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External Assessment and Refinement of a Population Pharmacokinetic Model to Guide Tacrolimus Dosing in Pediatric Heart Transplant

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Abstract

The immunosuppressant tacrolimus is a first-line agent to prevent graft rejection following pediatric heart transplant; however, it suffers from extensive inter-patient variability and a narrow therapeutic window. Personalized tacrolimus dosing may improve transplant outcomes by more efficiently achieving and maintaining therapeutic tacrolimus concentrations. We sought to externally validate a previously published population pharmacokinetic (PK) model that was constructed with data from a single site. Data were collected from Seattle, Texas, and Boston Children's Hospitals, and assessed using standard population PK modeling techniques in NONMEMv7.2. While the model was not successfully validated for use with external data, further covariate searching identified weight ($p<0.0001$ on both volume and elimination rate) as a model-significant covariate. This refined model acceptably predicted future tacrolimus concentrations when guided by as few as three concentrations (median prediction error = 7%;

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Author Contributions

JER constructed the population pharmacokinetic model and wrote the manuscript. AM helped with data formatting and manuscript review. BH, KPD, KDH, and AGC served as site PI for the external sites, aided data collection, and reviewed the manuscript. KMM developed the study and reviewed the manuscript.

Author Conflict of Interest

The authors declare no conflicts of interest.

median absolute prediction error = 27%), supporting the potential clinical utility of the model to provide personalized tacrolimus dosing guidance.

Keywords

Pharmacology; Decision Support Tool; Tacrolimus; Cardiac Surgery; Pediatric Surgery

Introduction

Heart transplantation is an accepted therapeutic option for children with end stage heart failure, congenital heart disease, and cardiomyopathy. More than 400 heart transplants are performed annually in children across the United States with improving outcomes in recent decades, though mortality from rejection, infection and coronary vasculopathy remain significant[1, 2]. Transplant survival in excess of 20 years following heart transplantation has been observed, with more than 70% of transplants expected to achieve greater than 5 year survival[1, 3]. Much of this success can be attributed to the use of immunosuppressive therapy to prevent the rejection of the transplanted heart.

The calcineurin inhibitors (CNI) tacrolimus and cyclosporine play a vital role in immunosuppressive therapy. Currently, tacrolimus is preferred in comparison to cyclosporine, due to its improved safety profile, especially with regards to hypertension and dyslipidemia[1]. However, tacrolimus suffers from extensive inter- and intra-patient pharmacokinetic (PK) variability, which necessitates frequent drug monitoring to guide therapeutic dosing. Several publications have explored the sources of variability in tacrolimus PK; however, few are targeted to the pediatric heart transplant population[4–15].

We previously published a population PK model of tacrolimus in pediatric heart transplant that demonstrated potential for guiding tacrolimus dosing[16]. One of the key limitations to this prior work was that it was conducted at a single center that serves a relatively homogenous Caucasian population. To address this limitation, we conducted a retrospective study with the primary objective of validating our previously published population PK model with data from external sites representing greater racial and ethnic heterogeneity. However, once the previously published model could not be validated, we utilized these external data to refine our population PK model, with the objective of better predicting future tacrolimus concentrations across all pediatric demographics.

Methods

Data Collection

Study approval was granted by each site's Institutional Review Board (IRB), including the combined IRB of the University of Utah, Intermountain Health, and Primary Children's Hospital (PCH)); Seattle Children's Hospital (SCH) IRB, Boston Children's Hospital (BCH) IRB, and Texas Children's Hospital (TCH) IRB. Data for the study included event times, dose amount, tacrolimus concentrations, demographics, and clinical laboratory values. Approaches for collecting these data differed by site, including by direct query of the site's electronic medical record or by manual chart review when necessary. Children were

eligible for study inclusion if they received tacrolimus during an inpatient stay within the first 6 weeks following heart transplant between the years of 2007 to 2018. The initial published population PK model was limited to <18 years; however, ongoing work using the model at our site has been limited to children between the ages of 6 months and 18 years. Therefore, children outside of this age range were excluded from the study *a priori*. Children meeting these criteria must have had at least one dose of tacrolimus and one subsequent tacrolimus concentration to be incorporated into the study. Tacrolimus was typically administered twice daily, either orally or enterally through a nasogastric or nasojejunal tube. Tacrolimus concentrations and clinical laboratory values were determined by each institution's laboratory service.

Pharmacokinetic Modeling

The initial single-site population PK model has been described previously[16] and is parameterized on elimination rate (k_e) and volume of distribution (V_d). Briefly, PK modeling utilized NONMEM software (v7.3, ICON Development Software, Ellicott City, MD, USA) interfaced with PDx-Pop (v5.0) and Pirana 2.9.2 (pirana-software.com). The first order conditional estimation with interaction (FOCE-I) method was used throughout model building and evaluation. Model selection was based on parsimony, objective function value (OFV), and visual diagnostic plots. After the base model was established, covariates were tested in the model using a stepwise forward inclusion ($p<0.05$) – backward exclusion ($p<0.01$) regression method using the stepwise covariate modeling (SCM) module available in Pirana. Median values of continuous covariates were determined within the module, and used to normalize covariate values at individual time points within the model. Time-varying covariates were updated for each observed tacrolimus concentration and analyzed using a standard covariate model approach, in which the impact of the covariate on the population parameter estimate is the same for a given covariate value [17]. Covariates were added to the model in a stepwise fashion, and allowed to remain in the model if covariate inclusion decreased the OFV by at least 3.84 ($p < 0.05$, χ^2, df = 1), and its exclusion increased the OFV by at least 6.63 ($p < 0.01$, χ^2, df = 1). Validation of our previously published model included a prediction corrected visual predictive check and bootstrapping accomplished using PsN 4.4.0 (psn.sourceforge.net) and Pirana, both using 1000 simulated datasets, as well as the use of an internal validation dataset. Subsequent refinements to the population PK model throughout this study used a similar approach.

