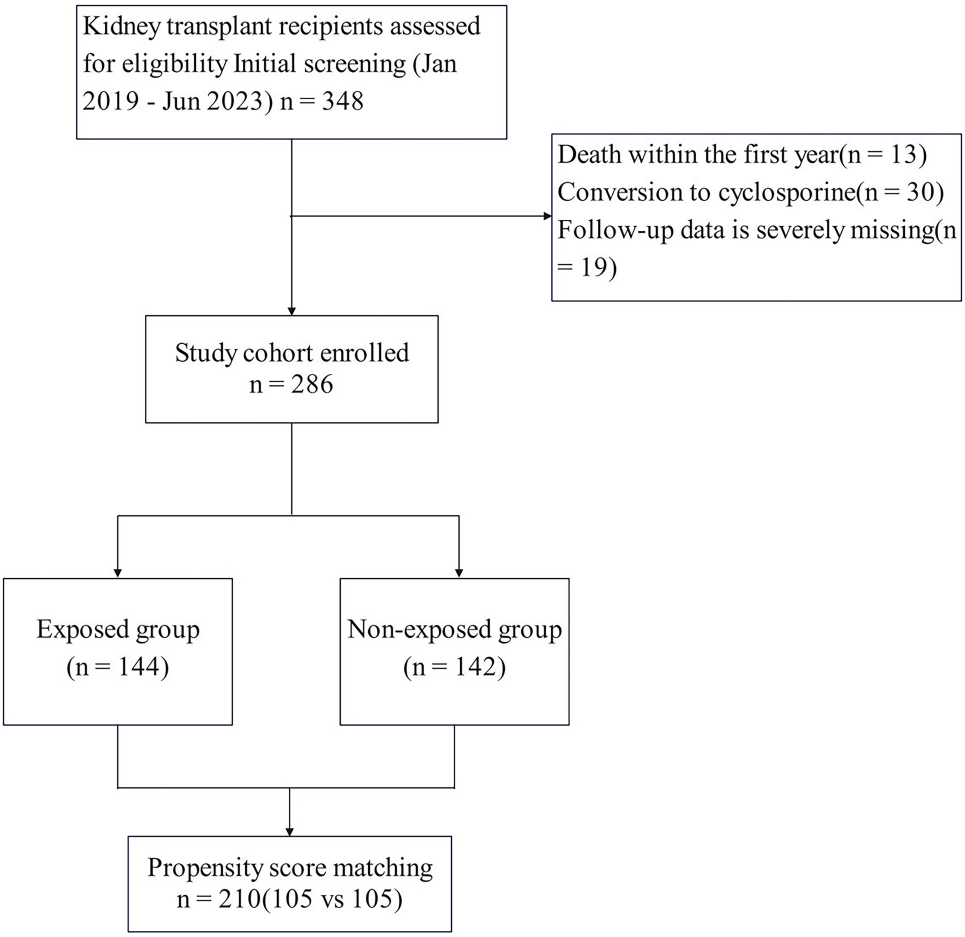

Figure 1 Flowchart.

Immunosuppressive Regimen

All patients received induction immunosuppression consisting of intravenous basiliximab (20 mg) administered intraoperatively and on postoperative day 4. Intravenous methylprednisolone was given as 1 g on day 1, 500 mg on day 2, and 250 mg on days 3 and 4. Oral methylprednisolone (20 mg/day) was started on day 1 and tapered to a maintenance dose (4 mg/day). Rabbit anti-thymocyte globulin (50 mg/day) could be added on days 1–3 based on individual clinical indications.

Maintenance immunosuppression was initiated on postoperative day 1: oral tacrolimus starting at 0.05–0.15 mg/kg/day administered every 12 h; enteric-coated mycophenolate sodium (MPS) at 0.5–0.75 g twice daily; and corticosteroids (methylprednisolone 4–8 mg/day or prednisone 5–10 mg/day).

Infection Prophylaxis

All patients received routine antibacterial prophylaxis with agents such as cefoperazone-sulbactam, meropenem, or linezolid. For cytomegalovirus (CMV) and Pneumocystis jirovecii pneumonia (PJP), oral valganciclovir was initiated within 10 days after transplantation and continued for 3–6 months. Postoperative prophylaxis with trimethoprim-sulfamethoxazole (TMP-SMX) was routinely recommended for 6–12 months.

