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Optimum tacrolimus trough levels for enhanced graft survival and safety in kidney transplantation: A retrospective multicenter real-world evidence study

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HIGHLIGTS

- A multicenter study employing institutional Clinical Data Warehouses (CDW) to ascertain optimal tacrolimus trough levels that balance efficacy and safety in kidney transplant recipients.
- Identified optimal tacrolimus trough levels of 5.0–7.9 ng/mL during the 2–12 month post-transplant period and 5.0–6.9 ng/mL during the 12–72 month period, correlating with improved graft outcomes and reduced risks of safety outcomes, including infections, cardiovascular events, malignancies, and mortality.
- Emphasizes the clinical significance of maintaining tacrolimus within these specified ranges, proposing an adjustment of immunosuppressive protocols in kidney transplantation to optimize graft longevity and minimize treatment-related adverse effects.

GRAPHICAL ABSTRACT

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ABSTRACT

Background:
The current study aimed to determine the optimal tacrolimus trough levels for balancing graft survival and patient safety following kidney transplantation.

Materials and Methods:
We conducted a retrospective cohort study involving 11,868 kidney transplant recipients from five medical centers. The association between tacrolimus exposures (periodic mean trough level, coefficient of variability, time in therapeutic range) and composite allograft outcome (de novo donor specific antibody, biopsy-proven rejection, kidney dysfunction, and graft failure), as well as safety outcomes (severe infection, cardiovascular events, malignancy, and mortality) were assessed. Data were sourced from Clinical Data Warehouses and analyzed using advanced statistical methods, including Cox marginal structural models with inverse probability treatment weighting.

Results:

Tacrolimus levels of 5.0–7.9ng/mL and 5.0–6.9ng/mL during the 2–12 month and 12–72 month post-transplantation periods, respectively, were associated with reduced risks of composite allograft outcomes. During the first post-transplant year, the adjusted hazard ratios (aHR) for composite allograft outcomes were: 0.69 (95% CI 0.55–0.85, p<0.001) for 5.0–5.9ng/mL; 0.81 (95% CI 0.67–0.98, p=0.033) for 6.0–6.9ng/mL; and 0.73 (95% CI 0.60–0.89, p=0.002) for 7.0–7.9ng/mL (compared to levels ≥8.0ng/mL). For the 6-year composite outcomes, aHRs were 0.68 (95% CI 0.53–0.87, p=0.002) for 5.0–5.9ng/mL and 0.65 (95% CI 0.50–0.85, p=0.001) for 6.0–6.9ng/mL. These optimal ranges showed reduced rates of severe infection (6 years), malignancy (6 years), and mortality (1 year).

Conclusion:

This multicenter study provides robust evidence for optimal tacrolimus trough levels during the periods 2–12 and 12–72 months following kidney transplantation.

KEYWORDS

Kidney transplantation; Tacrolimus; Graft rejection; Safety

INTRODUCTION

Kidney transplantation remains the gold standard for managing end-stage renal disease, offering superior patient survival and quality of life.[1,2] Advances in immunosuppressive regimens have led to decreased rates of acute rejection and better short-term outcomes.[3] Currently, the most common regimen combines tacrolimus with mycophenolate derivatives, and steroids.[4,5]

The principal challenge in immunosuppression is achieving a balance between under- and over-immunosuppression; under-immunosuppression can lead to graft rejection, while over-immunosuppression can lead to off-target toxicities and infection.[6] Tacrolimus, the cornerstone of modern immunosuppressive regimens,[4] has a narrow therapeutic index, and is associated with a range of adverse effects, including kidney dysfunction, hypertension, and dyslipidemia.[7–9] Though post-transplant tacrolimus trough levels are regularly monitored, optimal levels are not well defined.[10]

Historically, tacrolimus trough level targets were proposed to range from 5–20 ng/mL.[11–13] The Efficacy Limiting Toxicity Elimination (ELITE)-Symphony trial later advocated that targeting lower levels of 5–10 ng/mL tacrolimus led to superior effectiveness with acceptable adverse effects, compared to previous cyclosporine- or sirolimus-based regimens.[3,14] Following the Symphony trial, further studies, mostly of limited size and retrospective in nature, have produced conflicting results concerning tacrolimus trough levels and graft outcomes.[15–21] Larger registry studies, based on fragmented trough level data,[22,23] have failed to capture the dynamic fluctuations of tacrolimus over time. More recently, concepts of intrapatient variability (IPV) and time in therapeutic range (TTR) have been introduced to address the varying nature of tacrolimus trough levels;[24,25] however, target ranges of IPV and TTR are not well defined. Furthermore, data on optimal tacrolimus levels beyond the initial post-transplant year are remarkably scarce, highlighting a substantial gap in knowledge within the field.

The task of optimizing tacrolimus dosing in immunosuppression therapy extends beyond mere monitoring of drug levels; it requires a holistic consideration of a multitude of clinical factors, including patient age, cardiovascular health, infection status, renal function, recent graft rejections, and donor-specific antibodies. These factors are pivotal not only in immediate clinical decision-making regarding tacrolimus dosing but also significantly impact

both transplant and patient outcomes. The dynamic and multifactorial nature of these considerations emphasizes the need for an analytical approach capable of elucidating the complex relationships among these covariates, tacrolimus exposure, and transplant outcomes.

In response to this complexity, our study advocates for the utilization of Clinical Data Warehouses (CDW) as a pivotal resource. CDWs provide an advanced platform for big data analytics, facilitating the comprehensive analysis of longitudinal observational data that mirrors real-world clinical practice.[26,27] The CDWs from the participating five medical centers, upon which our database is based, are automatically updated with a one-day time lag, capturing a vast array of EHR data, including text medical records, anesthesia records, nursing notes, laboratory data, pathology reports, and unstructured imaging/diagnostic test interpretations. We utilized the CDWs to systematically collect data on 483 variables across our study cohort, thereby minimizing human error and reducing bias during data collection. The longitudinal data were comprehensive, covering from one-year pre-surgery to six years post-transplant or until the end of each patient's follow-up period.

In addition, to adequately model the intricate and time-varying interplay between covariates, tacrolimus exposure, and transplantation outcomes, we employed advanced statistical methodologies, specifically Cox Marginal Structural Models (MSM) that utilize stabilized weights calculated via the Inverse Probability of Treatment Weighting (IPTW) method.[28,29] This approach allows for adjustments of time-dependent confounders, thereby mitigating potential biases in estimating the impact of tacrolimus exposure on graft and patient outcomes.

By integrating comprehensive CDW data with sophisticated statistical analysis, we sought to elucidate the optimal tacrolimus levels that would balance graft survival with patient safety. By considering the complex clinical decision-making framework and the multifactorial influences on immunosuppression outcomes, our research aims to provide actionable insights for the refinement of tacrolimus management protocols in kidney transplantation.

MATERIALS AND METHODS

This retrospective multicenter cohort study (ClinicalTrials.gov, number) used CDW data to investigate the relationship between tacrolimus exposure and both short- and long-term graft

and patient outcomes in kidney transplant recipients. The study protocol was approved by the institutional review boards of the five participating centers in Korea.

Study Population

The study cohort included patients who underwent kidney-only transplants between January 2005 and December 2020. Patients were included if they were being administered oral tacrolimus at the start of the defined cohort time intervals, specifically 2 months post-transplant for the 1-year outcomes and 12 months post-transplant for the 6-year outcomes. We excluded patients who experienced graft failure or death before these time intervals, and those who received other solid organ transplants during the study period. In patients who received multiple kidney transplants during the study period, only the first transplant was included.