Predicting Tacrolimus Concentrations

The previously published single-site and refined final population PK model structure was used to investigate the model's ability to predict future tacrolimus concentrations and thus, guide tacrolimus dosing. In our previous publication, we determined that three prior tacrolimus concentrations were required to predict future concentrations accurately and with minimal bias[16]. Using a similar approach, we determined the accuracy and bias of the model for predicting future concentrations at the external sites. Briefly, this analysis was done by changing all but the first three concentrations for each patient to be a missing dependent variable (i.e. DV=−1), within the dataset. Next, an EVID (event identification) column was added to the dataset. For each row (corresponding to a time at which there was a study event, either a dose or concentration), the EVID column value was set to 0 for

the concentrations being used to determine the patient-specific post-hoc parameter estimates (i.e. MDV=0), 1 for all dosing events, and 2 when the concentration had been set to missing (i.e. MDV=1). For all rows where EVID=2, NONMEM will generate an individual predicted (IPRED) concentration based on the patient's post-hoc parameter estimate. This manipulated dataset was then analyzed in NONMEM and the output was used to compare predicted individual concentrations to the actual observed concentrations using median prediction error (MPE) and median absolute prediction error (MAPE). For a patient with 13 samples collected over the course of the study, the comparison between predicted and observed concentrations would utilize the final 10 samples (as the first three samples were used to generate the individual parameter estimates). MPE ≤ ±15% and MAPE ≤30% were targeted to accept the model as accurately and precisely predicting future tacrolimus concentrations.

Results

Study Population

Data were collected from a total of 285 patients receiving tacrolimus following heart transplant at the three study institutions. Of these, complete datasets from 57 subjects were excluded from the dataset for being less than 6 months or greater than 18 years of age (*a priori* inclusion/exclusion criteria, n=29), missing concentration and/or dose event data (n=6), having three or fewer concentrations total in the dataset (n=4), and/or a nonsensical data pattern (i.e. multiple concentrations without intervening doses over a span of several days or having multiple distinct dose amounts given at identical date/time stamps)(n=18). Therefore, concentrations data from 228 participants were used for the analyses. Demographics for these 228 participants are described in table 1. Study participants included 54 SCH, 105 TCH, 69 BCH, 94 female, 134 male, 159 white, 28 black, 161 non-Hispanic, and 65 Hispanic children. The median (interquartile, IQR) age and weight of study participants were 10.7 (3.7, 15.3) yr and 30.9 (14.3, 52.8) kg. A total of 1840 observed trough concentrations were obtained for these participants (an average of 8 samples per participant, with a maximum of 36). Tacrolimus trough concentrations ranged from 1.1 to 30.6 ng/mL, with a median value of 9.0 ng/mL. Target tacrolimus trough concentrations were 10 to 12ng/mL at all sites, in contrast to the initial publication, where the target trough concentrations were 10 to 14 ng/mL at PCH. The median (IQR) administered daily dose was 2.8 (1.2, 5.0) mg/day typically divided into two daily doses, though some younger participants received three daily doses. Oral capsule and suspension use was approximately even (46% vs. 54%). Data from TCH indicated use of the brand name (Prograf) tacrolimus, however, brand vs. generic (Sandoz) tacrolimus use was not available from other sites. In addition to tacrolimus, all participants received mycophenolate for immunosuppression. Outside of immunosuppressive therapy, study participants received extensive polypharmacy, with up to 180 unique medications used at a given site. The most common medications included anti-infectives and medications for managing pain, fluid retention, and blood pressure, more specific details are provided in supplementary table 1.

External Model Validation and Improvement

We first evaluated the previously published single-site population PK model to determine the bias (MPE) and accuracy (MAPE) of the model when applied to data collected at external

sites. PK parameters were fixed to the values determined in the previously published model, then concentrations were simulated based on the collected dosing information. For all sites, the MPE and MAPE (IQR) were −16% (−61%, 91%) and 66% (36%, 96%), indicating poor model performance when directly applied to the external dataset.

We next evaluated whether the model's failure to externally validate was a function of model parameter estimates or model structure. The potential for poor model parameterization was assessed first, by allowing the model to estimate parameters using the external dataset (of note, existing covariate relationship parameter estimates were fixed). In comparison to our previously published population PK model (k_e=0.0408 hr^{-1}, V_d=233 L), parameter estimates from the external dataset were k_e=0.0231 hr^{-1} and V_d=362 L. The improved model parameters yielded MPE and MAPE (IQR) of −2.6% (−24%, 18%) and 21% (10%, 39%), indicating adequate but variable model performance. This finding indicates that the inability to externally validate the model is most likely due to parameter estimates differing between the original population (Primary Children's Hospital) and the populations at external sites, rather than model structure (i.e. one vs. two compartment).

