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Tacrolimus time in therapeutic range and long-term outcomes in heart transplant recipients

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Abstract

Background: Little is known about the association between tacrolimus time in therapeutic range (TTR) within the guideline-recommended targets and heart transplant (HT) patient outcomes. This study evaluated the association of early tacrolimus TTR with rejection and other clinical outcomes during an extended follow-up after HT.

Methods: This was a single-center retrospective cohort study of HT recipients ≥18 years of age conducted at Michigan Medicine (1/1/2006-12/31/2017). The primary end point was the effect of tacrolimus TTR on time to rejection over the entire follow-up period.

Results: A total of 137 patients were included with a median follow-up of 53 months. Based on the median TTR of 58%, the patients were divided into the low tacrolimus TTR (n=68) and high tacrolimus TTR (n=69) cohort. The high tacrolimus TTR was associated with a significantly lower risk of rejection compared to the low tacrolimus TTR cohort (Hazard ratio [HR] 0.63, 95% confidence interval [CI] 0.41-

0.98; p=0.04). A *post-hoc* analysis revealed associations between rejection and TTR when high and low TTR groups were created at different levels. TTR <30% was associated with a 7-fold higher risk for rejection (HR 7.56; 95% CI 1.76-37.6; p<0.01) and TTR >75% was associated with a 77% lower risk of rejection (HR 0.23; 95% CI 0.08-0.627; p<0.01).

Conclusions: Patients in the higher tacrolimus TTR cohort had a lower risk of rejection. We observed correlations between higher risk of rejection with TTR <30% and lower risk of rejection with TTR >75%. Future studies should focus on validating the optimal TTR cutoff while also exploring a cutoff to delineate high-risk patients for which early interventions to improve tacrolimus TTR may be beneficial.

Introduction

The success of heart transplantation (HT) has been significantly impacted by the development of effective immunosuppression treatment regimens.[1] Tacrolimus is the cornerstone of these regimens in combination with an antimetabolite and corticosteroids. Regimens containing tacrolimus have shown lower rejection and mortality risk compared to those with cyclosporine.[2] However, the narrow therapeutic index of tacrolimus warrants monitoring of whole blood concentrations.

Though current guidelines recommend target tacrolimus trough concentrations, little is known about the association between time in therapeutic range (TTR) within those targets and patient outcomes.[3] A study of 67 adult HT recipients showed no difference in time to or time in therapeutic tacrolimus range with acute rejection, although the follow-up period was limited to the first 30 days after HT.[4] This contrasts with data in non-HT populations with longer follow-up. A study in 292 adult lung transplant recipients (LTRs) found that increasing tacrolimus TTR by 10% was associated with a significantly lower likelihood of acute cellular rejection (ACR) burden and chronic lung allograft dysfunction (CLAD) at 1 year.[5] Similar results have been shown in kidney transplant recipients where tacrolimus TTR <60% was associated with *de novo* donor-specific antibodies (dnDSAs) and acute rejection by 12 months and death-censored graft loss by 5 years.[6] Given these findings, this study evaluated the association of tacrolimus TTR with rejection and other clinical outcomes during long-term follow-up after HT.

Methods

Study Design

This was a single-center retrospective cohort study of HT recipients ≥18 years of age conducted at Michigan Medicine. Patients transplanted between January 1, 2006 to December 31, 2017 were included. Patients were excluded if they were transplanted by a pediatric program, received dual organs, died before tacrolimus initiation, or died within 30 days of HT. Information was collected through chart review on patient demographics; co-morbid conditions; medications; post-transplant coronary angiograms; echocardiograms; and positron emission tomography (PET) scans; and rejection history. The PET imaging protocol for diagnosis of cardiac allograft vasculopathy (CAV) has previously been published.[7] The study was approved by the University of Michigan Institutional Review Board. Informed consent was waived given the retrospective nature of this work.

Immunosuppression and Infection Prophylaxis Protocols

All patients received initial maintenance immunosuppressive therapy consisting of tacrolimus, mycophenolate mofetil (MMF), and corticosteroids. Induction therapy is not routine though rabbit anti-thymocyte globulin was given in patients if a delay in tacrolimus initiation was anticipated. Tacrolimus was initiated at the discretion of the transplant team. Initial dosing of MMF was 1500 mg twice daily with adjustments made in the event of renal dysfunction, or if the patient developed diarrhea or lymphopenia. All dose adjustments were done at the discretion of the provider. Corticosteroid therapy was initiated in the operating room with 500 mg of IV methylprednisolone followed by 125 mg IV every 8 hours for three doses on postoperative day 0. Weight-based prednisone dosing was initiated on postoperative day 1 at 0.50 mg/kg every 12 hours. When whole blood tacrolimus trough concentrations were between 7-8 ng/mL, prednisone was tapered by 0.05 mg/kg each day to 0.15 mg/kg every 12 hours over 2 weeks. Prednisone reductions were dictated by each negative endomyocardial biopsy. Patients were placed on appropriate antibiotic and antiviral prophylaxis against opportunistic infections per institutional protocol.