Outcome Variables and Study Endpoint

The primary endpoint was a composite of 1-year allograft outcomes, consisting of biopsy-proven rejection (BPR), kidney dysfunction (estimated glomerular filtration rate [eGFR] <30 mL/min/1.73m^2), the development of anti-human leukocyte antigen (HLA) de novo donor-specific antibodies (dnDSA), and death-censored graft failure, occurring 2–12 months post-transplant. Secondary endpoints were composite allograft outcomes at 12–72 months and safety outcomes (severe infection, cardiovascular events, malignancies, and mortality) occurring 2–12 months and 12–72 months post-transplant. Detailed definitions of the outcome variables are provided in the Supplementary methods, Supplemental Digital Content 1, http://links.lww.com/JS9/C773. Follow-up was censored at 1 or 6 years post-transplant, loss to follow-up, or by December 31, 2021, whichever occurred first.

Tacrolimus Exposure Variables

Tacrolimus exposure was assessed through several variables, including the periodic mean of tacrolimus trough level, the coefficient of variability (CV) as a measure of IPV, and TTR. The periodic mean was determined from multiple outpatient tacrolimus trough level measurements. If there were more than one measurement on the same day, the lower value was selected. Bi-monthly means within the first year post-transplant (2-12 months) were used for the analysis of its association with early post-transplant outcomes, and annual means during the subsequent five years (12-72 months) were used to evaluate 6-year outcomes. The periodic mean of tacrolimus trough levels was categorized into seven groups: <3.0 ng/mL,

3.0–3.9 ng/mL, 4.0–4.9 ng/mL, 5.0–5.9 ng/mL, 6.0–6.9 ng/mL, 7.0–7.9 ng/mL, and ≥8.0 ng/mL.

CV for tacrolimus trough concentrations was calculated as the standard deviation to mean ratio, categorized into quartiles. TTR was assessed using the Rosendaal method,[30,31] with therapeutic tacrolimus levels set at 7.0–10.0 ng/mL (2–6 months post-transplant), 6.0–8.0 ng/mL (6–12 months), and 5.0–8.0 ng/mL (after 12 months). TTR categorization used a 60% cut-off.

Data Collection

Data were retrieved from the institutional CDW of the five participating medical centers (Figure S1, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). To ensure relevance and consistency, investigators from all centers collaboratively defined the necessary variables and operational definitions. Custom extraction algorithms tailored to each CDW's structure facilitated automated data collection, yielding a dataset encompassing recipient and donor demographics, transplant details, and follow-up information. A rigorous multi-step quality control process was applied, involving data cleansing, missing value imputation, inconsistency resolution, and duplicate removal, supplemented by manual verification and augmentation. Longitudinal individual patient data were collected from 1 year preoperatively to 1 or 6 years postoperatively, or until the last date of follow-up, according to the cohort definition. The list of variables is provided in the Supplementary Methods, Supplemental Digital Content 1, http://links.lww.com/JS9/C773.

Statistical Analysis

Baseline characteristics were presented using descriptive statistics. The study population were grouped into seven tacrolimus trough level categories based on the tacrolimus trough level during the 2–4 months post-transplant period, and the between-group balance of baseline characteristics was checked using standardized mean differences. The Sankey diagram was used to visualize the changing patterns of the tacrolimus trough level over time.

Unadjusted survival analysis for the relationship between tacrolimus trough level and clinical outcomes was conducted using the standardized Cox proportional hazard model, with the periodic mean of tacrolimus trough level as the time-dependent covariate for all outcomes.

To control for confounding variables and obtain more accurate estimates of the effect of time-varying exposure (tacrolimus trough level) on the composite allograft outcome, we conducted

adjusted analysis using Cox MSM with stabilized weights calculated using the IPTW.[28,29] Tacrolimus levels at each time point are influenced not only by baseline patient characteristics but also by previous tacrolimus exposures and past clinical outcomes. The MSM approach allows for appropriate adjustment of these time-varying factors, providing more accurate causal estimates. This method adjusts for the confounding effect of imbalanced variables on both the probability that an individual will be allocated to the seven trough-level categories and the probability of the occurrence of the outcome. The stabilized weight at each time point consists of the product of the treatment weight and censoring weight. Treatment weights were calculated based on the inverse probability of each individual belonging to one of seven tacrolimus concentration categories at each observation time point, considering both time-dependent and time-independent covariates. Censoring weights were similarly calculated based on the probability of being censored at each time point. The covariates included for calculating stabilized weights were age, sex, previous dialysis months, immunosuppressant use other than tacrolimus, induction agent, desensitization, donor age, donor sex, and outcomes (rejection, renal dysfunction, and dnDSA) prior to the start of the cohort time and serum creatinine (time-dependent covariate). Detailed description of the method is provided in the Supplementary methods, Supplemental Digital Content 1, http://links.lww.com/JS9/C773.

We performed standard Cox proportional hazards analysis for the association between the CV or TTR of tacrolimus trough level and clinical outcomes. In the unadjusted Cox analysis for the association of periodic mean tacrolimus trough levels and outcomes, as well as the Cox analyses for CV or TTR and outcomes, trough levels beyond the occurrence of the outcome of interest were excluded when calculating tacrolimus periodic mean, CV, or TTR. Subgroup analyses were performed to explore potential variations in the association between tacrolimus exposure and primary endpoint within specific patient groups. These groups were defined by age (<18, 19–64, ≥65 years), diabetes, hypertension, donor type (living or deceased), desensitization status, and prior rejection history. Furthermore, for sensitivity analysis, we repeated the assessment of all outcomes using only tacrolimus levels below 25 ng/mL.

For all analyses, significance tests were two sided, and a *P*-value of <0.05 was considered significant. Analyses were performed using SAS version 9.4 for Windows (SAS institute, Cary, NC) and R (version 4.3.1). The current study has been reported in line with the

STROCSS criteria.[32] , Supplemental Digital Content 2, http://links.lww.com/JS9/C774.

RESULTS

A total of 11,868 patients underwent kidney transplant across five medical centers between 2005 and 2020 (Figure 1). Of these, 10,329 patients, who contributed a total of serial 153,065 tacrolimus trough levels measurements, met the inclusion criteria for the primary 1-year outcome analysis. For the analysis of 6-year outcomes, a subset of 4,488 patients who received transplants between 2005 and 2014 were included, contributing a total of 277,362 tacrolimus trough level measurements during the 2–6-year post-transplant period. Baseline characteristics are detailed in Table 1 (1-year cohort) and Table S1, Supplemental Digital Content 1, http://links.lww.com/JS9/C773 (6-year cohort).

Tacrolimus Trough Level Variations over Time

The most common tacrolimus trough level interval at 2 months post-transplant was ≥8.0 ng/mL (40.0%), followed by 7.0–7.9 ng/mL (20.4%), and 6.0–6.9 ng/mL (16.6%) (Table S2, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). The percentage of patients with a periodic mean tacrolimus trough level ≥6.0 ng/mL was over 60% until 10 months post-transplant, but decreased to about 50% thereafter, and to less than 40% after 3 years post-transplant. The Sankey diagram (Figure 2A and 2B) showed that periodic mean trough levels continued to oscillate across different tacrolimus concentration categories within 1-year and during 1–6 years post-transplant, manifesting the dynamic nature of tacrolimus concentration within this patient population.

