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Clinical Implications of Tacrolimus Time in Therapeutic Range and Intrapatient Variability in Urban Renal Transplant Recipients Undergoing Early Corticosteroid Withdrawal

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Background. Tacrolimus demonstrates wide intrapatient and interpatient variability requiring therapeutic drug monitoring. The utility of tacrolimus time in therapeutic range (TTR) after renal transplantation (RT) under an early corticosteroid withdrawal (ECSWD) protocol is unknown. The purpose of this study is to assess the impact of tacrolimus TTR in an ECSWD RT population. **Materials.** A retrospective analysis of adult RT recipients maintained on tacrolimus was conducted. Patients were excluded if they were on nonstandard protocol immunosuppression agents <12 months post-RT. Tacrolimus TTR was calculated using the Rosendaal method. Patients were divided into high (TTR-H) and low (TTR-L) TTR groups based on cohort median. The primary outcome was to compare the incidence of acute rejection 12 months post-RT. Secondary outcomes included comparing rejection subtypes, incidence of donor-specific antibody (DSA) and de novo DSA (dnDSA), risk factors for acute rejection and dnDSA development, and allograft function (serum creatinine and estimated glomerular filtration rate). **Results.** A total of 193 patients were analyzed (TTR-H = 98 and TTR-L = 95). There was no difference in the incidence of acute rejection (TTR-H 20.4% versus TTR-L 20.0%; $P = 0.944$). Positive DSA posttransplant (odds ratio [OR], 3.62; 95% confidence interval [CI], 1.41-9.26; $P = 0.007$) was associated with a higher acute rejection at 12 months post-transplant. Mycophenolate dose reduction (OR, 2.82; 95% CI, 1.13-6.97; $P = 0.025$) and acute rejection (OR, 2.99; 95% CI, 1.09-8.18; $P = 0.032$) were associated with dnDSA formation. No difference in serum creatinine or estimated glomerular filtration rate was observed ($P > 0.05$). **Conclusions.** Tacrolimus TTR was not significantly different with regards to acute rejection in an ECSWD population. Future studies are still needed to determine tacrolimus TTR thresholds post-RT and identify populations that may benefit from this intrapatient variability monitoring parameter.

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INTRODUCTION

The longevity of renal transplantation (RT) rests on the delicate balance of adequate immunosuppression to minimize immunologic allograft insult through rejection and donor-specific antibody (DSA) formation, as well as excess immunosuppression potentially leading to toxicities such as malignancy and infection.[1] Tacrolimus, a calcineurin inhibitor, is the mainstay of immunosuppression following RT. It is classified as a narrow therapeutic index medication, displaying wide interpatient variability and intrapatient variability (IPV) and requiring frequent therapeutic drug monitoring of trough levels.[2-5] Therefore, it is critical to maintain therapeutic levels to preserve allograft function.

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In addition to assessing adequate trough levels, tacrolimus IPV is another important consideration for allograft longevity.[5] Increased tacrolimus IPV, as measured by coefficient of variation (CV%) or SD, is associated with deleterious outcomes in RT recipients, including increased allograft fibrosis, rejection, development of DSA, and decreased allograft survival.[5-14] Several studies assessing time in therapeutic range (TTR) with tacrolimus have demonstrated that patients with lower TTR values have been associated with de novo DSA (dnDSA) development and inferior allograft outcomes.[15-17] However, many of these aforementioned studies were in the setting of triple immunosuppression therapy with immediate-release tacrolimus, antimetabolite, and long-term corticosteroid therapy. Patients on early corticosteroid withdrawal (ECSWD) protocol rely solely on tacrolimus and mycophenolate for maintenance immunosuppression, with mycophenolate doses being generally standardized and tacrolimus trough levels being titrated on the basis of targeted trough concentrations. Theoretically, the importance of tacrolimus TTR may be more critical within this population, as clinical efficacy in the prevention of rejection and DSA could depend more heavily on these tacrolimus concentrations and TTR compared with prior studies in the setting of triple immunosuppression.

Therefore, the purpose of this study was to evaluate the impact of tacrolimus TTR and IPV on renal allograft outcomes in the setting of an ECSWD protocol.

MATERIALS AND METHODS

Patient Population

This was a retrospective single-center cohort study. Adult (aged ≥18 y) isolated RT recipients at University of Illinois Hospital and Health Sciences System between January 1, 2015, and December 31, 2018, who were maintained on tacrolimus within the first year of transplant were evaluated. This study was approved by the institutional review board of the University of Illinois at Chicago. Patients were excluded if they underwent an ABO incompatible transplant, had a positive T-cell or B-cell flow crossmatch, died ≤90 days of transplantation, were lost to follow-up, transferred centers within the first 12 months posttransplantation, had <10 recorded tacrolimus trough levels, were not initiated on mycophenolate posttransplantation, were maintained on long-term corticosteroids posttransplant, or were maintained on nonstandard protocol immunosuppression (ie, eculizumab, mammalian target of rapamycin inhibitors) within the first 12 months posttransplantation. All patients included within the analysis underwent ECSWD a priori. Patients who do not meet criteria for ECSWD were the following: those patients who were on chronic corticosteroid maintenance therapy before transplantation, those with positive T-cell or B-cell flow crossmatch, and those recipients undergoing retransplantation whose initial allografts failed because of recurrent disease or rejection per transplant surgeon attending discretion. All patients who did not undergo ECSWD were excluded from this analysis.