Tacrolimus Evaluation

The tacrolimus TTR was determined using the Rosendaal linear interpolation method.[8] Therapeutic range was defined based on current guideline and institutional recommendations: 10-15 ng/mL from 0-2 months; 8-12 ng/mL from 3-6 months, and 5-10 ng/mL after 6 months.[3] All tacrolimus monitoring and dosage adjustments were left up to the discretion of the provider. The TTR was calculated using both inpatient and outpatient trough concentrations over a 6-month timeframe (postop day 1 through day 180). Since there is no consensus on optimal method and timing to measure TTR, we

chose the first 6 months following HT for this study given the study objective was to investigate the impact of early tacrolimus TTR. Tacrolimus doses were administered at 0900 and 2100 while in the hospital and patients were instructed to follow the same dosing schedule as outpatients. Any concentrations drawn before 0700 and after 1100 were excluded as they were not deemed to be accurate trough concentrations. Tacrolimus concentrations exceeding 20 ng/mL were chart reviewed and excluded if they were not valid troughs. Patients were divided into two cohorts for analysis (low and high tacrolimus TTR) based on the median TTR. A *post-hoc* analysis was completed to determine the impact of TTR value on rejection.

Clinical Events

Outcomes were adjudicated by two cardiologists after chart review. Rejection was diagnosed and graded according to the 2005 revised International Society for Heart and Lung Transplantation (ISHLT) criteria.[9] The primary end point was time to death-censored rejection. Significant rejection was defined as 2R or 3R cellular rejection, any antibody-mediated rejection, or treated biopsy-negative rejection for hemodynamic instability. Biopsy-negative rejection was defined as signs or symptoms of heart failure (HF) associated with one of the following (in the absence of another etiology for symptoms such as renal or liver failure): abnormal hemodynamics (low cardiac output or elevated filling pressures), decrease in ejection fraction, or treatment with intravenous diuresis. This excluded HF admissions during the first 90 days post-discharge as early graft dysfunction could have accounted for HF symptoms rather than rejection. Secondary end points included time to death; ISHLT CAV; clinical CAV, and a composite of clinical events of HF hospitalization, myocardial infarction (MI), revascularization, or all-cause mortality. Angiographic CAV was defined according to ISHLT nomenclature.[10] Moderate-severe CAV was defined as ISHLT CAV2 or CAV3 which requires at least one obstructive lesion in the proximal or middle third of either the left anterior descending, left circumflex, or right coronary artery or ISHLT CAV1 with allograft dysfunction or restrictive physiology. Clinical CAV was defined as the composite of myocardial flow reserve <2 on rest-stress rubidium-82 PET imaging, coronary revascularization, MI, or moderate-severe CAV on coronary angiography.[11]

Statistical Analysis

Baseline characteristics were summarized overall and by high and low TTR (median split) by counts and percentages or by means and standard deviations. Comparisons between high and low TTR were made with the Pearson chi square, Fisher exact, Student t, and Wilcoxon-Mann-Whitney tests, as appropriate. Associations of outcomes with TTR was investigated with Cox proportional hazard models. Inverse probability weighting (IPW) was used to balance the differences between groups. Variables in

the IPW model were selected if baseline differences between groups existed or if the variables were considered clinically important markers of the primary outcomes: gender, etiology of cardiomyopathy, panel reactive antibodies (PRA) class, low-density lipoprotein (LDL), and donor age. In a sensitivity analysis, the primary analysis was repeated with all other TTR splits to investigate the associations between outcomes and other TTR levels. All analyses were performed using SAS software, version 9.4 (SAS Institute, Cary, North Carolina).

Results

Patient characteristics

A total of 137 patients were included in the final cohort with a median follow-up of 53 months. Based on the median TTR of 58%, the patients were divided into the low tacrolimus TTR (n=68) and high tacrolimus TTR (n=69) cohorts (Table 1). The majority of patients were male (83%) and Caucasian (83%). More patients in the low TTR cohort had ischemic cardiomyopathy compared to those in the high TTR cohort (51% vs 33%; p=0.03, respectively). The mean TTR was 43±11% in the low TTR cohort and 72±9% in the high TTR cohort (p<0.01). No other statistically significant differences were noted between low and high TTR cohorts, including receipt of rabbit anti-thymocyte globulin induction and MMF dose at discharge.