Model Refinement Using External Data

While refining parameter estimates improved model performance, the variability around the MPE and MAPE reflect a potential need to identify additional covariates to explain variability in tacrolimus PK. Existing parameter-covariate relationships (k_e: creatinine clearance, fluconazole use; V_d: age) were retained in the model. During covariate modeling, parameters describing the impact of creatinine clearance and age were estimated, however, due to the extremely limited frequency of fluconazole use in the external dataset, this parameter was fixed to that of our prior published PK model. Covariate searching identified race and weight as significant covariates on both k_e and V after the backwards exclusion step, in addition to the covariates age (V), creatinine clearance (calculated using the Bedside Schwartz equation) (k_e), and fluconazole (k_e) included based on our prior work. Notably, excluding age (V) from this model increased the model's OFV by 7.49 (p=0.0062), while excluding CRCL (k_e) increased the OFV by 14.9 (p=0.0001), indicating the importance of these covariates from the prior model to the updated final model.

During covariate searching, race had three states: white/Caucasian (race=0), black/African-American (race=1), and other/unknown (race=2), however, parameter estimates for both k_e and V were similar whether race=1 or 2. Model instability was observed when race was allowed to have three states, which was lessened when race was allowed two states (either white or non-white). The difference in OFV between two state and three state race was minimal (ΔOFV=0.684). Therefore, race was coded as either white or non-white for the remaining analyses. Non-white race was found to decrease k_e by 33% (p=0.0013) and increase V by 93% (p<0.0001) relative to white race. However, the model continued to demonstrate some instability (i.e. an inability to successfully minimize the covariance matrix) was observed when race was included on both V and k_e, as well as when the covariate was included on either one or the other PK parameter. Moreover, the removal of race as a covariate did not significantly impact other parameter estimates in the model (i.e. all parameters changed by <5%), nor did it impact the model prediction analysis described

below (i.e. no change in MPE or MAPE between models with and without race). Therefore, based on parsimony, the race covariate was removed from the model.

Weight was included in the model using piecewise linear (V) and power (k_e) relationships, with $p<0.0001$ for both relationships. Changing these relationships to follow allometric scaling (i.e. $V \propto WT/70$ and $k_e \propto WT/70^{0.75}$) significantly worsened the model ($\Delta OFV=227$). Final model parameters were defined using the equations (theta abbreviations are spelled out in table 2):

$$k_e = \theta_1 \times \left(\frac{CRCL}{115.6}\right)^{\theta_4} \times \left(\frac{WT}{32.3}\right)^{\theta_5} \times (\theta_6[if \quad FLUC = yes])$$

$$V_d = \theta_2 \times \left(\frac{AGE}{5.7}\right)^{\theta_7} \times (1 + \theta_8 \times (WT - 32.3))$$

Final model parameter estimates are summarized in table 2. The appropriateness of the model fit was verified by model diagnostic plots (figure 1), visual predictive check (VPC, supplementary figure 1) and bootstrapping (table 2), though these analyses indicate a moderate level of variability remains associated with the model.

Predicting Concentrations from External Sites

We then assessed the refined model's ability to predict future concentrations for patients in the external dataset. Specifically, we investigated the accuracy and bias of model predictions when guided by between 2 and 5 prior concentrations for each individual patient (table 3). The use of two concentrations yielded MPE (14%) and MAPE (30%) which passed criteria, however, the associated 95% confidence interval (CI) indicate a wide range of variability around these estimates. The use of three concentrations substantially decreased MPE (7%) and improved the MAPE (27%), indicating better predictive ability when one additional concentration was used. Moreover, the use of a third concentration for the predictions reduced the variability in the predicted concentrations, as evidenced by the 95% CI of both the MPE and MAPE. Thus, the minimum number of values needed for acceptable model predictions is three concentrations. Plots showing an example of the model's predictive ability using three concentrations for refining individual parameter estimates for an individual at each site are shown in figure 2. The use of a fourth concentration moderately improved both the MPE and MAPE (as well as their associated 95% CI), while the use of a fifth concentration did not improve the model's predictive ability.

Discussion

Direct application and validation of our previously published population PK model to external data from three sites was unsuccessful. Further model refinement identified both race and weight as model significant covariates, though only weight performed with sufficient stability to be retained in the model. When guided by as few as three concentrations, this refined model successfully predicted future tacrolimus concentrations in a larger, more heterogenous population of children following heart transplant. Importantly,

while the model does accurately predict future concentrations, the associated variability in these estimates indicate a need for further investigation and refinement of the described model using a more richly sampled prospective approach.

The most likely explanation for the previously published population PK model failing validation is that the dataset used to construct the model was relatively homogenous. While PCH possesses a large regional catchment area of patients, the population is served by this hospital is predominantly white and rural. As such, only two of the participants in the initial study were of black race. In contrast, the external validation dataset consists of 28 participants of black race, in addition to 41 individuals of either unknown or other race. Consequently, the current study had the ability to test and identify as significant the role of race on tacrolimus PK. A primary clearance mechanism for tacrolimus is hepatic metabolism via CYP3A5[18–21], which is known to be polymorphic, with allele frequencies differing by race[22, 23]. Indeed, the *CYP3A5*1* allele associated with extensive CYP3A5 metabolism has been shown to occur more frequently in individuals of black race, whereas the inactive *CYP3A5*3* is the predominant allele in the white population[22, 23]. Genetic differences in CYP3A5 have previously been shown to greatly alter the required therapeutic tacrolimus dose in both children and adults[24–27]. Interestingly, in the absence of CYP3A5 genotype data due to the retrospective nature of the current study, the impact of race was observed to decrease, rather than increase, the elimination of tacrolimus in the current study population. However, the inclusion of race as a covariate was observed to cause model instability, and race was found to be potentially confounded with renal function ($p=0.03$), leading to its removal from the final model. Nonetheless, our data support the need for appropriately powered future work to reconcile the impact of *CYP3A5* genotype and race on tacrolimus PK.

