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Development and validation of a population pharmacokinetic model to guide perioperative tacrolimus dosing after lung transplantation

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BACKGROUND: Tacrolimus therapy is standard of care for immunosuppression after lung transplantation. However, tacrolimus exposure variability during the early postoperative period may contribute to poor outcomes in this population. Few studies have examined tacrolimus pharmacokinetics (PK) during this high-risk period.

METHODS: We conducted a retrospective pharmacokinetic study in lung transplant recipients at the University of Pennsylvania who were enrolled in the Lung Transplant Outcomes Group cohort. We used nonlinear mixed-effects regression to derive a population PK model in 270 patients and examined validity in a separate cohort of 114 patients. Covariates were examined with univariate analysis and a multivariable model was developed using forward and backward stepwise selection. The performance of the final model in the validation cohort was examined with calculation of prediction error (PE).

RESULTS: We developed a 1-compartment base model with a fixed rate absorption constant. Covariates improving model fit were postoperative day, hematocrit, transplant type, *CYP3A5* genotype, weight, and exposure to cytochrome p450 enzyme (CYP) inhibitor drugs. The strongest predictor of tacrolimus clearance was postoperative day, with median predicted clearance increasing more than 3-fold over the 14-day study period. In the validation cohort, the final model showed a mean PE of 36.4% (95% confidence interval 30.8%-41.9%) and a median PE of 7.2% (interquartile range −29.3% to 70.53%).

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CONCLUSIONS: Tacrolimus clearance is highly dynamic during the early postlung transplant period. Population PK models that include lung-transplant–specific covariates may enable precision dosing algorithms that account for this highly dynamic clearance. Future multicenters studies including a broader set of covariates are warranted.

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Background

Tacrolimus, a potent calcineurin inhibitor, is the standard of care for immunosuppression after lung transplantation.[1,2] Despite routine therapeutic drug monitoring in the early postoperative period, tacrolimus exposure during this vulnerable time frame is highly variable and may contribute to poor outcomes.[3,4] Tacrolimus is characterized by high interpatient and intrapatient variability of pharmacokinetic (PK) parameters and a narrow therapeutic index.[3] High concentrations can cause renal vasoconstriction[5] that may contribute to the development of acute kidney injury. Approximately 50% to 70% of patients develop acute kidney injury postoperatively, which is linked to increased early mortality and higher chronic kidney disease in survivors.[4,6,7] Low concentrations during the postoperative period may increase the risk for acute cellular rejection, which is especially concerning given that up to 30% to 50% of lung transplant recipients experience acute cellular rejection during the first posttransplant year.[8,9] Interventions to optimize early tacrolimus exposure thus constitute a significant unmet need in this population.

Optimization of early tacrolimus exposure is hindered by limited data on the patient-specific determinants of tacrolimus PK in lung transplant recipients, particularly during the early postoperative period. In the kidney transplant population, the impact of allelic variation in the expression of the cytochrome P450 3A5 (CYP3A5) drug-metabolizing enzyme has been studied extensively, with genetic variants causing higher CYP3A5 gene expression linked to approximately 50% higher tacrolimus dosing requirements.[10] However, we have shown that CYP3A5 genotype explains only 19% of the variability in tacrolimus concentration-dose ratio during the early postoperative period after lung transplantation, a time when other clinical factors may have a larger impact.[4]

While the PK of tacrolimus has been extensively investigated in kidney and liver transplant populations, few studies have developed population PK models in lung transplant recipients,[3,11,12] with limited data from the early postoperative period.[3] Compared to kidney transplantation, lung transplantation is a much greater physiologic insult, resulting in the need for mechanical ventilation and substantial hemodynamic support during the first few days after transplants. Consequently, the generalizability of PK data between kidney transplant patients and lung transplant patients is uncertain. We thus aimed to develop and validate a tacrolimus population PK model that includes clinical and genetic determinants of tacrolimus disposition during the initial 14 days after lung transplantation.