Immunosuppression

Rabbit antithymocyte globulin (1.5 mg/kg based on ideal body weight postoperative days [PODs] 0–4) was used in high immunologic risk living-donor and deceased-donor RT recipients. In total, patients received a total cumulative rabbit antithymocyte dose of 7.5 mg/kg (based on ideal body weight). Patients had dose adjustments for thrombocytopenia, leukopenia-neutropenia, and absolute lymphocyte count per center protocol. High immunologic risk was defined as panel reactive antibody >10%, repeat transplant, African American race, and donor factors determined to increase risk of acute tubular necrosis. Donor factors include the following: serum creatinine (SCr) >1.8 mg/dL, donor age >50 years, cold ischemic time >24 hours, donation after circulatory death, and Kidney Donor Profile Index ≥85%. All other patients received basiliximab or alemtuzumab induction therapy. For maintenance immunosuppression, tacrolimus was initiated immediately following RT on POD1. Patients were initiated on a tacrolimus immediate release of 0.05 mg/kg (based on ideal body weight) by mouth every 12 hours. For patients initiated on de novo tacrolimus extended release (XR), the started dose was 0.1 mg/kg (based on ideal body weight). Goal tacrolimus trough level between 0 and 2 months was 8–12 ng/mL and beyond 2 months was 5–10 ng/mL. Patients also received mycophenolic acid 720 mg twice daily. ECSWD by POD5 was accomplished in all included study patients.

Tacrolimus Level Assessment

Whole blood tacrolimus concentrations were determined using a microparticle immunoassay with an Architect 12000SR analyzer (Abbot Laboratories, Chicago, IL). Tacrolimus levels within 12 months of RT were extracted from the electronic medical record. Any tacrolimus level >20 ng/mL or levels that were outside of the inpatient or outpatient protocol time frames were individually examined for trough appropriateness. Patients were seen at scheduled times within the transplant clinic and obtained laboratory testing according to their time posttransplant. Clinic laboratory testing is as follows: month 1 posttransplant (clinic twice weekly, laboratory twice weekly), months 2–3 posttransplant (clinic every 1–2 wk, laboratory once a wk), months 4–6 posttransplant (clinic every 1–2 mo; laboratory every 2–4 wk), and months 7–12 (clinic every 2 mo, laboratory every 1 mo). Patients could be seen more frequently if there were complications in their care, per transplant team discretion. Tacrolimus level trough validation was examined by clinical pharmacist investigators (D.P. and A.L.) via electronic medical record review. Levels clearly drawn at inappropriate time points were not deemed appropriate and were also eliminated. All tacrolimus levels that were <2.0 ng/mL were considered 0.0 ng/mL. Tacrolimus values from POD21 onward were used to calculate IPV to eliminate initially high variability seen in the postoperative phase of care.[12,18]

The Rosendaal linear interpolation method was used to calculate TTR, which assumes a linear relationship exists between each measured value and then assigns a specific value for each day between tests.[19] Protocol tacrolimus goals were used to calculate 12-month TTR. The median tacrolimus TTR was 76.3% and the average TTR was 71.7% within the first 12 months post-RT. Given this information, study investigators used the cutoff of 75% within the context of this analysis, given the disparity of an established tacrolimus TTR. High tacrolimus TTR (TTR-H) was defined as being greater than or equal to a TTR of 75%. Low tacrolimus TTR (TTR-L) was defined as being <75%. In the absence of a clearly defined TTR cutoff in a RT population, median TTR was selected to divide and assess the population. IPV was assessed via

CV%, calculated as (SD/mean) × 100%. The whole cohort CV% median value was used to define the CV% threshold in analyses.

Clinical Outcomes

The primary outcome was to compare 12-month acute rejection between patients with high TTR and those with low TTR. Secondary outcomes included risk factors for low TTR, CV% between patients with high and low TTR, the incidence of dnDSA, patient and allograft survival, and allograft function via estimated glomerular filtration rate (eGFR).

Patients were considered to have acute rejection if they had biopsy-confirmed rejection or if they received empiric treatment for rejection. Biopsy-proven acute rejection included T-cell acute cellular rejection (ACR) and antibody-mediated rejection (AMR) and was diagnosed according to the Banff 2017 criteria in most cases.[20,21] Some patients were treated for rejection in the absence of histologic evidence of rejection in biopsy prohibitive circumstances, defined as: (1) acute kidney injury in the absence of other differential diagnoses and (2) response to high-dose corticosteroid therapy (methylprednisolone divided over 2–3 d) or antithymocyte globulin in which there was a return to baseline renal function. AMR was treated initially with plasmapheresis (1.5 plasma volume with anticoagulant citrate dextrose and 5% albumin or fresh frozen plasma replacement) and IVIG (150 mg/kg based on ideal body weight dosed after plasmapheresis sessions) with or without high-dose steroids and rabbit antithymocyte globulin. Salvage therapy with rituximab, bortezomib, or high-dose IVIG (2 g/kg ideal body weight) was per transplant team discretion and included 1 patient.