Tacrolimus Trough Levels and 1-Year Composite Allograft Outcomes

The primary endpoint, composite 1-year allograft outcome was observed in 11.2% (1,161/10,329) of the study population, with lower risks associated with tacrolimus trough levels of 5.0–5.9 ng/mL, 6.0–6.9 ng/mL, and 7.0–7.9 ng/mL. This composite included: BPR with an incidence of 8.8%; kidney dysfunction (eGFR < 30 mL/min/1.73m²) at 4.6%; dnDSA at 1.2%; and death-censored graft failure at 1.1% (Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/C773).

To evaluate the association between periodic mean tacrolimus trough levels and the primary

outcomes, we employed two distinct analytical approaches: an unadjusted time-varying Cox proportional hazards model, and the Cox MSM with IPTW for adjustment of confounding variables. The results for 1-year composite allograft outcomes were consistent across both methods (Figure 3A). For the tacrolimus trough level categories 5.0–5.9 ng/mL, 6.0–6.9 ng/mL, and 7.0–7.9 ng/mL, adjusted hazard ratios (aHR) for experiencing the composite allograft outcome were significantly lower when compared to the ≥8.0 ng/mL group (aHR 0.69, 95% CI [confidence interval] 0.55–0.85, *P* <.001; aHR 0.81, 95% CI 0.67–0.98, *P* =.033; aHR 0.73, 95% CI 0.60–0.89, *P* =.002, respectively). On the other hand, tacrolimus trough level categories <3.0 ng/mL and 3.0–3.9 ng/mL were associated with a higher risk of composite allograft outcomes (aHR 4.74, 95% CI 4.0–5.63, *P* < .001; aHR 1.40, 95% CI 1.05–1.87, *P* =.023, respectively).

Regarding the individual components of the composite allograft outcome, periodic mean tacrolimus trough levels of 5.0–5.9 ng/mL were associated with a reduced risk of developing BPR, and levels of 7.0–7.9 ng/mL were associated with a lower risk of kidney dysfunction (Table S4, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Conversely, levels of 3.0–3.9 ng/mL and 4.0–4.9 ng/mL were associated with a higher risk of dnDSA development and death-censored graft failure. Notably, tacrolimus trough levels <3.0 ng/mL were linked to the elevated risk of all individual components.

The detrimental effects of periodic mean tacrolimus trough levels <3.0 ng/mL and the beneficial impacts of levels of 5.0–5.9 ng/mL, 6.0–6.9 ng/mL, and 7.0–7.9 ng/mL were consistent across most major subgroups (Table S5, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). However, certain subgroups (those aged 65 years and older, diabetic patients, living donor kidney recipients, and patients who experienced BPR before 2 months post-transplant) did not show statistically significant benefits from tacrolimus trough levels of 5.0–5.9 ng/mL, 6.0–6.9 ng/mL, and 7.0–7.9 ng/mL.

Tacrolimus Trough Levels and 6-Year Composite Allograft Outcomes

The crude incidence of the composite allograft outcome during the 12–72 month period post-transplant was 23.1% (1,037/4,488; Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/C773), and tacrolimus levels of 5.0–5.9 ng/mL and 6.0–6.9 ng/mL were associated with lower risks of 6-year allograft outcome.

Patients with periodic mean tacrolimus trough levels <3.0 ng/mL had an increased risk (aHR

2.94, 95% CI 2.33–3.71, p<0.001) of experiencing the composite outcome. Contrastingly, levels of 5.0–5.9 ng/mL and 6.0–6.9 ng/mL were associated with a significantly reduced risk of the composite outcome (aHR 0.68, 95% CI 0.53–0.84, *P* =.002; aHR 0.65, 95% CI 0.50–0.85, *P* =.001, respectively; Figure 3B).

Further examination revealed that periodic mean tacrolimus tough levels ranging from 4.0 to 7.9 ng/mL were associated with a lower risk of kidney dysfunction (Table S6, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Additionally, levels between 5.0 and 6.9 ng/mL showed reduced hazards for death-censored graft failure. Levels below 3.0 ng/mL correlated with elevated risks across all individual outcomes, including BPR, kidney dysfunction, dnDSA development, and death-censored graft failure.

Tacrolimus Trough Levels and Safety Outcomes of Infection, Cardiovascular Events, Malignancy, and Mortality

The overall rates of severe infection, cardiovascular events, and mortality during the initial 2–12 months post-transplant were 8.2%, 0.1%, and 0.8%, respectively (Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Patients with the lowest periodic mean tacrolimus trough levels (<3.0 ng/mL) faced significantly higher risks of severe infection (aHR 5.49, 95% CI 4.52–6.68, *P* <.001), cardiovascular events (aHR 4.78, 95% CI 1.07–21.29, *P* =.040), and mortality (aHR 5.78, 95% CI 3.19–10.48, *P* <.001) when compared to those with levels ≥8.0 ng/mL (Figure 4, Table S7, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Conversely, the 5.0–5.9 ng/mL group had a reduced mortality risk (aHR 0.32, 95% CI 0.11–0.94, *P* =.038).

During the extended follow-up from 12–72 months post-transplant, the overall incidences of severe infection, cardiovascular events, malignancy, and mortality were 11.7%, 0.6%, 3.5%, and 2.9%, respectively (Table S3, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Tacrolimus trough levels ranging from 3.0 to 7.9 ng/mL were correlated with a lower risk of severe infection compared to ≥8.0 ng/mL (Figure 5, Table S8, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). For malignancy, levels below 3.0 ng/mL were associated with an elevated risk, while levels of 4.0–4.9 ng/mL and 5.0–5.9 ng/mL conferred reduced risk. No statistically significant associations were found for cardiovascular events or mortality.

Tacrolimus Coefficient of Variability, and Composite Allograft and Patient Safety

Outcomes

Additional analysis of the tacrolimus CV and outcome variables showed that the quartile groups with a higher CV had a higher incidence of both 1-year and 6-year composite allograft outcomes, severe infection, mortality, and 6-year malignancy (Table S9, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). A multivariate Cox analysis revealed that the groups with lower CV quartiles were associated with a lower risk of composite allograft outcome, severe infection, and patient mortality at 1 and 6 years (Table S10, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). The lowest versus highest CV quartile group also showed a significantly lower risk of malignancy 2–6 years post-transplant. No associations were found between tacrolimus CV and cardiovascular events.

Tacrolimus Time in Therapeutic Range, and Composite Allograft and Patient Safety Outcomes

Those who spent more time in predefined therapeutic range (high TTR; TTR≥60%) showed a significantly lower incidence rate of composite allograft outcome at 1 and 6 years (Table S11, Supplemental Digital Content 1, http://links.lww.com/JS9/C773), and were associated with a lower risk of developing composite allograft outcomes at 6 years (Table S12, Supplemental Digital Content 1, http://links.lww.com/JS9/C773). Regarding patient safety outcome variables, the low TTR group was associated with a higher risk of severe infection at both 1 and 6 years, and also a higher mortality at 6 years (Table S11, Supplemental Digital Content 1, http://links.lww.com/JS9/C773 and S12, Supplemental Digital Content 1, http://links.lww.com/JS9/C773).

DISCUSSION

Our study utilized a comprehensive CDW dataset and advanced methods, including Cox MSM with IPTW, to define optimal post-transplant tacrolimus trough levels. Findings (Table 2) demonstrate that levels of 5.0–7.9 ng/mL (2–12 months post-transplant) and 5.0–6.9 ng/mL (2–6 years post-transplant are associated with reduced allograft risks. The suggested 2–6 year concentration ranges were also associated with lower incidences of severe infection and malignancy.