Materials and methods

Study design and population

We conducted a retrospective PK study on lung transplant recipients at the University of Pennsylvania who were enrolled in the Lung Transplant Outcomes Group (LTOG) study, a multicenter prospective cohort study of primary graft dysfunction (PGD) after lung transplantation.[13] We merged LTOG data with electronic health record data (medications, tacrolimus concentrations, and other laboratory values) for the current analysis. We included LTOG subjects enrolled between November 2008 and August 2018. Exclusion criteria were age <18 years, combined organ transplantation other than heart-lung, exposure to cyclosporine (an alternative calcineurin agent), or treatment with intravenous tacrolimus. We randomly divided patients into derivation and validation cohorts, targeting an approximate 70% to 30% split, respectively. Tacrolimus dosing, concentration, and covariate data were collected from the initial 14 days after transplantation. The University of Pennsylvania institutional review board approved the study (IRB #806468), and patients provided informed consent for LTOG enrollment.

Tacrolimus dosing

Tacrolimus was initiated within 12 to 24 hours postoperatively and administered twice daily orally or sublingually. The standard administration times were at 6:00 AM and 6:00 PM. Dosing was at the discretion of the treating clinicians. Trough concentrations were obtained daily before the morning dose (obtained between 4:00 and 6:00 AM) for the first 2 postoperative weeks (i.e., the follow-up period). The target trough was 8 to 12 ng/ml and remained uniform during the study period. Tacrolimus concentrations were determined from whole blood samples analyzed in the Hospital of the University of Pennsylvania Toxicology Lab using liquid chromatography-tandem mass spectrometry.[14] It has an analytical range of 1 to 30 ng/ml, and samples >30 ng/ml are diluted up to 1:4 to quantify

higher levels. Within-run and between-run coefficients of variation are reported at < 9.2%.

Population PK analysis

We performed population PK analysis of tacrolimus concentrations using nonlinear mixed-effects regression[15] using NONMEM (version 7.5.1, ICON plc, Leopardstown, Dublin, Ireland), through the interface provided by PDx-POP (version 5.3, ICON plc, Leopardstown, Dublin, Ireland). Output was summarized using Stata (version 17.0, StataCorp, College Station, TX). All models used with the First-Order Conditional Estimation with Interaction method.[15]

Base model development

We a priori specified a one-compartment structural model for this dataset consisting mainly of trough concentrations, following other studies where only trough concentrations are available,[16] as the lack of concentrations in the early period after tacrolimus administration precluded estimation of intercompartmental transfer.[16] Similarly, the absorption rate constant (ka) was fixed to 4.5 liter/hour[16] [in a sensitivity analysis, we set ka to 0.58 liter/hour[3]]. Clearance (CL) and volume of distribution (Vd) are reported relative to bioavailability (F), that is, CL/F and Vd/F, respectively. We described random interindividual variability using an exponential variance model, with individual random effects for all parameters assumed to follow a multivariate normal covariance structure. Residual variability was evaluated using additive and proportional error models.

Genotypes

We examined CYP3A5 variants rs776746 [*CYP3A5*3*, (6986A > G in intron 3)]; rs10264272 [*CYP3A5*6*, (14690G > A in exon 7)]; and rs41303343 [*CYP3A5*7*, (27131-27132insT)]. We classified CYP3A5 activity following the Clinical Pharmacogenetics Implementation Consortium[10]: extensive metabolizers (CYP3A5*1*1), intermediate metabolizers (CYP3A5*1*3, *1*6, *1*7), poor metabolizers (CYP3A5*3*3, *6*6, *7*7, *3*6, *3*7, *6*7). We combined extensive and intermediate metabolizers into 1 group given small numbers in each category. We also genotyped CYP3A4*22 (rs35599367, g.15389C > T in intron 6), as this variant may explain residual variability beyond the effects of CYP3A5.[17,18] Genotyping was performed on peripheral blood samples using an Affymetrix Axiom genotyping array with Applied Biosystems Axiom 2.0 Reagents.[19]

Clinical covariates

Covariate included pretransplant health status (age, weight, race, sex, cystic fibrosis diagnosis); hematocrit (a marker for tacrolimus binding to red blood cells), modeled as a time-varying covariate[20-22]; factors associated with postoperative severity of illness [transplant type (single vs bilateral), postoperative day, and the occurrence of PGD[23] during the first 3 postoperative days]; and cytochrome p450 enzyme (CYP) enzyme inhibitors commonly administered in lung transplant patients (fluconazole, voriconazole, and amiodarone). We modeled drug exposures as time-varying covariates lagged by 24 hours, that is, the dates of administration were shifted by 24 hours to account for (1) the onset and offset of inhibition and (2) the timing of drug concentration monitoring. Because trough levels were typically obtained between 4:00 and 6:00 AM in the morning, the effect of inhibitors initiated later that same day would first manifest in the trough level for the following day.