Another possible explanation for the inability to validate the model is the discrepancy in hematocrit between the PCH population and this dataset from external sites. The PCH population studied to construct our published population PK model was found to have an average hematocrit of 43.2%, compared to average hematocrits of between 34–36% at the external sites. The distinct hematocrits between sites may be caused by differences in altitude between PCH (largely a high altitude population) and the external sites (predominantly sea-level), as prior research has demonstrated that individuals acclimated to living at higher altitudes are associated with having higher hematocrit levels than those at sea-level[28–30]. Aside from altitude, clinical approaches to transfusions and maintaining fluid balances can vary between sites, not only impacting hematocrit values between sites, but between individuals at a single site. Hematocrit was identified as a potential model-significant factor (on V_d) during the forward inclusion step of covariate searching, however, this relationship was removed during backwards elimination, supporting the role of hematocrit as a factor potentially impacting tacrolimus PK. It is important to note that the current study does not include study participants from PCH, which may have limited the model's ability to fully ascertain the impact of hematocrit on tacrolimus PK. It is well-established that tacrolimus sequesters into red blood cells[15, 18, 31, 32]. Increased red blood cell content in individuals with a high hematocrit may represent an additional drug storage depot, leading to elevated tacrolimus concentrations in these individuals. Given these findings, future research combining data should more closely evaluate the impact of

hematocrit on tacrolimus PK, especially in studies with sites at different altitudes, and the potential need to account for this difference when guiding tacrolimus dosing.

Another potential rationale for the model's poor performance when combined with external data is the different approaches for quantifying tacrolimus between sites. Tacrolimus concentrations obtained at PCH are generated using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) assay at ARUP® Laboratories, a national reference laboratory at the University of Utah. LC-MS/MS was utilized at both BCH and SCH, however, another approach would be to use an immunoassay to determine tacrolimus concentrations, as is standard practice at TCH. Importantly, immunoassay exhibits less analytical selectivity compared to LC-MS/MS assay, resulting in reported values that include signal from cross-reactive substrates (i.e. a tacrolimus metabolite). These values would therefore be greater than tacrolimus concentration values reported from the more selective LC-MS/MS approach[33]. The potential impact of immunoassay vs. LC-MS/MS determined concentrations on model parameter estimates was evaluated by including site as a potential covariate in the model. This covariate was found to be significant during a univariate screen (data not shown), however, it was not found to be model-significant during stepwise covariate model searching. Therefore, while the use of immunoassay vs. LC-MS/MS is unlikely to be a significant contributing factor to the inability to validate the previously published model, future multi-center studies should consider standardizing the analytical platform used to determine tacrolimus concentrations.

Though the previously published population PK model could not be validated for use with external datasets, the model was successfully refined using data from these sites, identifying weight as an additional model significant covariate. Weight represents an additional indicator of the child's maturation, as weight corresponds to organ growth and cardiac output [34, 35]. The refined model can predict future concentrations with acceptable accuracy and bias when guided by as few as three concentrations. Data driven approaches, such as population PK model guided dosing, allow dose optimization and individualization that can reduce the time to and increase the time at effective tacrolimus dosing, as well as reduce the number of blood draws and patient/parent/provider time devoted to managing tacrolimus dosing. Promptly achieving therapeutic tacrolimus concentrations is expected to improve transplant outcomes and longevity, as is maintaining therapeutic tacrolimus concentrations, particularly in the immediate post-transplant period[1, 36]. It is important to note that personalized dose recommendations following pediatric heart transplant are not a singular event in practice, rather they must continue to be refined as the patient recovers post-transplant (for example, in response to changes in renal function or fluid status). Therefore, the finding that the model's predictive accuracy and bias (and importantly, the precision of these errors) improves when more than three concentrations are used to guide model predictions lends support to the model's adaptability to guide dosing in the clinical setting. Studies testing the clinical implementation of our previously published model are ongoing at PCH, with early results indicating more rapid achievement of stable therapeutic tacrolimus concentrations compared to historical controls. More work is required to understand how long-term outcomes may improve in children who more rapidly achieve and maintain therapeutic tacrolimus concentrations. Combined, these results support the integration of personalized dosing tools into standard post-operative care.