Therapeutic Drug Monitoring and Genotyping

Tacrolimus whole-blood trough concentrations (C0) were monitored after steady state was achieved. Venous blood (1.0 mL) was collected in EDTA tubes immediately before the morning dose. C0 was measured using an enzyme-linked

immunosorbent assay (ELISA) kit on a Siemens Syva Emit-2000 analyzer, with reagents, quality controls, and calibration curves supplied by Siemens Healthcare Diagnostics (Shanghai). During the perioperative period (early postoperative hospitalization), C0 was measured three times per week. Monitoring frequency was increased if C0 exceeded 20 ng/mL or if related adverse events occurred. CYP3A5 genotyping was performed for all patients using the TaqMan probe method. Genomic DNA was extracted from EDTA-anticoagulated peripheral blood samples using a nucleic acid purification reagent. The CYP3A5*3 (6986A>G, rs776746) genotype was determined using a commercial assay kit (Beijing Huaxia Shidai Gene Co., Ltd., Medical Device Filing No. 20150009) based on fluorescence in situ hybridization with dual-labeled (FAM/HEX) cleavable oligonucleotide probes. Fluorescence signals were detected on a compatible instrument, with a signal intensity ≥1000 considered a valid result. All steps were performed according to the manufacturer's protocol.

Study Endpoints

Primary Endpoints

Renal graft function was compared between the exposed and non-exposed groups at 7 days, 1 month, 3 months, 6 months and 1 year after transplantation. Graft function was assessed using estimated glomerular filtration rate (eGFR) and serum cystatin C (CysC) levels. eGFR was calculated with the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation: eGFR (mL/min/1.73 m^2) = a × (Scr / b)^c × (0.993)^age, where a = 163 for Black men, 166 for Black women, 141 for non-Black men and 144 for non-Black women; b = 0.9 mg/dL for men and 0.7 mg/dL for women; c = -0.411 for men with Scr ≤ 0.9 mg/dL and -1.209 for men with Scr > 0.9 mg/dL, and c = -0.329 for women with Scr ≤ 0.7 mg/dL and -1.209 for women with Scr > 0.7 mg/dL. CysC was measured by latex-enhanced immunoturbidimetry on Roche Cobas 8000 or Beckman AU 5431 analyzers.

Secondary Endpoints

Secondary outcomes were evaluated according to physiological and clinical relevance, including: liver function markers (alanine aminotransferase [ALT], aspartate aminotransferase [AST]); hematological parameters (white blood cell count [WBC], hemoglobin [HGB], platelet count [PLT]); metabolic indicators (fasting blood glucose [Glu], triglycerides [TG], total cholesterol [TC], low-density lipoprotein cholesterol [LDL-C]); other renal function measures (serum uric acid, blood urea nitrogen, serum potassium [K+]); and the incidence of infection. Infection was defined based on clinical diagnosis supported by laboratory or imaging evidence, as documented in the electronic medical records.

Statistical Analysis

Categorical variables are presented as frequencies (percentages) and compared using Pearson's chi-square test or Fisher's exact test, as appropriate. Continuous variables are expressed as mean ± standard deviation and compared using the independent-samples t-test or the Mann–Whitney *U*-test after assessing normality (Shapiro–Wilk test) and homogeneity of variance (Levene's test).

To address baseline imbalance, we performed 1:1 propensity-score matching without replacement using the MatchIt package. Propensity scores were estimated via logistic regression, adjusted for age, sex, BMI, CRP, postoperative day 1 estimated glomerular filtration rate (eGFR), postoperative day 1 cystatin C (CysC), and induction regimen. A caliper width of 0.2 standard deviations of the logit propensity score was applied to ensure matching quality. The matching yielded a balanced cohort of 105 patients in group 1 and 105 patients in group 2 for the primary analysis.

Missing data were handled using multiple imputation via the mice package with the 2L.pan and 2L.norm methods, generating 20 imputed datasets over 30 iterations. For the primary renal outcomes (eGFR, CysC), linear mixed-effects models (LMMs) were fitted using the lme4 package, including group, time, group-by-time interaction, and adjusting for age, sex, BMI, tacrolimus concentration, mycophenolic acid concentration, and induction regimen, with a random intercept for participant ID. Longitudinal secondary outcomes were analyzed similarly. Results from the imputed datasets were pooled using Rubin's rules.

A multivariable Cox proportional-hazards model was developed to predict the risk of a high-tacrolimus event (C0 > 20 ng/mL) occurring within 7 days post-transplant. Predictor selection was performed using LASSO regression

via the glmnet package on each imputed dataset; variables retained in ≥50% of the imputed models were included in the final Cox model fitted with the survival package. The model's discrimination was assessed with the C-index, and internal validation was performed via 500 bootstrap resamples using the boot package. Calibration was evaluated with a grouped calibration curve.

For longitudinal group comparisons, the false-discovery rate (FDR) was controlled using the Benjamini–Hochberg method separately for each outcome family. Data manipulation and visualization were supported by the tidyverse package suite. All analyses were performed with R (version 4.5.0). A two-sided FDR-adjusted $P < 0.05$ was considered statistically significant.