We anticipated the effect of transplant type and PGD to vary over time as patients recovered from the surgical insult. We thus estimated time-varying effects for these covariates (i.e., estimating separate coefficients on CL during postoperative days 0-3 and 4-14). We categorized time at 3 days based on our prior study demonstrating an inflection point in tacrolimus concentration:dose ratio at postoperative day 3.[4] Although postoperative corticosteroid dosing may induce tacrolimus metabolism,[24] we did not examine this variable because all patients received corticosteroids, with limited variability in daily dose across patients: median 37 mg (interquartile range [IQR] 32-44). Age and cystic fibrosis were strongly correlated, as were race and CYP3A5 genotype. We selected cystic fibrosis and CYP3A5 for model inclusion, as these variables have the clearest biologic relationship with tacrolimus metabolism.[12]

Modeling procedures

We screened covariates to identify those significantly improving model fit (decrease in objective function value [OFV] > 3.84, which corresponds to p-value < 0.05). We then conducted a manual forward stepwise multivariable analysis with significant covariates from univariate screening. In this step, we retained covariates in the model that decreased OFV > 3.84. We then manually applied backward elimination to the full model, retaining covariates that resulted in an increase of OFV > 6.64 (p-value < 0.01) upon removal.

We coded discrete covariates as 1 indicating presence and 0 indicating absence of the covariate and applied a multiplicative function specified as $TVP = \theta_{TVP} \times (\theta_{cov})^{COV}$, where TVP is the typical value of the parameter, θ_{TVP} is the population parameter estimate, and θ_{cov} is the effect of the covariate.[15] We evaluated continuous covariates with normalized power models, specified as $TVP = \theta_{TVP} \times (\text{covariate value}/\text{covariate}_{med})^{\theta covariate}$, where the covariate value is the value at the time the PK samples were obtained, covariate_{med} is the median value of the covariate in the population, and $\theta_{covariate}$ is the effect of that covariate on the parameter of interest.[15]

Model evaluation in the derivation cohort

We assessed model adequacy using changes in OFV, visual inspection of diagnostic plots, plausibility of parameter

estimates based on prior literature,[16] precision of parameter estimates as measured by asymptotic standard errors, changes in Akaike Information Criterion, and the size of interindividual and residual variabilities for the specified model. Additionally, shrinkage was calculated for model parameters where interindividual variability was estimated, with shrinkage < 25% considered acceptable.[25] We evaluated the final model with a bootstrap analysis, wherein model parameters were re-estimated in 1,000 datasets randomly sampled from the derivation cohort with replacement. The median, 2.5th, and 97.5th percentiles from bootstrapped estimates were compared to the final model parameter estimates. Lastly, we conducted prediction-corrected visual predictive checks (pc-VPC)[26] to evaluate the predictive accuracy of the final model. To generate the pc-VPC, 500 simulation replicates of the original dataset were generated. Overlay plots of the observed concentrations vs time with the 95% prediction interval of the simulated data were generated.

Model evaluation in the validation cohort

We used the final model to calculate predicted concentrations for subjects in the validation cohort using each subject's tacrolimus dosing history and covariates. We calculated prediction error (PE),[27] defined as PE = $(C_{pred} - C_{obs})/C_{obs} \times 100\%$, where C_{pred} represents the population-predicted concentration and C_{obs} represents the observed concentration. We summarized PE with mean (95% confidence interval [CI]) and median (IQR). We a priori specified successful validation as showing both mean and median PE values to be less than 20%. Additionally, we calculated the percentage of predicted concentrations within 2 and 4 ng/ml of observed concentration as a measure of clinical utility. Lastly, we conducted a pc-VPC as described for the derivation cohort.