All HLA testing and antibody analyses were reviewed by the American Board of Histocompatibility and Immunogenetics board-certified HLA specialists in an American Society For Histocompatibility and Immunogenetics and College of American Pathologists accredited laboratory. Patients were typed by serology at HLA-A, -B, -Cw, and HLA-DR/DQ by sequence-specific oligonucleotide (LABType, One Lambda). Donors were typed by the organ recovery center and resolved in a similar manner by sequence-specific oligonucleotide, sequence-specific primers, and sequencing as needed. At the time of transplant, flow cytometry crossmatching was performed on all available sera up to 6 months before the transplant date. DSAs were expressed in mean fluorescence intensity (MFI) and measured using a Luminex single antigen bead assay platform (LabScreen Single Antigen; One Lambda, Inc., Canoga Park, CA). All samples were analyzed in neat serum and treated with dithiothreitol. Institutional positivity threshold for DSA was 700 MFI. All MFIs were normalized against negative control beads per the manufacturer's instructions. DSAs were monitored on the basis of transplant provider discretion, generally at the time of any allograft dysfunction or change in immunosuppression regimen.

SCr and eGFR, as calculated by the Modification of Diet in Renal Disease study equation, were collected at 1, 3, 6, and 12 months posttransplant.[22] Allograft loss was defined as a return to chronic dialysis.

Statistical Analysis

Data were assessed for normality with the Shapiro-Wilk test and then visually. Categorical variables were compared with either the Kruskal-Wallis test, χ^2 test, or Fisher exact test. Ordinal data and nonparametric continuous variables were compared with the Wilcoxon signed-rank test, Mann-Whitney U test, or the Kruskal-Wallis test. Parametric continuous data were compared with 1-way ANOVA or Student *t* test. Time to event analyses were assessed with Kaplan-Meier curves with log-rank comparisons. Median tacrolimus TTR was used to group patients into TTR-H and TTR-L. Additionally, TTR and CV% were assessed by receiver operating characteristic (ROC) curve analysis using univariate logistic regression and the Youden index in addition to assessing the whole cohort median.

Univariate and multivariate logistic regression models were used to assess the incidence of acute rejection within 1 year of RT and the development of dnDSA within 1 year of transplantation. Factors from the univariate models were then entered into the respective multivariate model if they achieved a $P < 0.20$. Model selection was completed using backward selection to optimize the Akaike information criterion. Model fit was confirmed with the Hosmer-Lemeshow goodness-of-fit test. An ROC curve was generated and area under the curve (AUC) was reported for the final multivariate model.

Statistical analysis was completed using STATA Version 14 Data Analysis and Statistical Software (StataCorp LP, College Station, TX). All *P* values <0.05 were considered statistically significant.

RESULTS

Baseline Demographics

The baseline demographics are demonstrated in Table 1. Patients were predominantly African American (51.8%) and male individuals (68.9%) with an average age of 51.7 years (SD, +13.2). A majority of the patients received a living-donor RT (60.6%) and received induction with rabbit antithymocyte globulin (59.1%). Patients were maintained predominantly on tacrolimus XR (43.0%).

The 2 groups were statistically similar with regards to age, race, transplant type, donor quality, and immunosuppression. Incidence rate of sensitized patient (ie, peak class I or class II panel reactive antibody >10%) was higher in the TTR-L groups ($P = 0.027$ and $P = 0.038$, respectively). There was also a higher incidence rate of steroid reintroduction in the TTR-L group (TTR-H 20.4% versus TTR-L 35.8%; $P = 0.017$). Out of the cohort, steroid reintroduction for infections/leukopenia (TTR-L 24 of 95 [25.3%] versus 13 of 78 [13.2%]; $P = 0.030$) was significantly different between the groups, but not steroid reintroduction for acute rejection (TTR-L 10 of 95 [10.5%] versus TTR-H 7 of 98 [1.5%]; $P = 0.267$). Table 1 highlights the baseline demographic and immunosuppression differences between the 2 groups.

Tacrolimus Intrapatient Variability

Over the course of the study, a total of 5894 tacrolimus levels were assessed. The median number of tacrolimus trough concentrations assessed per patient was 26 levels (interquartile range [IQR], 21–30). The overall 12-month TTR was 71.7% (SD, ±19.5%). Within the first 60 days of transplant, the whole cohort average tacrolimus TTR was lower compared with the TTR calculated from POD 61 to 365 (58.7% versus 74.3%; $P < 0.001$). Tacrolimus TTR was significantly different between the 2 groups when assessing values from POD 21 to 60 (TTR-H 66.0% versus TTR-L 51.2%; $P < 0.001$) and

TABLE 1.