Time to Event Analysis

Rejection occurred in 21 patients (30.9%) in the low TTR cohort and 12 patients (17.4%) in the high TTR cohort. The median time and interquartile range (IQR) to rejection for the low TTR cohort was 877 days (IQR: 150-1841 days) and 1358 days (IQR: 562-2273 days) in the high TTR cohort. The majority of rejection episodes were cellular rejection (91%) followed by treated biopsy-negative rejection (6%) and antibody-mediated rejection (3%). The distributions of rejection type were not significantly different between high and low TTR (p=0.69). There was a significant difference in time to the primary end point between the high tacrolimus TTR cohort and low TTR cohort (HR 0.63, 95% CI 0.41-0.98; p=0.04). No significant differences were observed between cohorts in time to death; ISHLT CAV; clinical CAV (37.8% low TTR vs 35.7% high TTR; p=0.87); or composite clinical events. Outcomes are summarized in Table 2.

Post-Hoc Analysis

A *post-hoc* analysis revealed associations between rejection and TTR when high and low TTR groups were created at different levels. TTR <30% was associated with a 7-fold higher risk for rejection (HR 7.56; 95% CI 1.76-37.6; p<0.01) and TTR >75% was associated with a 77% lower risk of rejection (HR 0.23; 95% CI 0.08-0.627; p<0.01) as shown in Figure 1.

Discussion

To the best of our knowledge, this is the first study to assess the effect of first 6-month tacrolimus TTR on long-term outcomes in HT recipients for over 4 years. Patients in the higher tacrolimus TTR cohort had a lower risk of rejection.

Previous literature has assessed the impact of tacrolimus TTR on outcomes in other solid organ transplant populations. A study in 538 kidney transplant recipients evaluated the risk of developing dnDSAs based on mean tacrolimus trough concentration and tacrolimus TTR.[6] Tacrolimus TTR of <60% was associated with the development of dnDSAs (odds ratio [OR] 2.05, 95% CI 1.28-3.30; p=0.003) and acute rejection (HR 4.18, 95% CI 2.31-7.58; p<0.001) by 12 months and death-censored graft loss by 5 years (HR 3.12, 95% CI 1.53-6.37; p=0.002). Variable outcomes associated with tacrolimus TTR in LTRshave been reported.[5] A single-center, observational, cross-sectional study of 292 adult LTRs evaluated the impact of tacrolimus TTR on ACR burden, CLAD, mortality, and infection rate. Increasing the TTR by 10% was associated with a significantly lower likelihood of high-burden ACR on univariable (OR 0.46, 95% CI 0.40-0.54; p<0.001) and multivariable (OR 0.64, 95% CI 0.47-0.86; p=0.03) assessment when controlled for age and induction agent. Additionally, increasing the TTR by 10% was associated with lower rates of CLAD (p<0.001) and mortality (p<0.001) at 1 year.

The optimal TTR cutoff from studies in other solid organ transplant populations is not well-defined. A study in 1241 living kidney transplant patients calculated an optimal TTR cutoff by the receiver operating characteristic curve analysis on the basis of acute rejection within 12 months.[12] The optimal TTR cutoff value was found to be 78%. Patients with TTR >78% had significant higher rejection- and infection-free survival. TTR <78% was associated with graft loss (OR: 3.2, 95% CI: 1.38-7.42) and patient death (OR: 6.54, 95% CI: 1.34-31.77). This value is similar to the results in our post-hoc analysis which revealed a lower risk for rejection in HT patients with a tacrolimus TTR >75%.

The role of tacrolimus TTR and HT outcomes in the first 30 days following transplantation has been reported.[4] A single-center, retrospective cohort study in 67 adult HT patients evaluated 30-day clinical rejection, 1R/1B, and ≥2R histologic occurrence for effect of time to and time in therapeutic tacrolimus range. The goal tacrolimus trough concentrations were 10-15 ng/mL. For clinical rejection versus no rejection groups, median (25^{th}, 75^{th} percentile) time to therapeutic tacrolimus level was 9.5 (8, 12.3) days compared to 9 (7, 13) days (p=0.623), respectively. The median time in therapeutic tacrolimus range was 34.1% (23.2, 42.4) versus 36.2% (19.9, 51.2), respectively (p=0.512); no differences in time to and time in therapeutic range were noted for patients who developed grade 1R/1B (p=0.650 rejection and p=0.725 no rejection) or grade ≥2R histology (p=0.632 rejection and p=0.933 no rejection). The

differences in outcomes reported in our study may be due to the importance of tacrolimus within the therapeutic range over time and impact on long-term outcomes which was not captured in the 30-day study period.