Results

Cohort characteristics

We included 384 patients (derivation cohort n = 270, validation cohort n = 114) who were genotyped and had at least 1 tacrolimus concentration measured (Table 1). Approximately two-thirds of patients in each cohort underwent bilateral lung transplantation. One in 5 patients had a CYP3A5 intermediate or extensive metabolizer genotype,

Table 1 Patient Characteristics

Characteristic	Derivation cohort (n = 270)	Validation cohort (n = 114)
Age, years, median (IQR)	61 (51, 66)	60 (52, 65)
Female sex, n (%)	107 (40)	44 (39)
Weight, kg, median (IQR)	75 (59, 88)	77 (61, 87)
Race, n (%)		
White	237 (88)	98 (86)
African American	23 (9)	10 (9)
Other	10 (3)	6 (5)
Cystic fibrosis, n (%)	29 (11)	8 (7)
Transplant type, bilateral, n (%)	177 (66)	78 (68)
Primary graft dysfunction, n (%)[a]	61 (23)	33 (30)
Hematocrit, (%), median (IQR)	33 (30, 38)	34 (30, 38)
CYP3A5 metabolizer genotype, n (%)[b]	53 (20)	22 (19)
CYP3A4 metabolizer genotype, n (%)[c]	29 (7)	10 (9)
CYP enzyme inhibitors, n (%)[d]		
Voriconazole	95 (35)	33 (29)
Fluconazole	20 (7)	4 (4)
Amiodarone	96 (36)	43 (38)
Follow-up duration, days, median (IQR)	13 (11, 14)	13 (10, 14)
Tacrolimus concentrations		
Total samples	3,143	1,279
Samples per patient, count, median (IQR)	13 (10, 14)	12 (10, 13)
Time after dose, hours, median (IQR)	11.1 (10.1, 11.9)	11.2 (10.4, 11.9)
Initial tacrolimus dose, mg, median (IQR)	2 (2, 2)	2 (2, 2)

Abbreviation: IQR, interquartile range.

[a]Diagnosed by postoperative day 3 or earlier.

[b]Presence of at least 1 allele encoding an extensive or intermediate metabolizer phenotype as defined by the Clinical Pharmacogenetics Implementation Consortium consensus guidelines.[10]

[c]Presence of at least 1 allele encoding a poor metabolizer phenotype.

[d]Ever exposed during the 14-day study period.

and CYP inhibitor exposure was common. Modest numerical differences between the cohorts were observed for median weight (77 kg vs 74 kg), frequency of cystic fibrosis (11% vs 7%), PGD (23% vs 30%), and voriconazole exposure (35% vs 29%). The median initial tacrolimus daily dose was 2 mg in both cohorts. The individual trend lines of tacrolimus concentrations in both cohorts are depicted in Figure 1, showing substantial variability relative to the target range.

Model building

Base model

Parameters of the 1-compartment base model are shown in Table 2. Goodness-of-fit plots are shown in Figure 2, with plots of predicted vs observed stratified by time (postoperative days 0-3 and 4-14). The base model tended to underpredict observed concentrations during the early time period, while overpredicting observed concentrations during the later time period.

Model incorporating patient-level covariates

Results of covariate modeling steps are shown in Table 3. Significant covariate effects on CL in univariate analysis included weight, CYP3A5 and CYP3A4 genotypes, transplant type, postoperative day, PGD, hematocrit concentration, and CYP inhibitor treatment (voriconazole, fluconazole, and amiodarone). Weight was also significantly associated with Vd. Transplant type and PGD significantly improved model fit when specified as time-varying effects (i.e., separate coefficients for days 0-3 and days 4-14), but not as time-invariant effects (i.e., a single coefficient for days 0-14). The final models for CL and Vd (Table 2) were