Demographic information

Variable	Whole cohort (n = 193)	High TTR (n = 98)	Low TTR (n = 95)	*P*
Age at transplant, mean (SD)	51.7 (±13.2)	51.7 (±12.5)	51.8 (±13.9)	0.829
Male, n (%)	133 (68.9)	73 (74.5)	60 (63.2)	0.089
African American, n (%)	100 (51.8)	47 (48)	53 (55.8)	0.276
BMI >35 kg/m^2, n (%)	73 (37.8)	40 (40.8)	33 (34.7)	0.384
Repeat transplant, n (%)	11 (5.73)	4 (4.1)	7 (7.4)	0.333
Deceased-donor renal transplant, n (%)	76 (39.4)	43 (43.9)	33 (34.7)	0.194
PRA class I >10%, n (%)	42 (21.8)	15 (15.3)	27 (28.4)	0.027
PRA class II >10%, n (%)	23 (11.9)	7 (7.1)	16 (16.8)	0.038
Pretransplant DSA, n (%)	29 (15.0)	8 (8.2)	21 (22.1)	0.007
KDPI, mean (SD)	47.3 (±24.1)	44.8 (±22.6)	50.1 (±25.6)	0.319
DCD donor, n (%)	13 (6.7)	6 (6.1)	7 (7.4)	0.656
Induction immunosuppression, n (%)				
Alemtuzumab	21 (12.2)	12 (12.2)	9 (9.5)	0.225
Rabbit antithymocyte globulin	114 (59.1)	52 (53.1)	62 (65.3)	
Basiliximab	58 (30.1)	34 (34.7)	24 (25.3)	
Tacrolimus formulation at POD21 posttransplant, n (%)				
Tacrolimus IR	54 (27.9)	31 (31.6)	23 (24.2)	0.115
Tacrolimus XL	59 (29.0)	32 (32.7)	24 (25.3)	
Tacrolimus XR	83 (43.0)	35 (35.7)	48 (50.5)	
Renal allograft function, n (%)				
Good immediate function	151 (78.7)	80 (82.5)	71 (74.7)	0.424
Slow graft function	19 (9.9)	8 (8.3)	11 (11.6)	
Delayed graft function	22 (11.5)	9 (9.3)	13 (13.7)	
Mycophenolate reduction or discontinuation within 12 mo of transplantation, n (%)	54 (27.9)	24 (24.5)	30 (31.6)	0.273
Reintroduction of steroids within 12 mo posttransplant, n (%)	54 (27.9)	20 (20.4)	34 (35.8)	0.017
Average 12 mo TTR, % (SD)	71.7 (19.5)	86.1 (6.5)	56.9 (17.2)	<0.001
Average TTR (POD 21–60), % (SD)	58.7 (25.2)	66.0 (23.1)	51.2 (25.2)	<0.001
Average TTR (POD 61–365), % (SD)	74.3 (21.8)	90.1 (7.0)	58.9 (17.2)	<0.001
Average 12 mo tacrolimus trough levels, ng/mL (SD)	8.7 (±1.1)	8.7 (+0.8)	8.6 (±1.4)	0.833
Average 12 mo tacrolimus levels, (SD)	2.9 (±0.8)	2.5 (±0.7)	3.2 (±0.7)	<0.001
Average 12 mo tacrolimus trough level CV%, % (SD)	33.3% (±9.9%)	28.6% (±6.7%)	38.2% (+10.5%)	<0.001
Death-censored graft loss at 12 mo, n (%)	1 (0.5)	0 (0)	1 (1.1)	0.492
Patient death at 12 mo, n (%)	3 (1.6)	1 (1.0)	2 (2.1)	0.542

CV%, coefficient of variation; DCD, donation after circulatory death; DSA, donor-specific antibody; IQR, interquartile range; KDPI, kidney donor profile index; POD, postoperative d; PRA, panel reactive antibody; TTR, time in therapeutic range; XR, extended release.

from POD 61 to 365 (TTR-H 90.1% versus TTR-L 58.9%; $P < 0.001$). Tacrolimus TTR appeared to be more stable further out from RT (whole cohort POD 21 to 60 tacrolimus TTR 58.7% versus whole cohort POD 61 to 365 tacrolimus TTR 74.3%; $P < 0.001$). There was no significant difference in TTR by tacrolimus formulation (tacrolimus IR 74.1% [SD, ±17.5%] versus tacrolimus XL 73.9% [SD, ±19.6%] versus tacrolimus XR 68.7% [SD, ±20.5%]; $P = 0.175$). There was no difference in the median number of tacrolimus levels collected per patients between the groups (TTR-H 26 levels versus TTR-L 25 levels; $P = 0.093$). There was no correlation between the number of tacrolimus trough levels and tacrolimus TTR ($R^2 = 0.013$; $P = 0.120$).