Although our study was not designed to identify optimal tacrolimus TTR cutoffs with confidence, we did observe correlations between higher risk of rejection with TTR <30% and lower risk of rejection with TTR >75%. Previously mentioned studies arbitrarily set up the TTR threshold as 30% in the lung transplant or 60% in the kidney transplant populations.[5,6] Other studies used receiver-operator curve analysis to determine the optimal tacrolimus TTR.[12,13] As noted above, the association with higher risk of rejection with TTR <30% has been previously reported in the lung transplant population.[5] The 75% cutoff found in our population is similar to the 78% threshold reported in the kidney transplant population.[12] Determining the optimal cutoff may be prudent to identify high-risk patients for which interventions may be implemented to improve tacrolimus TTR especially in the early period after transplantation; however, larger studies need to be completed to validate this optimal TTR.

Our study should be interpreted in the context of its limitations. First, the study was a single-center, observational study. However, inverse probability weighting was used to analyze statistics in an effort to account for differences between cohorts. Second, not all tacrolimus concentrations were confirmed to be drawn correctly as true trough concentrations to be included in the analysis though concentrations were excluded if drawn after the standard administration time at our institution presuming that the level was drawn after the dose was administered. Third, the current analysis was not done to identify a TTR cutoff of clinical significance though associations with outcomes were reported in the *post-hoc* analysis.

In conclusion, in a single-center study of HT recipients, higher tacrolimus TTR was associated with a lower risk of rejection. Future studies should focus on validating the optimal TTR cutoff while also exploring a cutoff to delineate high-risk patients for which early interventions to improve tacrolimus TTR may be beneficial.

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Table 1: Baseline Demographics