$$\text{CL/F (liter/hour)} = 3.69 * \text{postoperative day}^{0.67} * (\text{hematocrit}/33)^{-1.45} * [(1.0, \text{if single}) \textit{ or } (0.48, \text{if bilateral on days 1-3}) \textit{ or } (0.82, \text{if bilateral on days 4-14})] * [(1.0, \text{if poor CYP3A5 metabolizer}) \textit{ or } (1.78, \text{if intermediate or extensive CYP3A5 metabolizer})] * [(1.0, \text{if voriconazole untreated}) \text{ or } (0.39, \text{if voriconazole treated})] * [(1.0, \text{if amiodarone untreated}) \text{ or } (0.77, \text{if amiodarone treated})] * (\text{weight}/75)^{0.4}$$

$$\text{VD (liter)} = 642 * (\text{weight}/75)^{0.79}$$

The strongest predictor of tacrolimus CL was postoperative day, with median predicted CL increasing 3-fold over the study period (Figure 3). The final model results were similar when using a fixed ka of 0.58 liter/hour (Table S1).

Final model evaluation and validation

The bootstrap results are shown in Table 2, which were comparable with the typical values derived from the

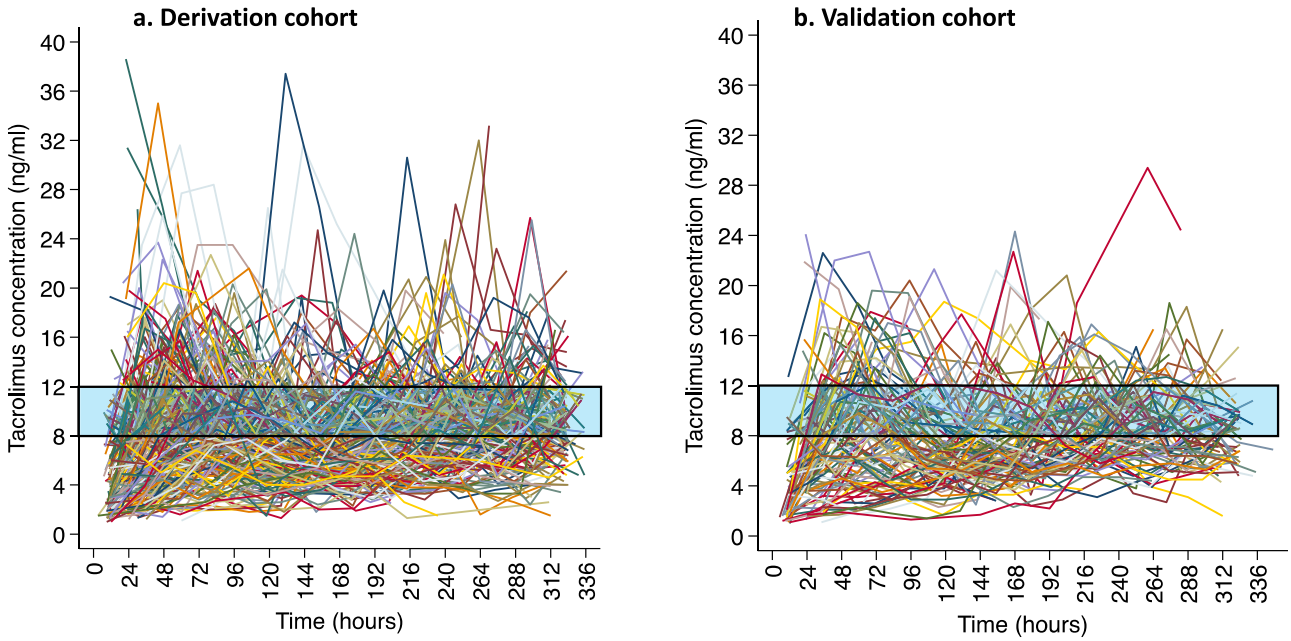

Figure 1 Individual trend lines of tacrolimus concentration over time in the derivation and validation cohorts. The shaded area represents the target tacrolimus concentration range (8-12 ng/ml). In the derivation cohort, there were a total of 3,143 concentrations, with 56% being below 8 ng/ml, 31% in the target range, and 13% above 12 ng/ml. In the validation cohort, there were a total of 1,279 concentrations, with 57% being below 8 ng/ml, 29% in the target range, and 14% above 12 ng/ml.