The overall cohort tacrolimus CV% average was 33.3% (SD, ±9.9). The low TTR group had a significantly higher CV% and SD compared with the high TTR group (TTR-H 28.6% versus TTR-L 38.2%; $P < 0.001$) and (TTR-H 2.5 versus TTR-L 3.2; $P < 0.001$). Average tacrolimus levels were similar between TTR groups ($P = 0.833$). Table 1 details tacrolimus IPV.

Rejection

There was no statistically significant difference between the incidence of acute rejection at 12 months (TTR-H 20.4% versus TTR-L 20.0%; $P = 0.944$). The incidence of biopsy-proven acute rejection (BPAR) was also statistically similar (TTR-L 12.2% versus TTR-L 15.8%; $P = 0.478$). When broken down by rejection subtype, there were no differences observed in ACR ($P = 0.207$), AMR ($P = 1.00$), and MAR ($P = 1.00$) between the groups. There was no difference in biopsy grade between the groups ($P = 0.495$). Time to first acute rejection episode did not differ by TTR group ($P = 0.214$). There was no difference in tacrolimus TTR between those who experienced acute rejection compared with those who did not (acute rejection TTR 69.6% [SD, ±22.7] versus no acute rejection TTR 72.2% [SD, ±18.73]; $P = 0.783$). Table 2 describes the rejection comparisons between the 2 groups.

In multivariate analysis, positive DSA posttransplant (odds ratio [OR], 3.62; 95% confidence interval [CI], 1.41-9.26; $P = 0.007$) was associated with a higher acute rejection incidence at 12 months posttransplant. Tacrolimus TTR was not significant in the univariate analysis and did not meet *P* value criteria for entrance into the multivariate model assessing risk of acute rejection. The AUC for the ROC curve for this multivariate analysis was 74.54%. Table 3 details the univariate and multivariate logistic regression for acute rejection at 12 months posttransplant.

TABLE 2.

Rejection, donor-specific antibody, and allograft outcomes within 12 mo post–renal transplantation

Variable	Overall (n = 193)	High TTR (n = 98)	Low TTR (n = 95)	*P*
Acute rejection at 12 mo, n (%)	38 (19.7)	20 (20.4)	18 (18.9)	0.799
All BPAR at 12 mo, n (%)	27 (13.9)	12 (12.2)	15 (15.8)	0.478
BPAR ACR at 12 mo, n (%)	5 (2.6)	1 (1.0)	4 (4.2)	0.207
BPAR AMR at 12 mo, n (%)	20 (10.4)	10 (10.2)	10 (10.5)	1.000
BPAR mixed at 12 mo, n (%)	2 (1.0)	1 (1.0)	1 (1.1)	1.000
Biopsy grade at first proven biopsy, n (%)				
Borderline	17 (8.8)	9 (9.2)	8 (8.2)	0.495
IA	0 (0)	0 (0)	0 (0)	
IB	3 (1.6)	2 (2)	1 (1.1)	
IIA	1 (0.5)	0 (0)	1 (1.1)	
IIB	1 (0.5)	0 (0)	1 (1.1)	
III	0 (0)	0 (0)	0 (0)	
Time to first acute rejection, d (IQR)	51.5 (16–206)	30 (13.5–146)	99 (21–210)	0.214
Time to first BPAR, d (IQR)	101 (52–221)	105 (52–261)	99 (23–210)	0.661
Pretransplant DSA, n (%)	29 (15.0)	8 (8.2)	21 (22.1)	0.007
DSA assessed posttransplant, n (%)	131 (67.9%)	59/98 (60.2)	72/95 (75.8%)	0.020
Posttransplant DSA (preexisting and de novo), n (%)	50/131 (38.2)	19/59 (32.6)	31/72 (43.1)	0.203
Multiple DSA, posttransplant, n (%)	12/50 (24.0%)	3/19 (15.6)	9/31 (29.0)	0.287
Posttransplant DSA loci (includes both preexisting and de novo DSA), n (%)				0.520
A	2/50 (4.0)	1/19 (5.3)	1/31 (3.2)	
B	9/50 (18.0)	2/19 (10.5)	7/31 (2.3)	
C	5/50 (10.0)	3/19 (15.8)	2/31 (6.5)	
DR	1/50 (2.0)	1/19 (5.3)	0/31 (0)	
DP	14/50 (28.0)	6/19 (31.6)	8/31 (25.8)	
DQ	19/50 (38.0)	6/19 (31.6)	13/31 (41.9)	
De novo DSA, n (%)	30/131 (22.9)	14/59 (23.7)	16/72 (22.2)	0.838
De novo DSA class, n (%)				
Class I only	5/30 (16.7)	4/14 (28.6)	1/16 (6.3)	0.258
Class II only	17/30 (56.7)	7/14 (50.0)	10/16 (62.5)	
Class I and class II	8/30 (26.7)	3/14 (21.4)	5/16 (31.3)	
Serum creatinine, mg/dL (SD)				
1 mo	1.73 (0.79)	1.79 (0.89)	1.67 (0.68)	0.259
3 mo	1.51 (0.71)	1.48 (0.54)	1.55 (0.85)	0.557
6 mo	1.38 (0.44)	1.39 (0.45)	1.37 (0.42)	0.641
12 mo	1.39 (0.48)	1.42 (0.55)	1.36 (0.39)	0.448
Estimated GFR, mL/min/1.73^2 (SD)				
1 mo	50.2 (19.1)	49.6 (19.2)	50.7 (19.0)	0.696
3 mo	56.9 (18.6)	57.9 (19.4)	55.9 (17.7)	0.489
6 mo	60.7 (19.0)	61.1 (20.2)	60.2 (17.7)	0.775
12 mo	60.5 (19.8)	60.9 (21.4)	60.1 (17.9)	0.809