While tacrolimus has become the cornerstone of immunosuppressive therapy, its ideal target

trough level for balancing efficacy and toxicity remains controversial.[4,5,10] In the ELITE-Symphony trial, patients maintained tacrolimus trough concentrations of 5.0–10.0 ng/mL (mean 6.0–8.0 ng/mL) throughout the first year, resulting in improved outcomes when compared to sirolimus or cyclosporine-based regimens.[3] However, a pooled analysis of three randomized trials, including the Symphony trial, failed to establish an optimal trough level, potentially due to limited sampling at only five discrete time points early post-transplant.[33] Our study adds to this dialogue by suggesting a lower limit of 5.0 ng/mL tacrolimus during the 2–12 months post-transplant period for both composite and individual graft outcomes. Our limit is consistent with those suggested by Wiebe et al.,[34] who showed that a greater proportion of patients developing HLA DR/DQ dnDSA had tacrolimus levels below 5.0 ng/mL. In contrast, Davis et al. recommended a higher limit, suggesting that a tacrolimus trough level less than 8.0 ng/mL within the first 6 or 12 months post-transplant led to higher risks of dnDSA development by 6 and 12 months.[35] The differences may be attributed to variations in study populations and analysis methodologies. Instead of dichotomizing or quartilizing the cohort based on mean tacrolimus level as in Davis et al., our approach offered a more granular analysis by comparing outcomes across multiple tacrolimus concentration groups based on a 2-month periodic mean. This was made possible by the sample size, which exceeded 10,000 patients and provided 430,427 serial tacrolimus concentrations.

Regarding the long-term impact of tacrolimus, there has been a paucity of studies examining optimal concentrations beyond the first-year post-transplant. Our study fills this gap by showing that tacrolimus trough levels 5.0–5.9 ng/mL and 6.0–6.9 ng/mL were significantly beneficial compared to levels above 8.0 ng/mL. This contrasts with Unagami et al.,[36] who did not find significant differences in dnDSA or kidney function among patients with different tacrolimus trough levels up to seven years post-transplant. Their study, however, was limited by its small sample size in the low (≤4.0 ng/mL) and high (>6.0 ng/mL) trough-level groups and its inability to capture the dynamic changes in tacrolimus concentrations. In support of our findings, another study using CTS registry data highlighted the potential adverse effects of maintaining low tacrolimus levels in the long term.[23] This study, which evaluated 6,638 patients, showed that a tacrolimus trough level below 4.0 ng/mL by the third year was associated with significantly lower graft survival 4–6 years post-transplant compared to patients with higher trough levels.

Our analysis revealed a non-linear relationship between tacrolimus trough levels and the risk

of individual components of the composite allograft outcomes. The main drivers for decreased risk within the 5.0–7.9 ng/mL concentration range for 1-year outcomes were rejection and kidney dysfunction. For 2-6 years graft outcome, the 5.0–6.9 ng/ml range proved beneficial, primarily due to reduced kidney dysfunction and graft failure. Notably, lower rates of kidney dysfunction in these concentration ranges, especially significant during the 2–6 year period, likely underscore the benefits of lower tacrolimus concentrations in mitigating drug-induced nephrotoxicity. This observation aligns with histological evidence suggesting that chronic changes are induced by long-term exposure to calcineurin inhibitors.[37]

Although there is significant concern about the long-term use of immunosuppressive medication for its systemic effects, it remains unclear whether certain tacrolimus levels are associated with adverse non-graft outcomes. Our results reveal a trend of lower risks of severe infection in the 6.0–7.9 ng/mL range in the first year (not significant) and a significantly lower risk in the 3.0–7.9 ng/mL ranges during 2–6 years transplant compared to those over 8.0 ng/mL. On the other hand, we observed an unexpectedly high risk of severe infection and mortality in the less than 3.0 ng/mL group. This may reflect the inclusion of patients who had been targeted for lower tacrolimus concentrations due to outpatient-managed infections before experiencing a severe infection requiring hospitalization.

Regarding malignancy risk, our adjusted analysis indicates that tacrolimus levels within the 4.0-4.9 ng/mL and 5.0-5.9 ng/mL ranges are associated with lower risks of malignancy compared to levels exceeding 8.0 ng/mL during 2 to 6 years post-transplant. This finding complements a nested case-control study that examined the association between early tacrolimus levels (at 6 and 12 months post-transplant) and the subsequent risk of malignancy after 3 years,[38] suggesting that higher tacrolimus levels may increase the risk of malignancy. Unlike the case-control study, our analysis specifically examines the relationship between tacrolimus levels during the 2-6 years post-transplant period and the incidence of malignancy within the same timeframe, further suggesting that long-term tacrolimus levels may also be important in determining individual malignancy risk.

A large patient population is indispensable for rigorous evaluation of the association between tacrolimus concentration and long-term outcomes, especially given the relatively lower frequency of events like dnDSA and rejection after the first year. While available real-world data (RWD) from registry studies serve this purpose, they are often limited by periodic

tacrolimus concentration measurements at fixed time intervals.[22,23] Emerging evidence suggests that time spent at low tacrolimus levels and the variability of tacrolimus concentration are crucial determinants of outcomes,[24,25] so continuous data capture is paramount. Our study successfully amalgamated the benefits of both large sample size and continuous data by conducting a multicenter investigation leveraging institutional CDW platforms. This entailed intricate data mapping, meticulous curation of data extraction algorithms tailored to each participating institution, and rigorous quality assessments. Building on the existing RWD literature, our study offers a more detailed analysis with a larger sample size and the use of consecutive tacrolimus concentration measurements.

Advancements in data integration and extraction technologies have made real-world big data including electronic health record data, claims data, and registry data more readily available. These data sources have the capability to produce valid and impartial real-world evidence, offering substantial reductions in cost and time compared to controlled trials.[39,40] Additionally, RWD can provide insights into treatment effects in scenarios where randomized controlled trials are not feasible due to technical, ethical, or economic reasons. However, despite these advantages of RWD, careful design and appropriate statistical methods are essential for drawing valid causal inferences.[39] As emphasized by Hernán and Robins' target trial framework, rigorous approaches are necessary to define causal questions akin to those addressed by controlled trials, specifying a hypothetical protocol and planning meticulously on how to mimic such studies using RWD.[41] Our study demonstrates the potential of these approaches in answering complex causal questions, such as the optimal tacrolimus trough level range for graft outcomes and safety after renal transplantation from RWD. By utilizing a multicenter CDW-based database and employing the Cox MSM with IPTW, we aimed to emulate the essential features of a controlled trials and estimate causal effects while addressing potential biases from time-varying confounders.

Our methodology of IPTW with stabilized weights in a time-varying analysis to mitigate confounding also deserves mention. The inherent challenge in studying the effects of tacrolimus levels on outcomes is their intentional modulation based on various covariates; for example, a history of acute rejection within the first two months post-transplant could influence both the risk of subsequent rejection (outcome) and the tacrolimus concentration a clinician targets (exposure). By incorporating both baseline and time-dependent covariates into the calculation of stabilized weights, we aimed to control for the confounding effects of

these variables on the relationship between tacrolimus trough levels and relevant outcomes. This methodological rigor facilitated balanced comparisons between different treatment groups and mitigated bias from potential confounders. Additionally, the Cox MSM with IPTW helped address attrition bias by accounting for differential loss to follow-up. Overall, the utilization of stabilized weights within the IPTW framework reinforced the methodological integrity of our study, minimizing biases and enhancing the reliability of our findings regarding the association between tacrolimus trough levels and post-transplant outcomes.