Results

Patient Characteristics

The baseline characteristics after propensity score matching are shown in Table 1. No statistically significant differences were observed between the two groups regarding demographics, baseline renal function, most laboratory parameters, or induction regimen (all $p > 0.05$). The exposed group exhibited higher white blood cell count (12.2 ± 4.0 vs 11.1 ± 4.3 $\times 10^9$/L, p=0.030), fasting glucose (8.6 ± 2.6 vs 7.5 ± 2.4 mmol/L, p=0.003), and daily tacrolimus dose (8.2 ± 1.5 vs 7.7 ± 1.5 mg/d, p=0.014). The proportion of patients carrying the *CYP3A5 *3/*3* genotype was higher in the exposed group (68.3% vs 30.2%, p<0.001). Baseline characteristics before matching are shown in Supplementary Table S1. Missing data for key variables were quantified (Supplementary Tables S2 and S3) and handled using multiple imputation.

Table 1 Characteristics of Kidney Transplant Recipients (After PSM)

Variable	Exposed Group (n = 105)	Non-Exposed Group (n = 105)	P
Gender			0.882
Male, n%	70(66.7%)	72(68.6%)	
Female, n%	35(33.3%)	33(31.4%)	
Age (years)	43.3±11.5	43.1±11.3	0.889
BMI	21.9±3.1	22.0±3.2	0.818
BSA (m2)	1.6±0.2	1.6±0.2	0.885
Weight (kg)	60.7±12.2	60.4±11.0	0.877
WBC (×109/L)	12.2±4.0	11.1±4.3	0.030
HGB (g/L)	109.4±16.5	105.9±16.5	0.106
PLT (×109/L)	186.5±60.3	192.9±63.4	0.462
HCT (L/L)	0.3±0.1	0.3±0.1	0.094
eGFR (mL/min/1.73m2)	7.7±3.9	7.8±3.9	0.599
CysC (mg/L)	3.9±1.5	3.7±1.7	0.279
Urea (mmol/L)	19.3±6.2	19.5±5.7	0.762
UA (μmol/L)	482.8±137.3	468.6±136.0	0.450
Urine output (mL)	3803.8±2186.9	3877.6±1993.4	0.369
ALT (U/L)	15.9±14.4	14.5±11.4	0.969
AST (U/L)	19.2±13.0	17.1±8.8	0.328
ALB (g/L)	35.9±4.0	36.9±5.6	0.368
TBIL (umol/L)	10.6±7.0	9.4±6.8	0.102
GLU (mmol/L)	8.6±2.6	7.5±2.4	0.003
CRP (mg/L)	29.6±24.4	30.1±25.0	0.977
TAC dose (mg/d)	8.2±1.5	7.7±1.5	0.014
MPS dose (mg/d)	946.3±174.8	913.3±183.7	0.191
GC dose (mg/d)	1495.2±125.6	1508.1±63.6	0.675

(*Continued*)

Table 1 (Continued).

Variable	Exposed Group (n = 105)	Non-Exposed Group (n = 105)	P
Immune induction			0.889
Monotherapy, n%	58(55.2%)	60(57.1%)	
Dual Therapy, n%	47(44.8%)	45(42.9%)	
Systolic blood pressure (mmHg)	148.3±11.9	146.4±10.6	0.675
Diastolic blood pressure (mmHg)	90.0±8.9	89.1±10.0	0.463
Diabetes, n%	18(17.1%)	19(18.1%)	1.000
Hypertension, n%	84(80.0%)	74(70.5%)	0.150
CYP3A5			<0.001
*1/*1+*1/*3	19(31.7%)	44(69.8%)	
*3/*3	41(68.3%)	19(30.2%)	

Notes: Monotherapy, Basiliximab or rabbit anti-human thymocyte globulin; Dual Therapy, Basiliximab combined with rabbit anti-thymocyte globulin.*1/*1 and *1/*3 are rapid/intermediate metabolizers, while *3/*3 are slow metabolizers.

Abbreviations: BMI, body mass index; BSA, Body Surface Area; UA, Uric acid; GLU, Glucose; HCT, Hematocrit; ALB, albumin; TBIL, Total bilirubin; TAC, Tacrolimus; MPS, mycophenolate sodium; GC, Glucocorticoids.

Primary Outcomes

The longitudinal trajectories of graft renal function (eGFR and CysC) are presented in Supplementary Figure S1 (eGFR) and S2 (CysC). At all postoperative time points (7 days, 1, 3, 6, and 12 months), the mean eGFR was higher in the non-exposed group (eg, 64.0 ± 21.9 vs 57.1 ± 25.6 mL/min/1.73 m^2 at 1 year), while the mean CysC was lower (eg, 1.58 ± 0.48 vs 1.92 ± 0.93 mg/L at 1 year).