Descriptions of pediatric heart transplant PK data and/or models in the literature are limited. Furthermore, recent work by Pasternak et al. found that tacrolimus models developed in pediatric kidney transplant populations did not extend well to pediatric heart transplant recipients, suggesting a need for organ-specific PK models[37]. In addition to demonstrating that *CYP3A5*1* genotype conferred a greater dose requirement in pediatric heart transplant recipients, Gijsen et al. found that the weight-normalized dose requirement decreased with increasing age[24]. On the surface, this finding contrasts with our model which determined that increased age increases volume of distribution, which would suggest a greater dose requirement in older children. However, the impact of age in our model is confounded by the inclusion of weight on both elimination rate and volume of distribution, hampering a straightforward analysis of the impact of age on dose requirement from our model. Population PK models reported in the literature are summarized in a review by Brooks et al. for liver and kidney transplants, in both children and adults[38]. The review demonstrates that there exists a wide range of published parameter estimates, covariates, and variability associated with tacrolimus PK. As might be expected from the variation in published models, external validations of these models have frequently failed[39–42], as was the case in our current study. This supports the continued need to pursue a broadly generalizable population PK model yielding accurate and precise Bayesian forecasting of tacrolimus doses.

Though the described results are supportive of the potential benefit of implementing personalized dose guidance clinically, there are limitations to consider. The first is that the model was built using retrospective data. Retrospective data is expected to, but may not, contain a full and accurate accounting of tacrolimus dose, concentration, and associated lab and/or demographic data. Our observation of the collected data showed that some individuals had data that was either missing, replicated, or non-sensical, and thus, these data and/or individuals were removed from the analysis. It is possible that removing these data and/or individuals may impact the described results, however, it is likely that the impact would be observed as slight shifts in the parameter estimates and not as changes to the structural or covariate model. Furthermore, we opted to use a data-inclusive approach, wherein data that did not demonstrate a clear rationale for biasing the analysis were retained in the dataset. This approach likely increases the imprecision of parameter estimates, leading to an overestimation of between-subject variability. Furthermore, these data represent trough data collected from therapeutic drug monitoring data, which inherently limits the model's ability to precisely estimate structural and error model parameters, particularly volume and inter-individual variability. This likely explains the poor precision of the age on V covariate relationship, as well as the high shrinkage value on k_e (which is confounded with V, as k_e=CL/V). A model using the same covariate structure, but parameterized on clearance (CL) rather than k_e, had shrinkages of 10% and 21% for clearance and volume, respectively, further supporting that the study design limits the ability to precisely determine V. However, conducting a more richly sampled prospective study that would better define V within this fragile population that is already undergoing substantial blood sampling presents many challenges, particularly as it relates to the safe blood volume to collect from these children. The use of retrospective data obtained from standard clinical practice also prevents the collection of *CYP3A5* genotype for analysis, precluding the investigation of

whether this genotype affects the association of race and tacrolimus PK. An additional limitation is that the model includes creatinine clearance as a covariate, which is intriguing given the predominant clearance mechanism for tacrolimus is hepatic metabolism, and not urinary excretion. Moreover, it is unclear how the model is impacted by this covariate when patient receives some form of dialysis, which not only artificially alters the observed serum creatinine values used to calculate creatinine clearance, but may also represent an additional mechanism of drug clearance. More work is needed to understand the role of renal function, creatinine clearance, and dialysis on tacrolimus PK.