Table 2 Parameter Estimates of the Base Model and Final Model

Parameter	Base model (95%CI) [shrinkage %]	Final model (95%CI) [shrinkage %]	Bootstrap analysis Median (2.75th-97.5th percentile)
CL/F, liter/hour	14.06 (12.7, 15.3)	3.69 (2.93, 4.45)	3.68 (2.77, 4.47)
Vd/F, liter	454 (399.0, 509.0)	642 (575, 709)	644 (604, 779)
Ka, liter/hour	4.5 Fixed	4.5 Fixed	4.5 Fixed
Covariates on CL			
Postoperative day	–	0.67 (0.58, 0.76)	0.71 (0.55, 1.06)
Hematocrit, %	–	−1.45 (−1.84, −1.06)	−1.39 (−1.74, −0.96)
Transplant type[a]			
Days 1-3	–	0.48 (0.35, 0.61)	0.53 (0.45, 0.93)
Days 4-14	–	0.82 (0.70, 0.94)	0.83 (0.68, 0.98)
CYP3A5 genotype	–	1.78 (1.44, 2.12)	1.64 (1.44, 2.23)
Voriconazole exposure	–	0.39 (0.32, 0.47)	0.37 (0.31, 0.63)
Amiodarone exposure	–	0.77 (0.69, 0.85)	0.79 (0.73, 0.96)
Weight, kg	–	0.40 (0.11, 0.69)	0.40 (0.10, 0.73)
Covariates on Vd			
Weight, kg	–	0.79 (0.41, 1.18)	0.79 (0.47, 1.14)
Interindividual variability			
CL/F	0.37 (0.30, 0.44) [5.2]	0.29 (0.23, 0.34) [4.8]	0.30 (0.20, 0.42)
Vd/F	0.42 (0.28, 0.56) [26.2]	0.54 (0.43, 0.65) [7.6]	0.55 (0.42, 0.70)
Residual variability			
Proportional, %	13.01 (0.09, 0.17) [5.9]	7.73 (6.23, 9.23) [7.5]	8.08 (7.69, 10.37)
Additive, ng/ml	3.79 (1.20, 6.38) [16.6]	1.80 (1.08, 2.52) [14.4]	1.82 (1.11, 2.56)

Abbreviations: 95%CI, 95% confidence interval; CL, clearance; F, bioavailability; ka, absorption rate constant; Vd, volume of distribution.
[a]Single vs bilateral.

original population PK analysis. Diagnostic plots of the final model are shown in Figure 4 (derivation cohort) and Figure 5 (validation cohort). In both cohorts, the symmetry of PE around the line of identity is improved compared to the base model, but predicted concentrations continued to underestimate observed values to some extent in the early postoperative time period. The results of the pc-VPC (Figure 6) suggest the model has good fit of observed concentrations during the first week of follow-up, with suboptimal fit during the second week of follow-up. Similar results for the pc-VPC were observed in the validation cohort. Mean PE of the final model in the validation cohort was 36.4% (95%CI 30.8%-41.9%) and the median PE was 7.2% (IQR −29.3% to 70.53%). The percentage of population-predicted concentrations within 2 or 4 ng/ml observed was 34.7% and 59.1%, respectively.

Discussion

We developed a population PK model tailored to the early postoperative time period after lung transplantation. In addition to CYP3A5 genotype, our final model included key clinical factors relevant to lung transplantation, such as transplant type and commonly encountered drug-drug interactions. Nevertheless, we observed substantial PE when our model was applied to the validation cohort, and the pc-VPCs suggest the model is suboptimally fit for the second week of the study period. This suggests that a broader assessment of clinical covariates, paired with rich interdose sampling is needed to optimally explain tacrolimus exposure variability in this setting.