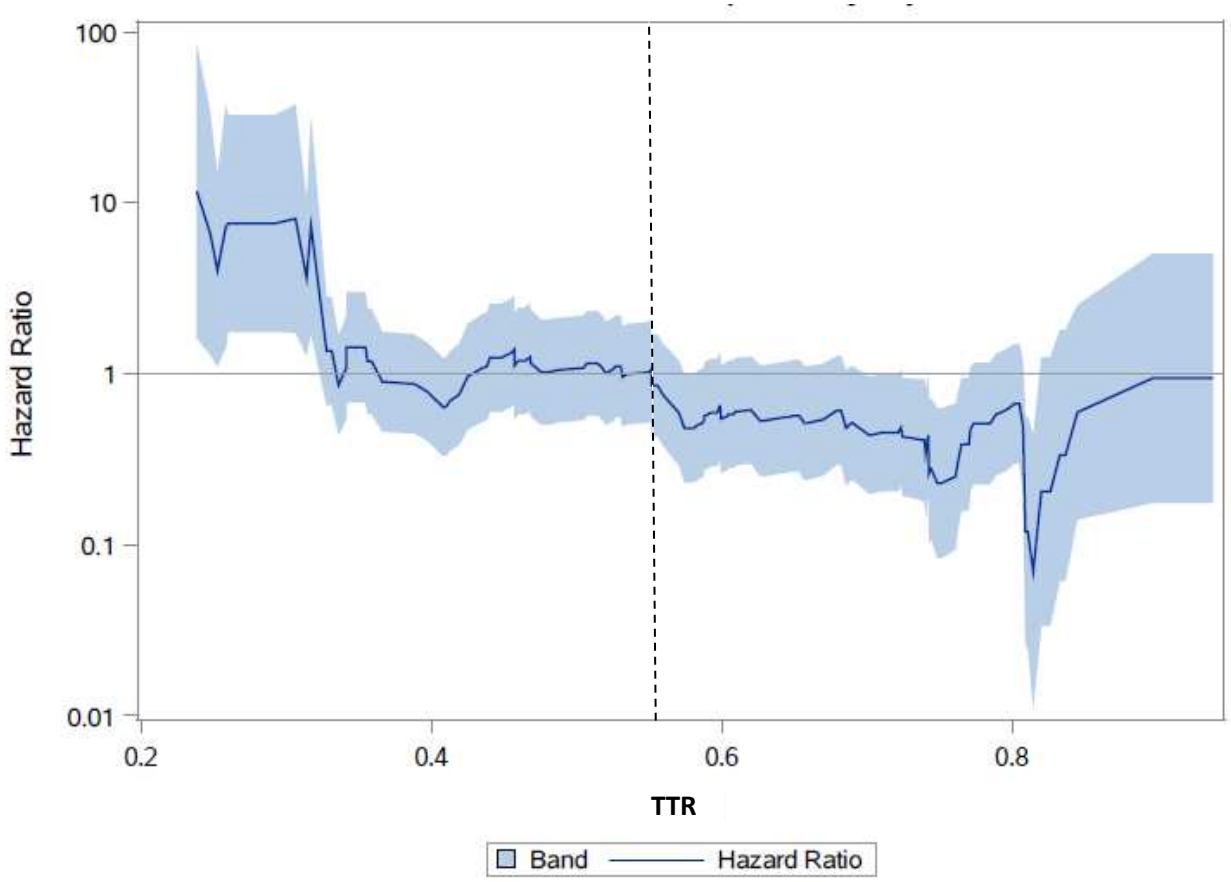
	Low TTR (n=68)	**High TTR (n=69)**	**P-value**
Age (years) – mean ±SD	53.8±12.5	54.3±10.3	0.80
Male – no. (%)	56 (82%)	58 (84%)	0.79
Caucasian – no. (%)	57 (84%)	56 (81%)	0.36
HTN – no. (%)	44 (65%)	38 (55%)	0.25
Diabetes – no. (%)	24 (35%)	21 (30%)	0.54
Ischemic cardiomyopathy – no. (%)	35 (51%)	23 (33%)	0.03
CMV serostatus – no. (%) D+/R+ D-/R- D+/R- D-/R+	 21 (31%) 13 (19%) 16 (24%) 18 (26%)	 16 (23%) 15 (22%) 19 (28%) 19 (28%)	0.78
History of CMV infection – no. (%)	9 (13%)	5 (7%)	0.24
Baseline eGFR <60 mL/min/1.73 m^2 – no. (%)	36 (53%)	41 (60%)	0.38
Donor age (years) – mean ±SD	34.3±12.3	33.1±11.2	0.56
BMI (kg/m^2) – mean ±SD	28.6±3.9	29.1±4.6	0.49
LDL baseline (mg/dL) – mean ±SD	80.8±29.2	86.0±31.2	0.32
PRA class I – mean ±SD	4.8±14.4	4.1±11.3	0.76
PRA class II – mean ±SD	3.4±11.8	3.5±10.9	0.95

Rabbit anti-thymocyte globulin induction – no. (%)	23 (33.8%)	16 (23.2%)	0.14
MMF dose at discharge (mg) – mean ±SD	2597±666	2731.9±474	0.17
TTR (%) – mean ±SD TTR range (minimum, maximum)	43±11 (8.3-57.4)	72±9 (58.0-93.8)	<0.01
Follow-up (days) – median (IQR)	1350 (733, 2516)	1782 (927, 2550)	0.21
Abbreviations: TTR=time in therapeutic range; HTN=hypertension; CMV=cytomegalovirus; eGFR=estimated glomerular filtration rate; BMI=body mass index; LDL=low-density lipoprotein; PRA=panel reactive antibodies; MMF= mycophenolate mofetil			

Table 2: Outcomes

	Low TTR (n=68)	**High TTR (n=69)**	**HR (high TTR vs low TTR) [95% CI]**	**P-value**
Rejection – no. (%)	21 (30.9%)	12 (17.4%)	0.63 [0.41-0.98]	0.04
Death – no. (%)	17 (25.0%)	9 (13.0%)	0.49 [0.23-1.07]	0.07
ISHLT CAV – no. (%)	2 (7.7%)	3 (6.1%)	0.62 [0.19-2.04]	0.43
Clinical CAV – no. (%)	17 (37.8%)	20 (35.7%)	0.90 [0.49-1.83]	0.87
Composite clinical events – no. (%)	24 (35.2%)	17 (24.6%)	0.63 [0.33-1.16]	0.14
Abbreviations: TTR=time in therapeutic range; HR=hazard ratio; ISHLT=International Society for Heart and Lung Transplantation; CAV=cardiac allograft vasculopathy				

Figure 1: Hazard Ratio (High TTR vs Low TTR) for Rejection based on Tacrolimus TTR Breakpoints



The hazard ratio for the primary outcome of rejection based on tacrolimus TTR is shown in solid line. The shaded band represents the 95% confidence interval. The dotted vertical line shows the median TTR (58%) for the study population.