ACR, acute cellular rejection; AMR, antibody-mediated rejection; BPAR, biopsy-proven acute rejection; DSA, donor-specific antibody; GFR, glomerular filtration rate; IQR, interquartile range.

Donor-specific Antibody

The incidence of preexisting DSA was higher in patients with low TTR (TTR-H 8.2% versus TTR-L 22.1%; $P < 0.001$) before RT. Out of the whole cohort, 131 patients (67.9%) were assessed for DSA within the first year posttransplant, which differed by TTR group (TTR-H 59 of 98 patients [60.2%] versus TTR-L 72 of 95 patients [75.8%]; $P = 0.020$). Out of those checked for DSA posttransplant, the whole cohort incidence of posttransplant DSA was 38.2% (50 of 131 patients) at 12 months posttransplant. Patients in the TTR-H group had numerically lower incidence of any DSA posttransplant (both preexisting and dnDSA) relative to those in the TTR-L group but this was not statistically different (TTR-H 19 of 59 patients [32.6%] versus TTR-L 31 of 72 patients [43.1%]; $P = 0.203$). A total of 12 out of 50 (24%) patients within the whole cohort possessed multiple DSA posttransplant. There was no significant difference in the presence of multiple DSA posttransplant between the groups (TTR-H 3 of 19 patients [15.6%] versus TTR-L 9 of 31 patients [29.0%]; $P = 0.287$). Out of the dnDSA, HLA class II formed more often than HLA class I, but there was no difference between the groups ($P = 0.520$). Table 2 details DSA outcomes.

Whole cohort incidence rate of dnDSA development within the first 12 months of transplant was 22.9% (30 of 131 patients). The incidence rate of dnDSA formation was similar between the 2 groups (TTR-H 14 of 59 [23.7%] versus TTR-L 16 of 72 [22.2%]; $P = 0.838$). Time to dnDSA detection was similar between the groups (TTR-H 108 d [IQR, 74–168] versus TTR-L 140 d [IQR, 55.5–235.5]; $P = 0.803$).

Out of the 131 patients who were assessed for DSA in the posttransplant setting, a logistic regression was constructed to assess the risk of dnDSA production. In multivariate analysis,

TABLE 3.

Multivariate logistic regression analysis for assessing acute rejection 12 mo posttransplant

Univariate analysis			Multivariate analysis		
Variable	**OR (95% CI)**	***P***	**Variable**	**OR (95% CI)**	***P***
Tacrolimus TTR% (increasing by 10%)	0.94 (0.79-1.12)	0.513			
Tacrolimus CV% (continuous variable)	1.74 (0.53-57.23)	0.754			
Age at transplant (continuous variable)[a]	0.98 (0.95-1.00)	0.174	Age at transplant (continuous variable)	0.97 (0.93-1.00)	0.124
Female[a]	1.83 (0.88-3.82)	0.104			
Black race[a]	2.05 (0.98-4.30)	0.057	Black race	1.82 (0.68-4.94)	0.235
BMI (continuous variable)[a]	1.02 (0.98-1.06)	0.166	BMI (continuous variable)	1.03 (0.99-1.08)	0.113
Deceased-donor renal transplant	0.85 (0.41-1.73)	0.650			
HLA match	0.99 (0.78-1.24)	0.946			
Peak PRA >10%	1.92 (0.87-4.24)	0.105			
Pretransplant DSA	1.44 (0.56-3.69)	0.447			
Lymphodepleting induction[a]	2.69 (1.05-6.84)	0.038			
Mycophenolate dose reduction or discontinuation[a]	0.90 (0.40-2.00)	0.799			
DSA positive posttransplant (preexisting and de novo)[a]	4.12 (1.66-10.21)	0.005	DSA positive posttransplant (preexisting and de novo)	3.62 (1.41-9.26)	0.007
De novo DSA	1.21 (0.38-3.88)	0.742			

[a]Variables selected for inclusion into the multivariate model.