Our study has several limitations. Despite the substantial sample size, the study was limited by its retrospective design and the variability in data structures across participating institutions. The study also had to forgo the inclusion of viral infection status as a time-varying covariate due to significant data gaps caused by differences in surveillance protocols. While the large sample size enhanced the generalizability of our findings, it is essential to note that the results may not extend to settings with different medical resources and protocols, as the participating centers were all large tertiary care hospitals. In addition, the ethnic homogeneity of our study could affect the generalizability of our findings to non-Asian populations. Previous studies have suggested that pharmacogenetic differences may influence the metabolism and efficacy of tacrolimus across different ethnic groups.[42] Therefore, further studies are needed to extrapolate our results to populations with diverse ethnic backgrounds. Lastly, the extended study duration from 2005 to 2020 could introduce potential time effects related to changes in baseline patient characteristics and post-transplant management protocols. Although our analysis adjusted for these factors, there may still be residual time-related influences that were not fully accounted for.

CONCLUSION

This multicenter study provides evidence for optimal tacrolimus trough levels during the 2–12 and 12–72 months post-transplantation. Our findings suggest that maintaining tacrolimus levels within 5.0–7.9 ng/mL for the first year and 5.0–6.9 ng/mL for years 2–6 correlates with high graft survival and optimal safety outcomes. Optimizing tacrolimus use may improve graft and patient outcomes, enhance overall renal transplantation success rates, and extend benefits to a greater number of patients. This is critical given the ongoing imbalance between organ demand and supply. Improving graft survival and reducing complications will directly support the goal of transplant surgeons to treat end-stage organ disease and maximize the

therapeutic potential of transplantation.

Provenance and peer review

Not commissioned, externally peer-reviewed

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Figure 1. Flow chart of study patient selection.

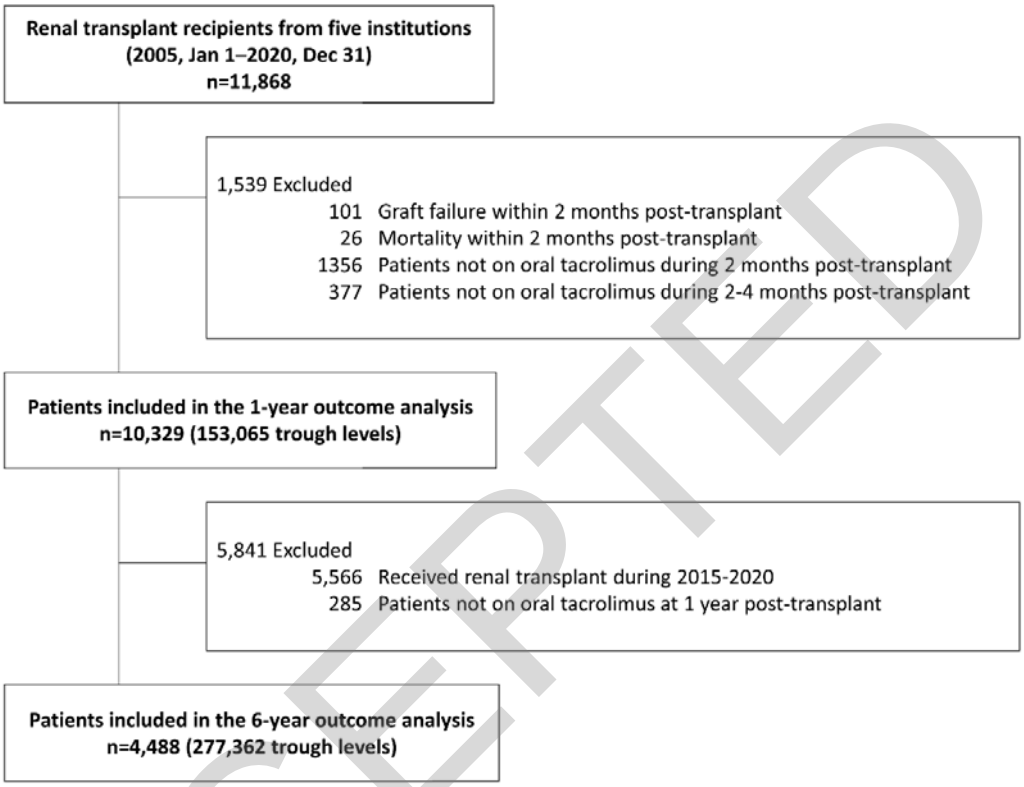
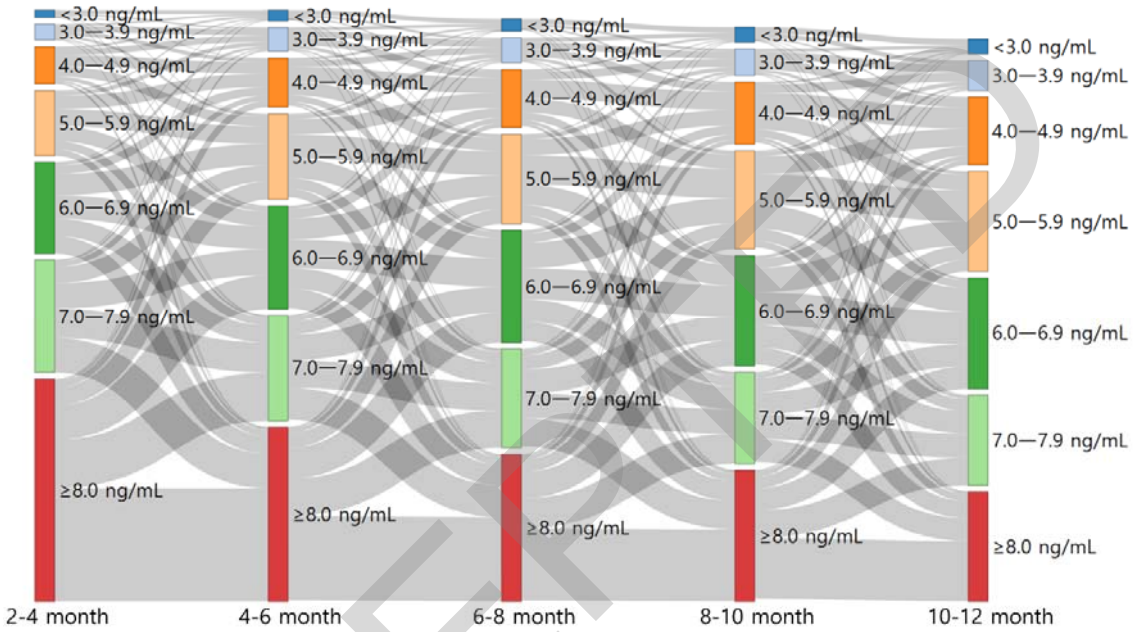


Figure 2. Sankey diagram of tacrolimus trough level changes. (A) Within 1-year post-transplant. **(B)** During 1- to 6-years post-transplant.