Our findings add to prior studies evaluating tacrolimus PK in postoperative lung transplant patients. Sikma

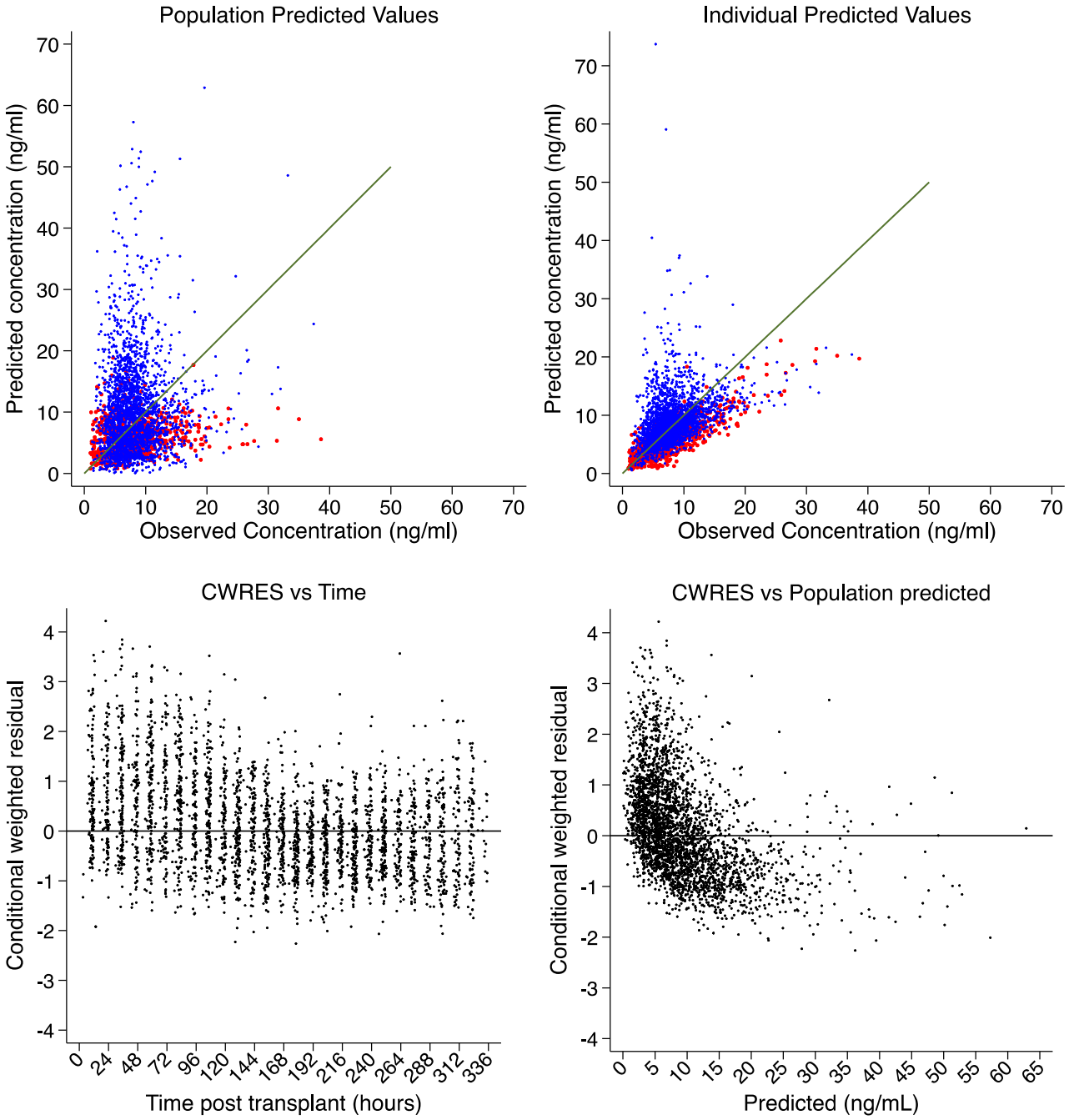

Figure 2 Diagnostic plots of the base model in the derivation cohort. Observed vs predicted plots stratified by postoperative day (red days 0-3, blue days 4-14). CWRES, conditional weighted residual error.

et al developed a tacrolimus population PK model in 20 lung and 10 heart recipients, but no clinical covariates were included in the final model. Two other studies have evaluated tacrolimus PK after lung transplantation, although neither study focused on the immediate postoperative period. Saint-Marcoux et al included 22 patients who were studied between 3 and 116 months after transplant.[11] Monchaud et al included 182 PK profiles from 78 patients