BMI, body mass index; CI, confidence interval; CV%, coefficient of variation; DSA, donor-specific antibody; OR, odds ratio; PRA, panel reactive antibody; TTR, time in therapeutic range.

mycophenolate dose reduction/discontinuation (OR, 2.82; 95% CI, 1.13-6.97; $P=0.025$) and acute rejection within 12 months posttransplant (OR, 2.99; 95% CI, 1.09-8.18; $P=0.032$) were associated with dnDSA formation posttransplantation (Table 4). The AUC for the ROC curve for this multivariate analysis was 71.12%.

Renal Function

There was no statistically significant difference in SCr and eGFR at 1, 3, 6, or 12 months posttransplantation. When assessing postoperative allograft function, there was no difference between the 2 TTR groups ($P=0.424$). Table 2 details patient allograft function.

DISCUSSION

This study demonstrates that there was no difference in allograft rejection between TTR-H and TTR-L within an early corticosteroid withdrawal RT population. The incidence of DSA, dnDSA, and allograft function over time was similar between the 2 TTR groups. In multivariate analysis, the presence of posttransplant DSA was associated with the development of acute rejection within the first year posttransplantation. Furthermore, reduction/discontinuation of mycophenolate and acute rejection were associated with the development of dnDSA within the first year posttransplant.

The first analysis of tacrolimus TTR using the Rosendaal method was assessed in lung transplant recipients.[17] Ensor et al demonstrated that increasing TTR by 10% increments was associated with a decreased risk of ACR (OR, 0.46; 95% CI, 0.40-0.54; $P<0.001$), as well as lower rates of 1-year allograft dysfunction and mortality. However, this study was done in predominantly Caucasian lung recipients who were maintained on long-term steroids. They also had a much lower TTR cutoff of <30% as compared to our cutoff of <75%. Different TTR cutoffs were examined via ROC curve analysis;

TABLE 4.

Multivariate logistic regression for the development of de novo DSA at 12 mo posttransplant

Univariate analysis			Multivariate analysis		
Variable	**OR (95% CI)**	***P***	**Variable**	**OR (95% CI)**	***P***
Tacrolimus TTR% (increasing by 10%)	0.94 (0.77-1.14)	0.540			
Tacrolimus CV% (continuous variable)	2.79 (0.07-109.09)	0.583			
Age at transplant (continuous variable)	0.99 (0.97-1.03)	0.832			
Female	0.71 (0.29-1.78)	0.472			
Black race[a]	2.12 (0.90-4.98)	0.084	Black race	1.51 (0.61-3.74)	0.376
BMI (continuous variable)	1.02 (0.98-1.06)	0.251			
Deceased-donor renal transplant	0.88 (0.38-2.03)	0.766			
HLA match	0.98 (0.71-1.33)	0.885			
Peak PRA >10%	0.72 (0.27-1.96)	0.522			
Lymphodepleting induction	1.12 (0.46-3.11)	0.709			
Mycophenolate dose reduction or discontinuation[a]	2.61 (1.12-6.02)	0.025	Mycophenolate dose reduction or discontinuation	2.82 (1.13-6.97)	0.025
Acute rejection within 12 mo posttransplant[a]	2.65 (1.05-6.72)	0.039	Acute rejection within 12 mo posttransplant	2.99 (1.09-8.18)	0.032

[a]Variables selected for inclusion into the multivariate model.

BMI, body mass index; CI, confidence interval; CV%, coefficient of variation; DSA, donor-specific antibody; OR, odds ratio; PRA, panel reactive antibody; TTR, time in therapeutic range.

however, our ROC AUC was 48.56% for the assessment of tacrolimus TTR and acute rejection. Thus, it was determined that using the population tacrolimus TTR median of 75% (yielding a sensitivity 52.63% and specificity 49.68%) was the most appropriate approach in the absence of an established literature cutoff.

Davis et al[15] were the first to describe the implications of low TTR post-RT. They found that tacrolimus troughs of <8 ng/mL were associated with dnDSA formation at 6 months (OR, 2.51; 95% CI, 1.32-4.79; $P = 0.005$). They further went on to analyze TTR with a cutoff of <60%, which was extrapolated from warfarin studies. This cutoff was associated with dnDSA formation (OR, 1.05; 95% CI, 1.28-3.30; $P = 0.003$), acute rejection (hazard ratio [HR], 4.18; 95% CI, 1.53-6.37; $P = 0.002$), and death-censored graft loss at 5 years posttransplant (HR, 3.12; 95% CI, 1.53-6.37; $P = 0.002$). However, this analysis included patients with varying tacrolimus off-protocol trough goals, such as those on mammalian target of rapamycin inhibitors or belatacept conversion and long-term steroids. In this way, extrapolation to ECSWD maintenance immunosuppression protocols is difficult.

In a subsequent evaluation by the same study group, it was reported that patients with CV% >44.2% and TTR <40% had an increased risk of dnDSA (OR, 4.93; 95% CI, 2.02-12.06; $P < 0.001$).[16] Additionally, patients with CV% >44.2% and TTR <40% had an increased association with death-censored graft loss at 5 years (HR, 4.00; 95% CI, 1.31-12.24; $P = 0.015$). This study identified their CV% and TTR thresholds based on ROC curve analysis.[16] However, this study cohort also included both kidney and pancreas transplant recipients with various tacrolimus off-protocol trough goals, which increased the potential for lower TTR compared with a more homogenous immunosuppression cohort.