In summary, our previously published PK model was refined and successfully applied to external datasets consisting of a more heterogeneous population than is present at our local institution. The presented data support the further development of personalized dosing tools using the described model to more efficiently achieve therapeutic tacrolimus concentrations. It is anticipated that more efficiently achieving and effectively maintaining therapeutic tacrolimus concentrations will decrease care events (blood draws, therapeutic drug monitoring assays, patient/provider interactions) and may also improve long-term transplant outcomes and longevity, though further studies with larger patient populations are needed to confirm this hypothesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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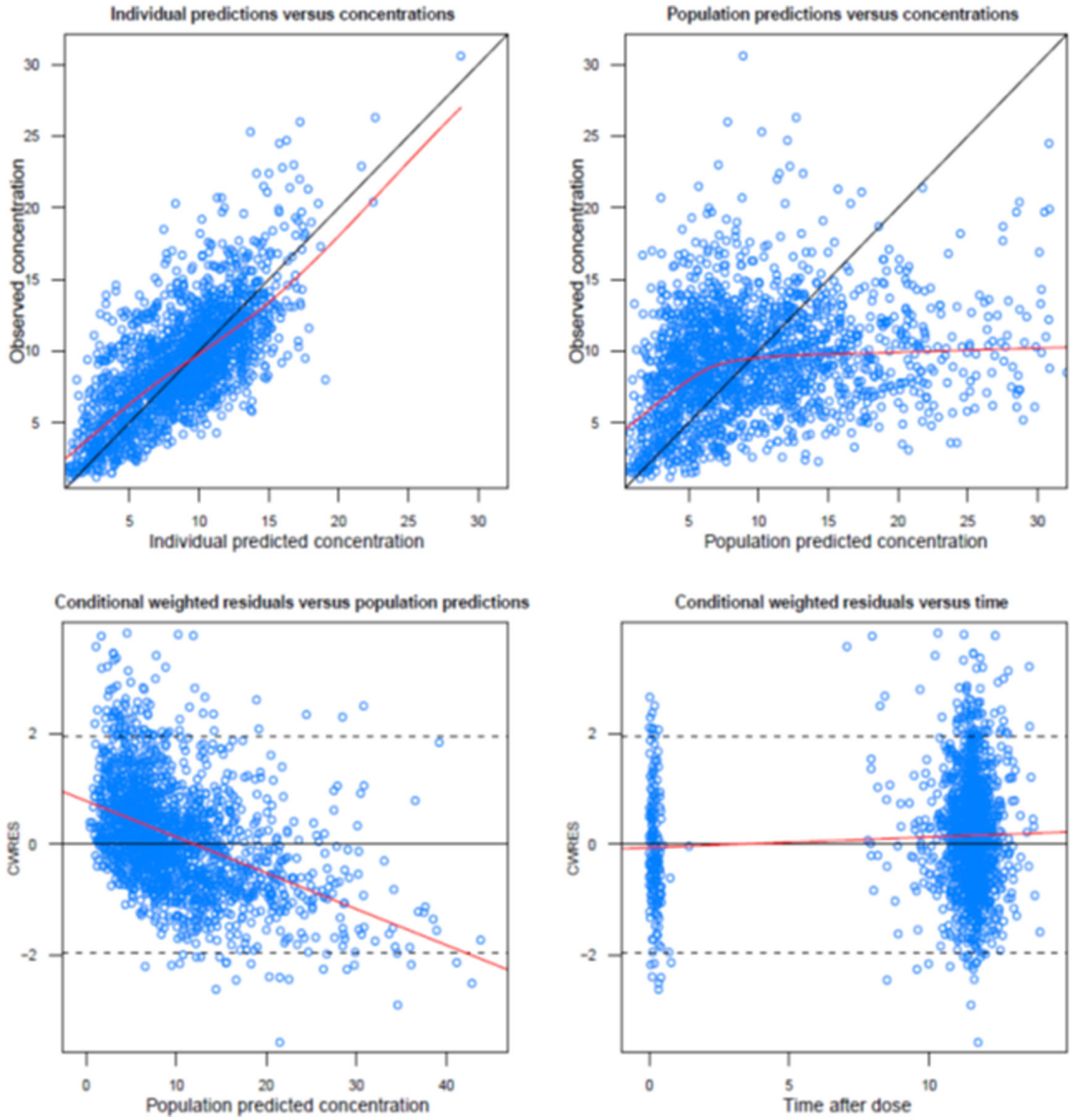
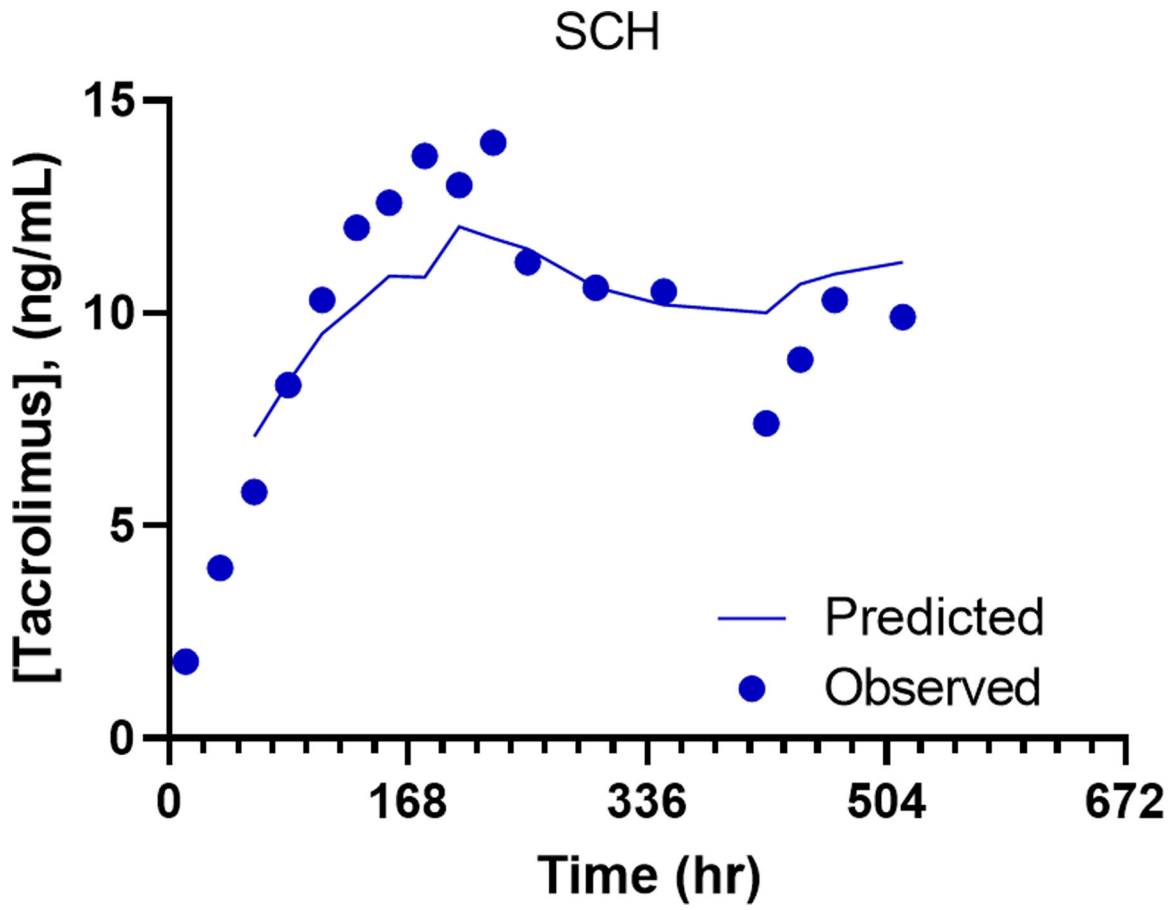
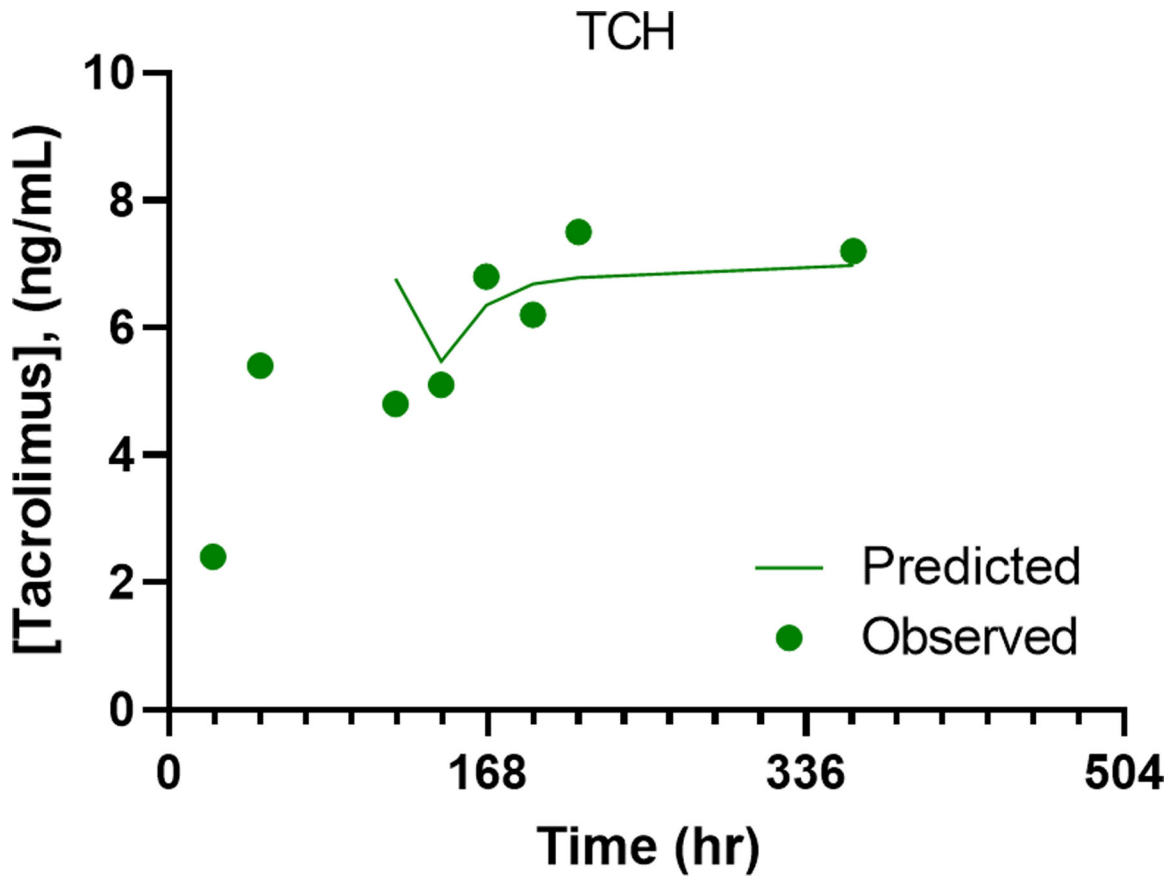