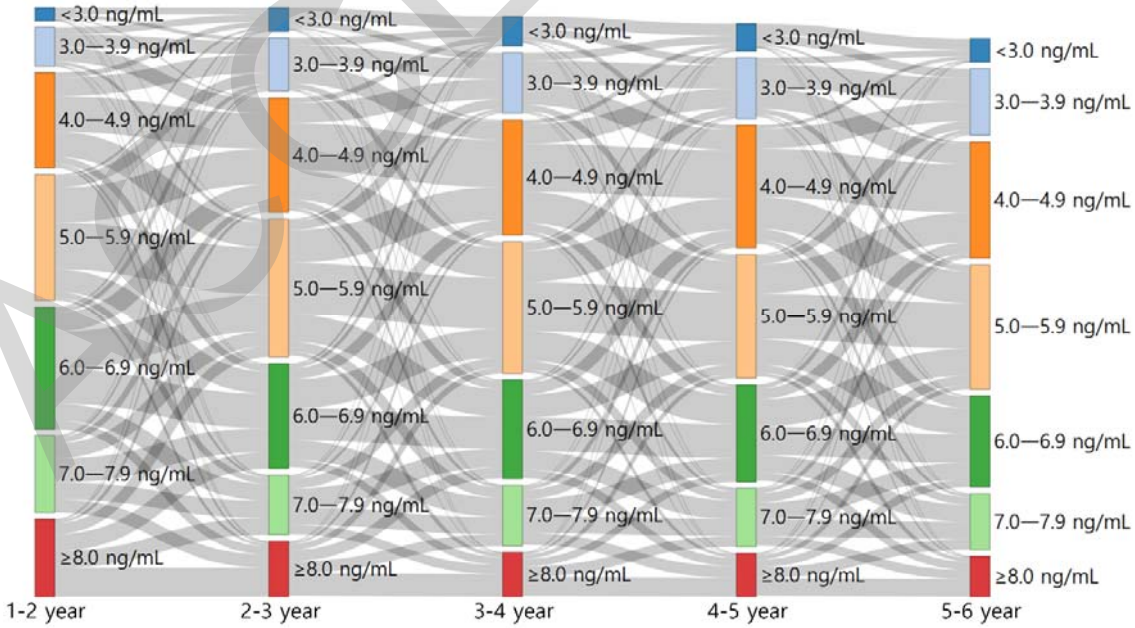
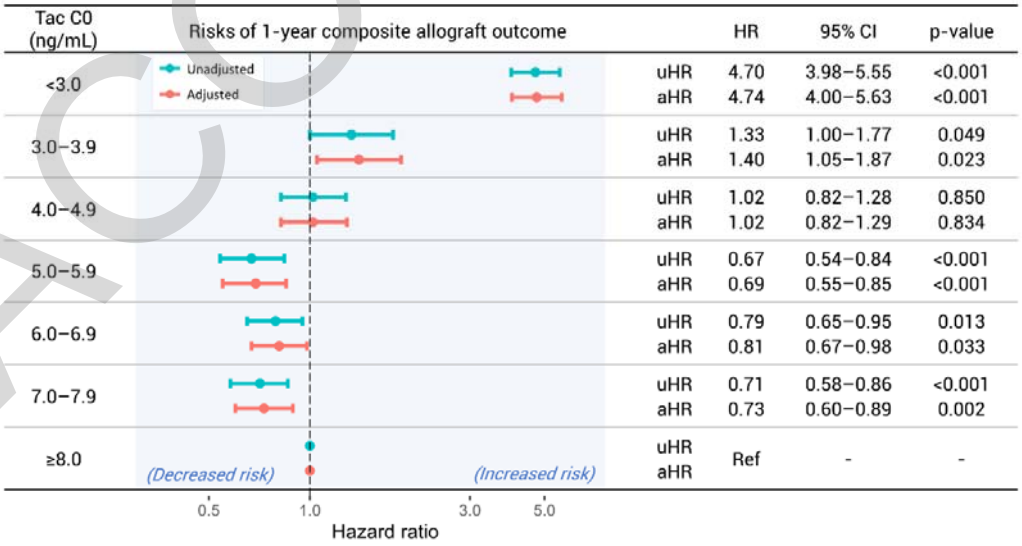


Figure 3. Risks of composite allograft outcome of biopsy-proven acute rejection, renal dysfunction, de novo donor-specific antibody development, and death-censored graft failure by periodic mean tacrolimus trough level. (A) Relative hazards of 1-year composite allograft outcome. (B) Relative hazards of 6-year composite allograft outcome. aHR, adjusted hazard ratio; C0, trough concentration; CI, confidence interval; HR, hazard ratio; uHR, unadjusted hazard ratio. For the unadjusted analysis, the p-value was calculated using the time-varying Cox proportional hazard model with periodic mean tacrolimus as time-varying variable. The adjusted analysis incorporated the inverse probability of treatment weighting (IPTW) method with stabilized weight, which included: sex; age; previous dialysis months; use of immunosuppressants other than tacrolimus; use of induction agents; desensitization; donor age; donor sex; donor-specific antibody at baseline; rejection/renal dysfunction status at baseline; and serum creatinine (time-dependent covariate) for the calculation of stabilized weights.



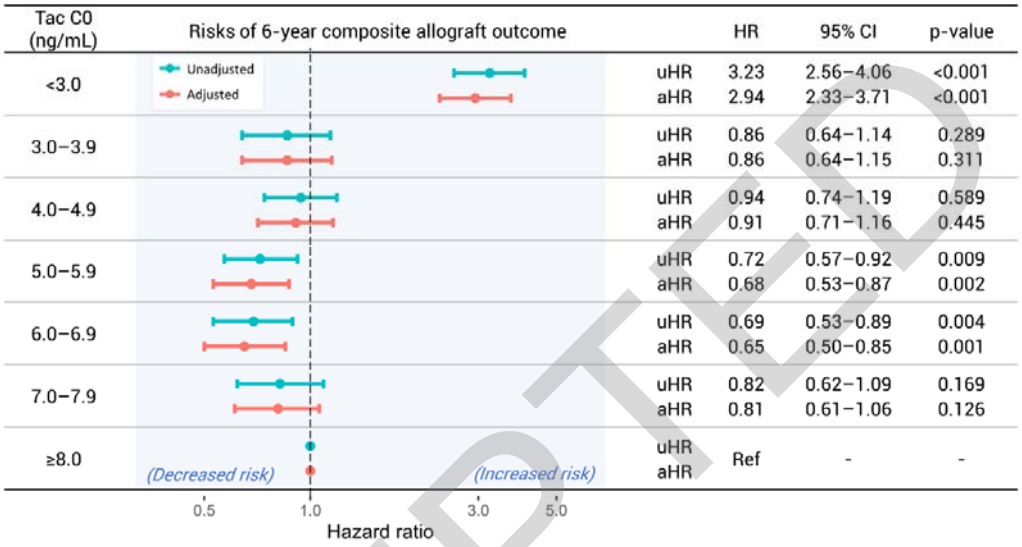


Figure 4. Association of tacrolimus trough levels and the risk of safety outcomes (severe infection, cardiovascular events, and mortality) 2–12 months post-transplant. All hazard ratios used ≥8 ng/mL as the reference. The adjusted analysis incorporated the inverse probability of treatment weighting (IPTW) method with stabilized weight, and included the following covariates: sex; age; previous dialysis months; use of immunosuppressants other than tacrolimus; use of induction agents; desensitization; donor age; donor sex; and serum creatinine (time-dependent covariate) for the calculation of stabilized weights. Hazard ratios for cardiovascular events for tacrolimus trough concentrations 3.0–3.9 ng/mL were not estimable due to the small number of events.