An additional study was done in heart transplant comparing the tacrolimus TTR in patients with and without clinical rejection.[23] Baker et al concluded that there was no significant difference between the median TTR (34.1% versus 36.2%; $P = 0.512$) or the time to therapeutic tacrolimus levels (9.5 versus 9.0 d; $P = 0.623$) between those patients who experienced rejection and those who did not experience rejection, respectively. This single-center retrospective cohort study was done in primarily Caucasian heart transplant recipients maintained on chronic steroids. However, this study highlights that tacrolimus variability is only a component of rejection risk and might not be a sole predictor in and of itself.

Through examination of past studies, the clinical utility of tacrolimus variability and TTR has yet to be fully elucidated in RT recipients. In tacrolimus dry-blood level assessment of stable, adherent transplant recipients, the median CV% was 15.2% (range, 4.8%–10%).[8] In this analysis, there were no differences in CV% by allograft type or tacrolimus formulation and multivariate analysis did not identify any demographic characteristics associated with a CV% >30%.[8] In this way, establishing thresholds for tacrolimus variability remains nebulous. Taber et al[10] assessed the impact of African American race on tacrolimus variability and found that a 10% increase in tacrolimus CV% increased the risk of acute rejection by 20% (adjusted HR, 1.20; $P < 0.001$) and the risk of graft loss by 30% (adjusted HR, 1.30; $P < 0.001$). However, in our particular analysis, neither TTR nor CV% was the factor impacting acute rejection in multivariate modeling.

Within the context of this evaluation, pharmacogenomics testing is an important consideration in tacrolimus trough level monitoring. The "Clinical Pharmacogenetics Implementation Consortium Guidelines for CYP3A5 Genotype and Tacrolimus Dosing" do not recommend for or against CYP3A5 screening, but rather how best to use this information within the context of clinical practice.[24] Knowing this pharmacogenetic information upfront could potentially guide therapy and also the aggressiveness of tacrolimus dose titration. Furthermore, CYP3A5 pharmacogenetics also have the potential to impact TTR in RT recipients, but the widespread clinical use of this has not been proven and is a significant cost burden to institutions at this point in time with unclear benefit.[25,26]

In addition, this study highlights the cautiousness needed regarding mycophenolate dose adjustments in the setting of an ECSWD population. Alterations in mycophenolate dosing or even discontinuation should be balanced through the reintroduction of a steroid as a component of the immunosuppression regimen. Development of protocols or monitoring guidance would be potentially beneficial within the context of a program, regardless of the dose adjustment indication.

There were several limitations of this study. First, this is a single-center, retrospective study, so missing data and variable follow-up could impact the analyses. There were differences between the groups at baseline, including differences in pretransplant DSA, that could affect the results. Second, we calculated CV% over the entire year, starting from POD21 and including all inpatient levels. Tacrolimus pharmacokinetics and pharmacodynamics can be altered in the setting of the acute transplant phase and also in the setting of infection; therefore, these data are likely not extrapolatable to chronic RT recipients when only outpatient tacrolimus IPV is assessed.[12] Additionally, tacrolimus dose adjustment is subject to provider preference and introduces heterogenicity. Furthermore, low TTR could be influenced by both patients with subtherapeutic levels and those with supratherapeutic levels. As such, this can impact the utility of the TTR as a measurement of risk in regard to allograft outcomes. Despite this, our patient population had a relatively high TTR compared with previous experiences. Finally, there is also no protocol for preemptively monitoring DSA at standard time points posttransplant, which may underestimate the incidence of DSA in the study cohort. However, DSAs were checked in a majority of the patients (67.9%)

This study also has notable strengths. This was the first study assessing TTR in RT recipients at a predominantly ECSWD center in patients maintained on tacrolimus and mycophenolate alone. Within this analysis, a high proportion of Black RT recipients, which are more commonly to be CYP3A5 *1 expressors compared with the general population and who were not as well represented within past studies. The presence of CYP3A5*1 can increase tacrolimus variability relative to CYP3A5 nonexpressors.[10,11,13] These varying tacrolimus levels have the potential to have a larger impact on clinical outcomes, potentially more so in a ECSWD population. Additionally, patients were on a variety of tacrolimus formulations, as opposed to analyzing the immediate release tacrolimus formulation alone. In the setting of different tacrolimus formulations, there was no observed difference in tacrolimus TTR or CV%.

In conclusion, we found that there was no difference in acute rejection or BPAR when assessing 12-month tacrolimus

TTR. There was a higher incidence of DSA at 1 year post-transplant in those with reduced mycophenolate dosing and history of acute rejection. Future studies are still needed to determine TTR thresholds and ideal populations for this particular tacrolimus variability measurement. Additionally, further studies are needed to assess the impact of mycophenolate dose reduction and discontinuation in an ECSWD population.

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