Analysis using linear mixed-effects models based on multiply imputed data revealed associations between early high exposure and renal function parameters (Table 2). After adjustment for covariates, the exposed group had lower eGFR values at 7 days, 1 month, and 3 months (adjusted mean difference: −11.32, −10.20, −10.75 mL/min/1.73 m^2, respectively; all FDR-adjusted $P < 0.05$) and higher CysC levels (adjusted mean difference: 0.83, 0.36, 0.36 mg/L, respectively; all FDR-adjusted $P < 0.05$). At 6 months and 1 year, the negative differences in eGFR persisted (adjusted mean difference: −8.01 and −7.54 mL/min/1.73 m^2), with the difference at 1 year remaining statistically significant (FDR-adjusted $P = 0.038$). At 1 year, CysC levels were also higher in the exposed group (adjusted mean difference: 0.40 mg/L, FDR-adjusted $P = 0.043$).

Table 2 Adjusted Differences in Renal Function Parameters Between Exposure Groups Over Time After Transplantation (Multiple Imputation)

Time Point	Outcome	Adjusted Mean Difference (95% CI)	Unadjusted P	FDR-Adjusted P
7 days	eGFR	−11.32 (−18.37 to −4.28)	0.003	0.010
	CysC	0.83 (0.50 to 1.16)	<0.001	<0.001
1 month	eGFR	−10.20 (−17.15 to −3.25)	0.006	0.010
	CysC	0.36 (0.03 to 0.68)	0.033	0.043
3 months	eGFR	−10.75 (−17.72 to −3.77)	0.004	0.010
	CysC	0.36 (0.03 to 0.69)	0.035	0.043
6 months	eGFR	−8.01 (−15.01 to −1.01)	0.027	0.034
	CysC	0.28 (−0.04 to 0.61)	0.086	0.086
1 year	eGFR	−7.54 (−14.62 to −0.46)	0.038	0.038
	CysC	0.40 (0.06 to 0.73)	0.022	0.043

Notes: Adjusted mean difference (95% confidence interval) represents the difference of exposure group compared to non-exposure group (reference). FDR adjustment using Benjamini-Hochberg method for 5 hypothesis tests.All linear mixed models were adjusted for age, sex, BMI, tacrolimus concentration, and mycophenolic acid concentration, with random intercepts set for participant ID. Multiple imputation was performed with 20 iterations.

To assess robustness, a complete-case analysis was performed (Supplementary Table S4). Results were consistent with the primary analysis in both direction and statistical significance. Detailed fixed-effect parameters from the linear mixed-effects models are provided in Supplementary Tables S5 (eGFR) and S6 (CysC).

Secondary Outcomes

Results for secondary outcomes are summarized in supplementary tables. Analyses employed linear mixed-effects models adjusted for covariates, with multiple imputation (20 iterations).

Analysis of other renal function parameters (Supplementary Table S7) showed that serum urea was lower in the non-exposed group at 7 days (adjusted mean difference: −4.94 mg/dL, FDR-adjusted P = 0.0008). At 1 year, serum potassium was lower in the non-exposed group (adjusted mean difference: −0.17 mmol/L, FDR-adjusted P = 0.174). No other significant differences were found.

Liver enzymes (ALT and AST, Supplementary Table S8) and metabolic parameters (including glucose and lipids, Supplementary Table S9) showed no statistically significant differences between groups after FDR adjustment (all FDR-adjusted P > 0.05).

For hematological parameters (Supplementary Table S10), no sustained significant differences were observed in white blood cell or platelet counts (all FDR-adjusted P > 0.05). For hemoglobin, a lower level was observed in the non-exposed group at 7 days before multiple testing correction (adjusted mean difference: 5.92 g/L, uncorrected P = 0.044), but this was not significant after FDR adjustment (adjusted P = 0.220).

Regarding infection outcomes, the exposed group had a higher one-year postoperative infection rate (80.0% [84/105] vs 52.3% [55/105]). After adjustment, the exposed group was associated with increased odds of infection (adjusted OR = 4.27, 95% CI: 2.22–8.52, P < 0.001; Supplementary Table S11). Pathogen-specific infection rates are detailed in Supplementary Table S12.

Drug Concentrations

Trends in tacrolimus (TAC) and mycophenolic acid (MPA) blood concentrations are shown in Supplementary Figure S3 (TAC) and S4 (MPA). TAC concentrations declined over time in both groups.