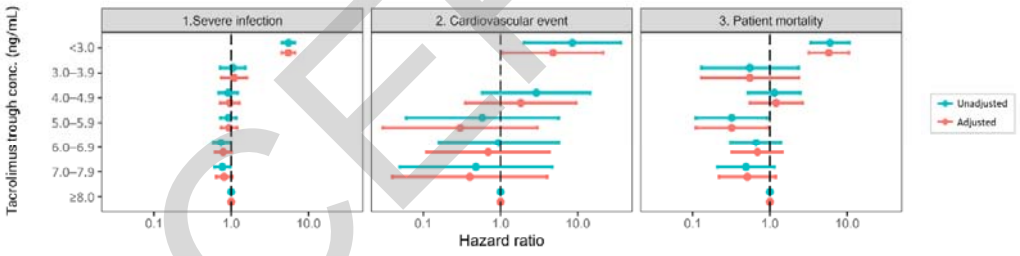


Figure 5. Association of tacrolimus trough levels and risk of safety outcomes (infection, cardiovascular events, malignancy, and mortality 12–72 months post-transplant. All hazard ratios used ≥8.0 ng/mL as the reference. The adjusted analysis incorporated the inverse probability of treatment weighting (IPTW) method with stabilized weight, and included the following covariates: sex; age; previous dialysis months; use of immunosuppressants other than tacrolimus; use of induction agents; desensitization; donor age; donor sex; donor-specific antibody at baseline; rejection/kidney dysfunction status at baseline; and serum creatinine (time-dependent covariate) for the calculation of stabilized weights.

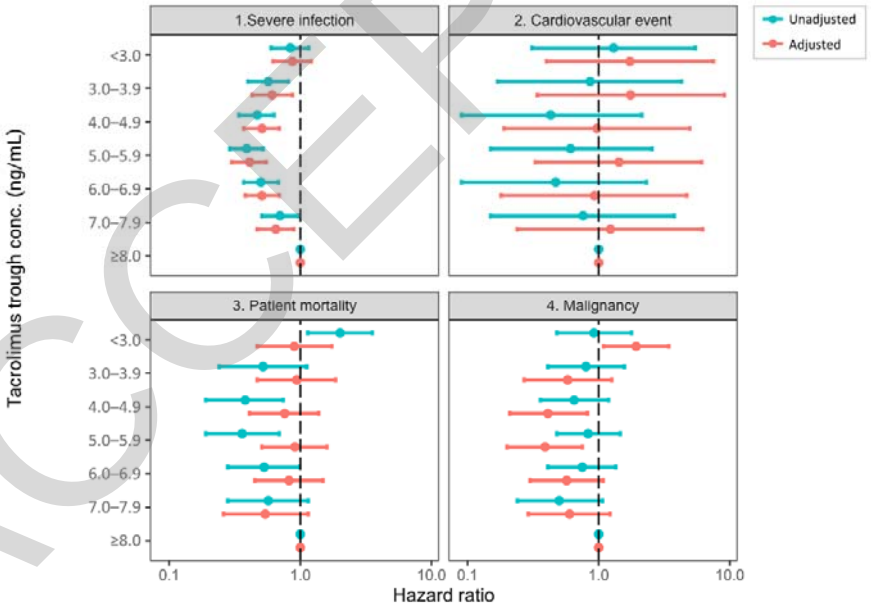


Table 1. Demographic and clinical characteristics of the 1-year analysis cohort (n=10,329)

	Total (n=10,329)	Tacrolimus trough level (ng/mL)							*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)		
Recipient characteristics										
Age, years, mean±SD	46.0±13.6	48.5±12.3	46.3±12.6	46.1±13.2	46.5±13.5	46.1±13.7	45.4±13.9	45.9±13.5	0.0658	2.10
Male sex, n (%)	5,993 (58 .0)	95 (61.7)	155 (52)	356 (50.4)	620 (50.7)	935 (54.6)	1,189 (56.5)	2,643 (64.0)	0.0000	8.87
BMI, kg/m^2, mean±SD	23.0±25.5	24.5±13.7	22.8±4.6	22.9±5.9	22.6±4.7	22.7±5.2	23.8±5.3	22.9±5.8	0.7742	0.09
Hypertension, n (%)	6,951 (67.3)	63 (40.9)	137 (46.0)	388 (54.9)	774 (63.3)	1,174 (68.5)	1,488 (70.7)	2,927 (71.0)	0.0000	12.85
Diabetes mellitus, n (%)	2,254 (21.8)	20 (13.0)	34 (11.4)	111 (15.7)	239 (19.6)	352 (20.6)	461 (21.9)	1,037 (25.1)	0.0000	8.22
Primary etiology of ESRD, n (%)									0.0000	5.60
Diabetes	2,045 (19.9)	35 (22.9)	45 (15.2)	123 (17.6)	219 (18.0)	309 (18.2)	407 (19.4)	907 (22.1)		

	Total (n=10,329)	Tacrolimus trough level (ng/mL)							*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)		
Hypertension	1,001 (9.8)	11 (7.2)	23 (7.8)	51 (7.3)	118 (9.7)	181 (10.6)	201 (9.6)	416 (10.1)		
GN	1,738 (16.9)	17 (11.1)	41 (13.9)	105 (15.0)	230 (18.9)	291 (17.1)	386 (18.4)	668 (16.3)		
PKD	561 (5.5)	4 (2.6)	19 (6.4)	49 (7.0)	67 (5.5)	98 (5.8)	112 (5.3)	212 (5.2)		
IgA nephropathy	1,229 (12.0)	26 (17.0)	31 (10.5)	87 (12.4)	121 (10.0)	200 (11.8)	265 (12.6)	499 (12.2)		
Others	1,361 (13.3)	8 (5.2)	32 (10.8)	107 (15.3)	199 (16.4)	256 (15)	300 (14.3)	459 (11.2)		
Unknown	2,326 (22.7)	52 (34.0)	105 (35.5)	178 (25.4)	262 (21.5)	367 (21.6)	424 (20.2)	938 (22.9)		
Repeat transplant, n (%)	818 (7.9)	12 (7.8)	17 (5.7)	53 (7.5)	79 (6.5)	143 (8.4)	161 (7.6)	353 (8.6)	0.1951	2.13
Pre-transplant dialysis, n (%)	8,468 (82.0)	106 (68.8)	225 (75.5)	532 (75.2)	993 (81.3)	1,419 (82.8)	1,752 (83.3)	3,441 (83.5)	0.0000	6.41
Transplant characteristics										

	Total (n=10,329)	Tacrolimus trough level (ng/mL)							*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)		
PRA, %, mean±SD										
Class I	13.8±27.1	13.6±25.7	12.2±26.9	14.5±28.1	15.2±28.9	15.7±29.2	14.6±27.7	11.9±24.8	0.0003	3.58
Class II	13.4±27.2	16.2±30	14.4±29.1	14.4±28.6	14.9±29	14.5±28.3	14.1±27.8	11.7±25.3	0.0042	4.21
HLA-A/B/DR antigen mismatches, mean±SD	3.3±1.5	3.4±1.8	3.4±1.6	3.5±1.5	3.3±1.6	3.3±1.6	3.4±1.5	3.3±1.5	0.2513	1.94
Desensitization, n (%)	2,457 (23.8)	53 (34.4)	70 (23.5)	212 (30.0)	327 (26.8)	453 (26.4)	495 (23.5)	847 (20.5)	0.0000	6.90
Pre-DSA positivity, n (%)	1,177 (16.3)	27 (19.4)	25 (10.6)	76 (14.0)	145 (16.8)	222 (18.5)	270 (18.3)	412 (14.8)	0.0013	0.19
ABO incompatible, n (%)	1,282 (14.8)	28 (21.4)	33 (14.5)	72 (15.3)	129 (14.7)	224 (16.7)	273 (15.0)	523 (13.8)	0.0777	1.22
Crossmatch positivity,	730 (7.1)	12 (7.8)	23 (7.7)	89 (12.6)	147 (12.0)	158 (9.2)	135 (6.4)	166 (4.0)	<0.0001	10.25