Figure 1:

Model diagnostic plots for the refined model, indicating that the individual predicted concentrations match well with observed concentrations, and that residuals are not correlated with time after dose.





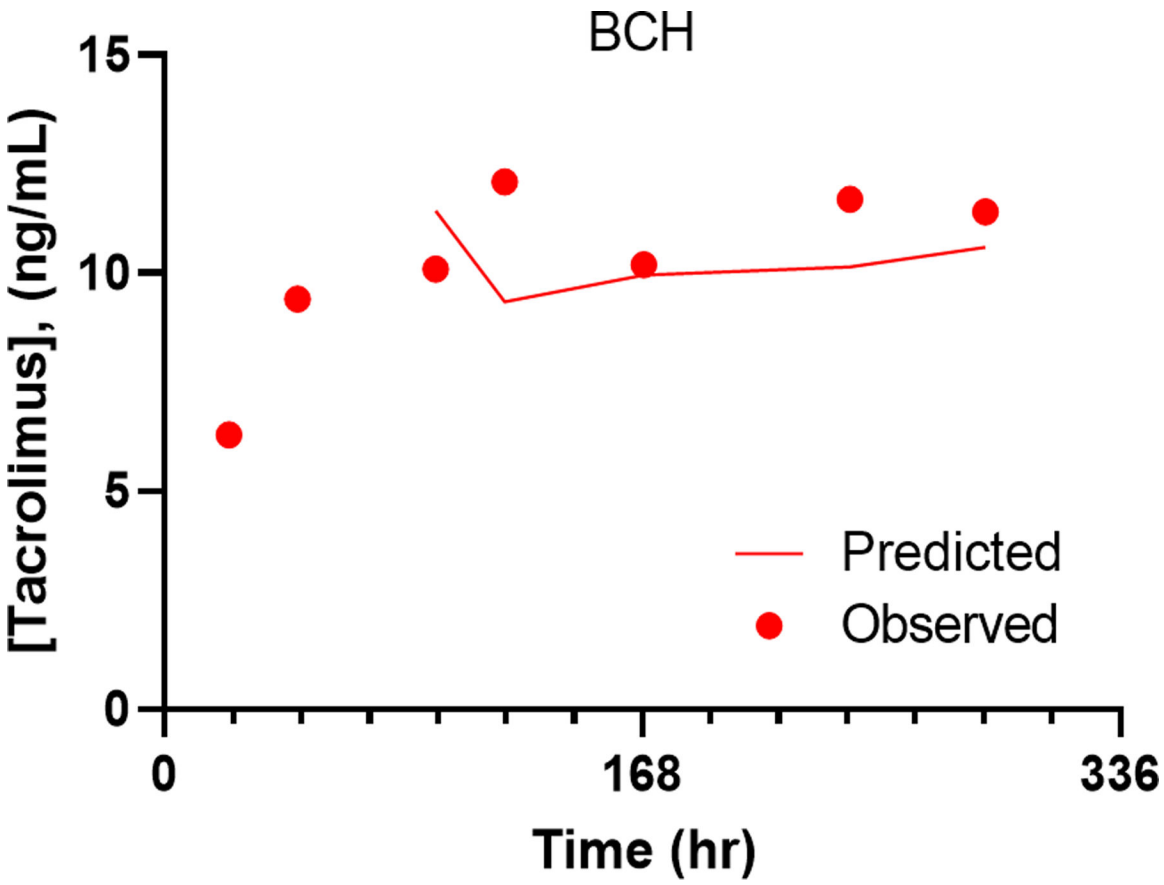


Figure 2:
Observed concentration data (filled dots) and model predicted concentrations (connected lines) for individuals from each of the three study sites: (A) Seattle Children's Hospital [SCH], (B) Texas Children's Hospital [TCH], and (C) Boston Children's Hospital [BCH], depicting typical concordance between observed and predicted concentration data for each site.

Table 1:

Demographics of the study participants.

Participant # by Site	54 (24%) Seattle Children's Hospital 105 (46%) Texas Children's Hospital 69 (30%) Boston Children's Hospital
Sex	94 (41%) Female 134 (59%) Male
Race	159 (70%) White 28 (12%) Black 41 (18%) Unknown/Other
Ethnicity	161 (71%) Non-Hispanic 65 (29%) Hispanic 2 (<1%) Unknown/Other
Age (yr)	10.7 (3.7, 15.3)
Weight (kg)	30.9 (14.3, 52.8)
Creatinine Clearance (mL/min/1.73m^2, Bedside Schwartz)	116 (82.1, 125)
ALT (IU/L)	32.0 (24, 46)
AST (IU/L)	37.0 (29, 74)
Hemoglobin (g/dL)	11.8 (11.0, 12.7)
Hematocrit (%)	35.7 (34.5, 36.7)
Tacrolimus Trough Concentration (ng/mL)	9.00 (6.60, 11.5)
Total Daily Dose (mg)	2.8 (1.2, 5.0)

* Continuous variables are reported as median (interquartile range)

Table 2:

Parameter estimates from the previously published model, the final model constructed using external data, and the bootstrap analysis. Model parameter estimates are reported as population mean (% relative standard error) [η-shrinkage] while results from the bootstrap analysis represent the mean (95% confidence interval).

	Published Model	Final Model	Bootstrap Analysis (906/1000 successful)
Parameter			
K_e (1/hr) (θ_1)	0.0408 (15%)	0.0255 (11%)	0.0256 (0.0198, 0.0312)
V_d (L) (θ_2)	233 (17%)	314 (16%)	325 (216, 412)
K_a (1/hr) (θ_3)	3.43 (fixed)	3.43 (fixed)	3.43 (fixed)
K_e:CRCL (θ_4)	0.850 (24%)	0.232 (43%)	0.241 (0.031, 0.434)
K_e:WEIGHT (θ_5)	n/a	−0.471 (21%)	−0.470 (−0.653, −0.289)
K_e:FLUCONAZOLE (θ_6)	0.657 (5%)	0.657 (fixed)	0.657 (fixed)
V_d:AGE (θ_7)	0.775 (13%)	0.353 (63%)	0.330 (−0.003, 0.708)
V_d:WEIGHT (θ_8)	n/a	0.025 (33%)	0.025 (0.013, 0.037)
Between-Subject Variability			
ω_{Ke}^2	0.262 (40%)	0.118 (25%) [43%]	0.114 (0.055, 0.181)
ω_{Vd}^2	0.329 (35%)	0.303 (13%) [13%]	0.297 (0.227, 0.380)
Residual Error			
Additive (ug/L)	3.69 (13%)	3.13 (7%) [7%]	3.13 (2.91, 3.35)

Table 3:

MPE and MAPE (95% confidence interval) when between 2 and 5 concentrations are used to guide the model's predictions.

Concentrations	MPE (95%CI)	MAPE (95%CI)
2	14% (−11%, 58%)	30% (12%, 61%)
3	7% (−17%, 40%)	27% (13%, 49%)
4	5% (−19%, 33%)	24% (12%, 46%)
5	5% (−17%, 33%)	23% (11%, 44%)