At 7 days post-transplant, TAC concentration was higher in the exposed group (12.88 ± 5.46 vs 10.12 ± 2.70 ng/mL, p < 0.001). At subsequent time points (1, 3, 6, and 12 months), mean TAC concentrations were comparable between groups (eg, 7.72 ± 2.13 vs 7.67 ± 1.67 ng/mL at 1 year), with no statistically significant differences (all p > 0.05).

Throughout follow-up, MPA concentrations showed no statistically significant differences between groups at any time point (all p > 0.05). The concentration ranges for MPA overlapped between groups (exposed: 2.68–3.44 μg/mL; non-exposed: 2.77–3.49 μg/mL).

Sensitivity Analysis

A sensitivity analysis was conducted by increasing the exposure threshold to 25 ng/mL. A balanced cohort of 75 exposed and 75 non-exposed patients was constructed using propensity score matching (baseline in Supplementary Table S13). After multiple imputation, renal function outcomes were re-analyzed.

Under this higher threshold, the exposed group continued to show lower eGFR and higher CysC levels (Supplementary Table S14). Specifically, eGFR was lower at 7 days, 3 months, and 1 year (adjusted mean difference: −12.53, −8.79, −8.90 mL/min/1.73 m^2, respectively; all FDR-adjusted P < 0.07), and CysC was higher at 7 days (adjusted mean difference: 0.98 mg/L, FDR-adjusted P < 0.001). These trends align with the primary analysis, supporting the robustness of the associations.

Prediction Model

Results of the Cox proportional hazards model are as follows. The *CYP3A5 *3/*3* genotype was associated with the highest risk (HR = 2.19, 95% CI: 1.44–3.33, p = 0.0004). Weight-standardized daily tacrolimus dose (HR = 1.58, 95% CI: 1.27–1.97, p < 0.0001) and elevated CRP on postoperative day 1 (HR = 1.27, 95% CI: 1.07–1.52, p = 0.0085) were also associated with increased risk. Serum albumin showed a trend toward a protective effect (HR = 0.82, 95% CI:

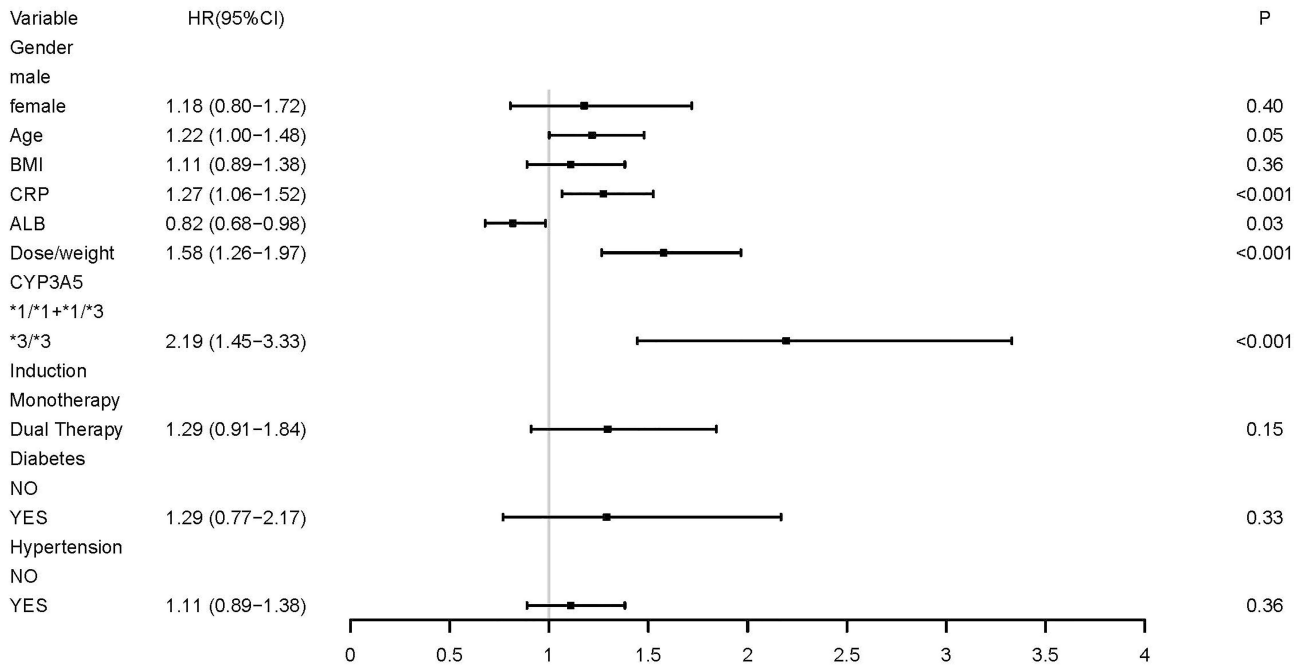

Figure 2 Forest Plot for Risk Prediction Model of Early Tacrolimus Overexposure in Kidney Transplant Recipients.
Notes: dose/weight: Weight-standardized daily tacrolimus dose (mg/kg); CYP3A5*3/*3: Homozygous variant genotype (reference: *1/*1+ *1/*3); $h(t \mid \mathbf{X}) = h_0(t)\exp(0.20 \cdot \text{Age} + 0.24 \cdot \text{CRP} + 0.46 \cdot \text{TAC dose/weight} + 0.79 \cdot \text{CYP3A5*3/*3} + 0.39 \cdot \text{Hypertension} + 0.26 \cdot \text{Dual induction} + 0.16 \cdot \text{Female} - 0.20 \cdot \text{Albumin} + 0.13 \cdot \text{Total bilirubin} + 0.26 \cdot \text{Diabetes} + 0.10 \cdot \text{BMI})$ *1/*1 and *1/*3 are rapid/intermediate metabolizers, while *3/*3 are slow metabolizers.

0.68–0.98, p = 0.033). Age, sex, diabetes, hypertension, BMI, and induction regimen did not show independent predictive value (all p > 0.05). Variable selection was performed using LASSO regression, which applies coefficient shrinkage. Hazard ratios are visualized in Figure 2.

The model's discrimination, measured by the C-index, was 0.716. Internal validation via 500 bootstrap resamples yielded an optimism-corrected C-index of 0.728 (optimism = −0.012). The calibration curve for the 7-day risk prediction (Supplementary Figure S5) demonstrated good agreement between predicted and observed risks, with a mean absolute error (MAE) of 0.027. It is noted that validation was internal only.

Discussion

This retrospective cohort study, utilizing propensity score matching and multiple imputation to address confounding and missing data, demonstrates that kidney transplant recipients with early postoperative exposure to high tacrolimus trough concentrations (C_0 > 20 ng/mL within 7 days) were associated with significantly poorer short- to medium-term renal allograft function compared to a matched non-exposure group. The association was evidenced by consistently lower eGFR and higher cystatin C (CysC) levels up to one year post-transplant. Furthermore, early high exposure was significantly associated with a markedly increased risk of infection within the first year (80.0% vs 52.3%; adjusted OR = 4.27, 95% CI: 2.22–8.52, P<0.001). In contrast, no significant associations were found with parameters of glucose or lipid metabolism, or with liver function. These findings suggest that early supratherapeutic TAC exposure may confer a dual risk: potential nephrotoxicity affecting graft function and heightened susceptibility to infections, likely stemming from its potent immunosuppressive effects.

When interpreting these associations, several methodological limitations of this study must be acknowledged. First, in the original cohort, approximately 70% of patients in the non-exposed group maintained tacrolimus concentrations below 20 ng/mL throughout the entire first postoperative year. This means the comparison in this study essentially involves an "early single episode of high exposure" group versus a control group predominantly composed of patients with "long-term stable low exposure," which may introduce selection bias and overestimate the true effect size. Second, despite balancing measurable covariates via propensity score matching, the possibility of residual confounding from unmeasured

or inadequately measured factors (eg, donor-recipient characteristics, detailed medication adherence behaviors, etc.) cannot be excluded. These inherent limitations of the observational study design warrant caution when inferring causal relationships. Nonetheless, consistent direction of effects and statistical significance across multiple time-point analyses and sensitivity analyses, coupled with biological plausibility, support the clinical relevance of the observed.

The observed mean eGFR in our propensity score-matched non-exposure cohort at one year post-transplant (64.0 mL/min/1.73 m^2) aligns with the reported multicenter average of 51.1 ± 18.7 mL/min/1.73 m^2,[19] indicating that our matched cohort achieved a representative and satisfactory level of graft function. In contrast, the exposure group exhibited a lower mean eGFR (57.1 ± 25.6 mL/min/1.73 m^2). The significant, adjusted difference in eGFR favoring the non-exposure group persisted across multiple time points, as detailed in our primary analysis. This consistent pattern suggests that early high tacrolimus exposure is associated with a measurable and sustained detriment in renal allograft function within the first post-transplant year.