	Total (n=10,329)	Tacrolimus trough level (ng/mL)						*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)	
n (%)									
Induction therapy, n (%)								0.0000	0.64
None	268 (2.6)	7 (4.5)	5 (1.7)	8 (1.1)	17 (1.4)	39 (2.3)	35 (1.7)	157 (3.8)	
Basiliximab	7,919 (76.7)	114 (74.0)	237 (79.5)	561 (79.3)	949 (77.7)	1,318 (76.9)	1,629 (77.4)	3,111 (75.4)	
ATG	2,105 (20.4)	33 (21.4)	56 (18.8)	138 (19.5)	254 (20.8)	351 (20.5)	434 (20.6)	839 (20.3)	
Others	35 (0.3)				2 (0.2)	5 (0.3)	8 (0.4)	20 (0.5)	
IS at 2 months post-transplant, n (%)[a]									
Tacrolimus	7,664 (74.2)	122 (79.2)	263 (88.3)	620 (87.7)	960 (78.6)	1,245 (72.7)	1,439 (68.3)	3,015 (73.0)	0.0000	9.01
Once daily	245 (2.4)	17 (11.0)	33 (11.1)	53 (7.5)	61 (5.0)	32 (1.9)	28 (1.3)	21 (0.5)	0.0000	17.15
Twic	7,426	106	230	568	900	1,214	1,412	2,996	0.000	3.0

	Total (n=10,329)	Tacrolimus trough level (ng/mL)							*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)		
e daily	(71.9)	(68.8)	(77.2)	(80.3)	(73.6)	(70.9)	(67)	(72.6)	0	5
Cyclosporine	29 (0.3)	7 (4.5)		3 (0.4)	3 (0.2)	4 (0.2)	6 (0.3)	6 (0.1)	0.0000	4.37
MMF or EC-MPA	6,141 (59.5)	76 (49.4)	184 (61.7)	468 (66.2)	770 (63.0)	1,086 (63.4)	1,264 (60.0)	2,293 (55.5)	0.0000	5.28
Steroid	4,260 (41.2)	87 (56.5)	167 (56.0)	380 (53.7)	519 (42.5)	700 (40.9)	793 (37.7)	1,614 (39.1)	0.0000	8.65
Others	363 (3.5)	23 (14.9)	17 (5.7)	32 (4.5)	40 (3.3)	60 (3.5)	58 (2.8)	133 (3.2)	0.0000	4.87
Donor information										
Age, years, mean±SD	44.7±13.3	45.9±13.7	45.1±13.7	45.3±13.2	45±13.1	44.8±13.3	45.1±13.3	44.1±13.3	0.0264	2.97
Male sex, n (%)	5,349 (51.8)	65 (42.2)	141 (47.3)	362 (51.2)	616 (50.4)	909 (53.1)	1,080 (51.3)	2,176 (52.7)	0.0669	2.40
BMI, kg/m^2, mean±SD	24.6±43.7	23.5±3.4	23.5±3.3	23.6±3.3	23.7±3.4	26.0±83.0	25.1±59.5	24.2±13.7	0.7945	0.42
Hypertension, n (%)	808 (11.5)	16 (11.1)	18 (6.7)	59 (9.4)	100 (10.4)	150 (12.4)	173 (12.6)	292 (12.1)	0.0363	3.34
Donor									0.000	1.1

	Total (n=10,329)	Tacrolimus trough level (ng/mL)							*P*-value[a]	SMD
		<3.0 (n=154)	3.0–3.9 (n=298)	4.0–4.9 (n=707)	5.0–5.9 (n=1,222)	6.0–6.9 (n=1,713)	7.0–7.9 (n=2,106)	≥8.0 (n=4,129)		
type, n (%)									0	0
Living related	5,145 (49.9)	88 (57.1)	131 (44.0)	363 (51.4)	611 (50.0)	831 (48.5)	1,072 (51.0)	2,049 (49.7)		
Living non-related	2,056 (19.9)	38 (24.7)	87 (29.2)	173 (24.5)	270 (22.1)	353 (20.6)	360 (17.1)	775 (18.8)		
Deceased	3,118 (30.3)	28 (18.1)	80 (26.9)	170 (24.1)	340 (27.8)	529 (30.9)	672 (31.9)	1,299 (31.5)		

ATG, anti-thymocyte globulin; BMI, body mass index; DSA, donor-specific antibody; EC-MPA, enteric-coated mycophenolic acid; ESRD, end-stage renal disease; GN, glomerulonephritis; IgA, Immunoglobulin A; IS, immunosuppression; MMF, mycophenolate mofetil; PKD, polycystic kidney disease; PRA, panel reactive antibodies; SD, standard deviation; SMD, standardized mean difference.

Table 2. Summary of the study findings; association between tacrolimus trough levels and 1-year and 6-year outcomes showing a significant increase (↑) or decrease (↓) of risk based on hazard estimates. Hazard ratios (values in brackets) are derived from adjusted analysis using the inverse probability inverse probability of treatment weighting (IPTW) method with stabilized weights.

	Tacrolimus trough level (ng/mL)						
	<3.0	3.0–3.9	4.0–4.9	5.0–5.9	6.0–6.9	7.0–7.9	≥8.0
1-year outcome							
Allograft composite outcome	↑ (4.74)	↑ (1.40)	-	↓ (0.69)	↓ (0.81)	↓ (0.73)	Ref
BPR	↑ (2.97)	-	-	↓ (0.71)	-	-	Ref
Kidney dysfunction	↑ (6.85)	↑ (1.55)	-	↓ (0.68)	-	↓ (0.58)	Ref
dnDSA	↑ (14.32)	↑ (5.47)	↑ (4.31)	-	-	-	Ref
DCGF	↑ (12.42)	↑ (4.80)	↑ (2.50)	-	-	-	Ref
Severe infection	↑ (5.49)	-	-	-	-	-	Ref
MACE	↑ (4.78)	-	-	-	-	-	Ref
Mortality	↑ (5.78)	-	-	↓ (0.32)	-	-	Ref
2–6-year outcome							
Allograft composite outcome	↑ (2.94)	-	-	↓ (0.68)	↓ (0.65)	-	Ref
BPR	↑ (2.66)	-	-	-	-	-	Ref
Kidney dysfunction	↑ (2.46)	↓ (0.65)	↓ (0.56)	↓ (0.42)	↓ (0.46)	↓ (0.52)	Ref
dnDSA	↑ (2.67)	-	-	-	-	-	Ref
DCGF	↑ (3.27)	-	-	↓ (0.58)	↓ (0.53)	-	Ref

Severe infection	-	↓ (0.61)	↓ (0.51)	↓ (0.41)	↓ (0.51)	↓ (0.65)	Ref
MACE	-	-	-	-	-	-	Ref
Malignancy	↑ (1.93)	-	-	↓ (0.41)	↓ (0.39)	-	Ref
Mortality	-	-	-	-	-	-	Ref

BPR, biopsy-proven rejection; DCGF, death-censored graft failure; dnDSA, de novo donor-specific antibody; MACE, major adverse cardiovascular events, Ref, references.

Dashes (-) indicate hazard ratios that were not statistically significant

The data that support the findings of this study are available from the corresponding author upon reasonable request. However, due to the nature of the data and the policies of the institutional review board (IRB), some restrictions may apply to the availability of these data.