The association between early high TAC exposure and renal allograft dysfunction was evident across multiple post-transplant time points in our cohort. This association was robustly demonstrated by significantly reduced eGFR at all intervals (7 days to 1 year) and elevated cystatin C (CysC) at most time points (except 6 months) in the exposure group compared to the matched controls. This observed pattern is mechanistically plausible, as it aligns with the known pharmacological effects of TAC, including afferent arteriolar vasoconstriction and vascular endothelial cell apoptosis,[20] which are implicated in both functional impairment and potential structural renal damage. Our clinical findings are consistent with prior evidence. A retrospective cohort study by Cosio et al[21] reported that eGFR changes within the first 3 weeks post-transplant under relatively high TAC levels were associated with early nephron injury. Furthermore, Steegh et al[22] found that the loss of peritubular capillaries in the initial post-transplant period correlated with long-term outcomes, a histopathological finding that could underlie the sustained functional deficits we observed. Supporting the biological plausibility of concentration-dependent effects, in vitro experiments have demonstrated that high TAC concentrations can induce significant damage to renal cells.[23]

A nuanced finding in our study was the partial dissociation between eGFR and CysC trends at 6 months, where the eGFR difference remained significant but the CysC difference did not. This apparent divergence does not contradict the overall association but rather highlights the complementary roles of these biomarkers. Creatinine-based eGFR and CysC reflect glomerular filtration through different biological filters and can be differentially influenced by clinical conditions. Evidence suggests that GFR-estimating equations incorporating CysC offer improved accuracy in transplant recipients.[24] Therefore, the combined longitudinal profile of both markers is likely to provide a more comprehensive assessment of graft health. The sustained eGFR reduction is suggestive of persistent functional impairment, while the dynamic CysC profile may be indicative of evolving or subtler aspects of injury. Collectively, this evidence supports the interpretation that early high TAC exposure is associated with a sustained detrimental effect on allograft function within the first year, with the manifestation of this association varying across complementary biomarkers.

Analysis of other renal parameters revealed a statistically significant elevation of serum urea only at postoperative day 7 in the exposure group (adjusted mean difference: 4.94 mg/dL; FDR-adjusted P = 0.0008), which likely reflects the prevailing renal functional status at this very early phase rather than a direct TAC effect, consistent with literature suggesting urea's responsiveness to significant functional impairment.[25,26] For serum potassium, a numerical trend toward lower levels was observed in the exposure group at one year (adjusted mean difference: −0.17 mmol/L). However, this difference was not statistically significant after FDR correction (FDR-adjusted P = 0.174). It is noteworthy that hypokalemia is documented as a potential adverse effect in the prescribing information for tacrolimus and has been reported in clinical studies,[27] with proposed mechanisms including tubular dysfunction leading to renal tubular acidosis.[28,29] While the direction of the observed trend is consistent with this pharmacological profile, the absence of statistical significance in our analysis precludes any definitive conclusion regarding an association in our cohort.

Beyond its immunosuppressive role, tacrolimus exhibits complex pharmacodynamic effects, including potential influences on hematopoiesis and infection risk. In our cohort, an initial numerical difference in hemoglobin (HGB) levels was observed at postoperative day 7, with higher levels in the exposure group (unadjusted P = 0.044). However, this difference was not statistically significant after FDR correction for multiple comparisons (FDR-adjusted P = 0.220). While in vitro studies have suggested that high concentrations of TAC might exhibit dose-dependent stimulatory effects

on certain progenitor cells,[30] the transient and non-significant trend in our clinical data is not sufficient to establish an association between early high TAC exposure and a clinically meaningful stimulatory effect on erythropoiesis.

Consistent with the potent immunosuppressive effect of tacrolimus, our analysis indicated that early high exposure was strongly associated with an increased risk of infection within the first post-transplant year. In the propensity score-matched cohort, the incidence of infection was significantly higher in the exposure group compared to the non-exposure group (80.0% vs 52.3%; adjusted OR = 4.27, 95% CI: 2.22–8.52, P<0.001). A numerical trend toward higher rates of viral infection was also observed in the exposure group. This clinical finding aligns with previous reports in which elevated tacrolimus concentrations have been correlated with an increased risk of opportunistic infections. Manitpisitkul et al[31] found that high tacrolimus concentrations were significantly associated with BK polyomavirus-associated nephropathy risk in renal transplant patients, and a meta-analysis by Datrinoa et al[32] noted that cytomegalovirus infection was predominant under tacrolimus-based immunosuppressive therapy.

Post-transplant hyperglycemia is a well-recognized complication,[33] and tacrolimus is associated with a higher risk of new-onset diabetes compared to some other immunosuppressants.[33] However, in our cohort, no statistically significant differences in fasting glucose levels were observed between the exposure and non-exposure groups at any post-transplant time point after FDR correction (all FDR-adjusted P > 0.05). This finding may be contextualized by the known dose-dependent effect of tacrolimus on glucose metabolism, where its impact on glycemic control is reported to be more pronounced at blood concentrations exceeding 15 ng/mL.[34] In our study, although the exposure group had higher concentrations initially, the mean tacrolimus trough levels in both groups remained at or below 15 ng/mL from postoperative day 7 through the 1-year follow-up. This sustained maintenance within a lower concentration range may partly explain the absence of a significant between-group difference in glucose metabolism outcomes in our analysis.

To assess the robustness of our primary findings, a sensitivity analysis was performed using a higher exposure threshold of 25 ng/mL. The results reinforced the main association, particularly in the immediate postoperative period. At this stricter cutoff, early high exposure remained significantly associated with reduced eGFR and elevated cystatin C at postoperative day 7. While statistical significance was attenuated at later time points after rigorous multiple-testing correction, the directional trends for both eGFR and cystatin C across the follow-up period remained consistent with those observed in the primary analysis. The diminished significance at later intervals in this sensitivity analysis may be attributed to two interrelated factors. First, applying a higher threshold reduced the sample size of the exposed cohort, consequently lowering statistical power to detect sustained differences. Second, from a clinical standpoint, a trough concentration exceeding 25 ng/mL is highly likely to prompt immediate dose reduction, thereby truncating the duration of exposure and potentially mitigating its longer-term measurable impact on renal function. Collectively, the consistency in the direction of effect across analyses, coupled with the strong early signal even at a more stringent threshold, supports the conclusion that the association between early supratherapeutic tacrolimus exposure and graft dysfunction is robust and not an artifact of a specific numerical cutoff.

This study also aimed to develop a prediction model for early high TAC exposure. It is worth noting that while existing tools, such as population pharmacokinetic (PPK) models, have greatly enhanced our understanding of factors like CYP3A5 genotype and body size that influence tacrolimus disposition,[35] their primary strength often lies in describing and predicting steady-state pharmacokinetics. In contrast, the model presented here was specifically designed to provide a very early risk assessment using data readily available on postoperative day 1. A particular aspect we sought to explore was the role of the immediate postoperative inflammatory response. We found that an elevated CRP level at this early time point was a significant predictor in our model. This observation is consistent with prior research by Chavant et al in liver transplant patients, which also reported an association between elevated CRP and tacrolimus overexposure.[36] Additionally, the model indicated a potential role for serum albumin, which aligns with the known pharmacokinetics of tacrolimus as a highly protein-bound drug.[37] Thus, our model may complement existing tools by focusing on the dynamic and complex perioperative period.

Regarding potential clinical application, if further validated, this model could be conceptualized as a supportive tool to aid early clinical decision-making. In practice, by inputting readily available parameters from the first postoperative day—such as the CYP3A5 genotype, the weight-adjusted tacrolimus dose, CRP, and albumin levels—the model might provide an early indication of a patient's risk for imminent high exposure. This could prompt clinicians to consider strategies such as more vigilant monitoring or cautious dose titration in the subsequent few days, potentially helping to avoid the high trough levels associated

with adverse outcomes in our study. We wish to emphasize that this represents a potential future direction; the model requires rigorous external validation and assessment of its impact on clinical endpoints before any consideration of routine use.

Limitation

This study has several important limitations. The potential for selection bias, arising from the asymmetric exposure definition and the composition of the non-exposed cohort (as elaborated in the Discussion), alongside residual confounding from unmeasured factors despite propensity score matching, must be acknowledged. Furthermore, the retrospective, single-center design may limit the generalizability of our findings and is susceptible to information bias in outcome ascertainment. Finally, the clinical prediction model we developed, while promising, requires external and prospective validation in diverse populations before its clinical utility can be established. Despite these constraints, our findings offer robust observational evidence and a practical conceptual framework for early post-transplant risk assessment.

Conclusion

In this single-center retrospective cohort study of kidney transplant recipients, we observed that early postoperative tacrolimus trough concentrations exceeding 20 ng/mL were associated with poorer renal allograft function and a significantly higher incidence of infections during the first post-transplant year, compared to a propensity score-matched non-exposed cohort. These findings suggest that early supratherapeutic tacrolimus exposure may contribute to adverse clinical outcomes in this patient population.

To facilitate early risk identification, we developed a prediction model for such high exposure events using readily available data from the first postoperative day. The model, which incorporates *CYP3A5 *3/*3* genotype, elevated C-reactive protein, and a higher weight-adjusted tacrolimus dose, demonstrated promising discriminative ability upon internal validation. It represents a potential tool for stratifying patient risk, which could guide more vigilant monitoring and personalized dose titration in the immediate postoperative period.

Collectively, our data highlight the clinical relevance of avoiding early tacrolimus overexposure. The proposed prediction model, after further external validation, might aid clinicians in optimizing initial tacrolimus therapy, with the aim of improving graft outcomes and reducing infection-related complications.

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Disclosure

The authors declare that they have no competing interests in this work.

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