Table 3 Model Building Steps

Covariate	OFV	Delta OFV	AIC	Delta AIC
Base model	12,086.34	–	12,100.30	–
Univariate covariate effects on clearance (CL)				
Total body weight	12,079.71	−6.63	12,095.70	−4.60
Postoperative day	11,107.33	−979.01	11,123.30	−977.00
Transplant type (single vs bilateral)				
Time-invariant effect	12,083.47	−2.87	12,099.50	−0.80
Time-varying effect (early vs late)	11,509.63	−576.71	11,527.60	−572.70
PGD				
Time-invariant effect	12,086.26	−0.08	12,102.30	2.00
Time-varying effect (early vs late)	11,945.08	−141.26	11,963.10	−137.20
CYP3A5 genotype	12,042.01	−44.33	12,058.00	−42.30
CYP3A4 genotype	12,078.81	−7.54	12,094.80	−5.50
Cystic fibrosis	12,086.24	−0.10	12,102.20	1.90
Voriconazole coexposure	12,053.02	−33.32	12,069.00	−31.30
Fluconazole coexposure	12,076.32	−10.02	12,092.30	−8.00
Amiodarone coexposure	12,059.75	−26.59	12,075.70	−24.60
Hematocrit	11,849.44	−236.90	11,865.40	−234.90
Univariate covariate effects on volume of distribution (Vd)				
Weight	12,063.97	−22.37	12,080.00	−20.30
Multivariable models: forward selection				
Model 1: postoperative day on CL	11,107.36	–	11,123.40	–
Model 2: Model 1 + hematocrit on CL	11,025.08	−82.28	11,043.10	−80.30
Model 3: Model 2 + transplant type on CL	10,949.50	−75.58	10,971.50	−71.60
Model 4: Model 3 + CYP3A5 genotype on CL	10,916.60	−32.90	10,940.60	30.90
Model 5: Model 4 + voriconazole on CL	10,585.86	−330.74	10,611.90	−328.7
Model 6: Model 5 + amiodarone on CL	10,541.02	−44.85	10,569.00	−42.9
Model 7: Model 6 + CYP3A4 genotype on CL	10,539.65	−1.36	10,569.70	0.70
Model 8: Model 6 + fluconazole on CL	10,537.95	−3.06	10,567.60	−1.40
Model 9: Model 6 + total body weight on CL	10,532.47	−8.55	10,562.50	−6.50
Model 10: Model 9 + PGD on CL	10,528.97	−3.50	10,562.60	0.10
Model 11[a]: Model 9 + total body weight on Vd	10,515.47	−17.00	10,547.50	−15.00
Multivariable models: backward selection				
Model 12: Model 11 – total body weight on Vd	10,523.93	8.46	10,553.90	6.40
Model 13: Model 11 – amiodarone on CL	10,561.03	45.55	10,591.00	43.50
Model 14: Model 11 – voriconazole on CL	10,853.49	338.01	10,883.50	336.00
Model 15: Model 11 – CYP3A5 genotype on CL	10,552.36	36.89	10,582.40	34.90
Model 16: Model 11 – transplant type on CL	10,590.46	74.99	10,618.50	71.00
Model 17: Model 11 – hematocrit	10,645.67	130.20	10,675.70	128.20
Model 18: Model 11 – postoperative day on CL	11,153.97	638.49	11,184.00	636.50

Abbreviations: AIC, Akaike Information Criterion; CL, clearance; OFV, objective function value; PGD, primary graft dysfunction diagnosed within 3 days of transplant; Vd, volume of distribution.

[a]Final model.

who were studied during the first year after transplant,[12] with 24/182 PK profiles obtained between postoperative days 7 and 14, and the remainder obtained > 30 days after transplant. Thus, these studies provide limited information regarding factors influencing the dynamic CL of tacrolimus over the first 7 to 14 days following the transplant procedure, where patients have varying degrees of critical illness physiology, characterized by inflammation and organ

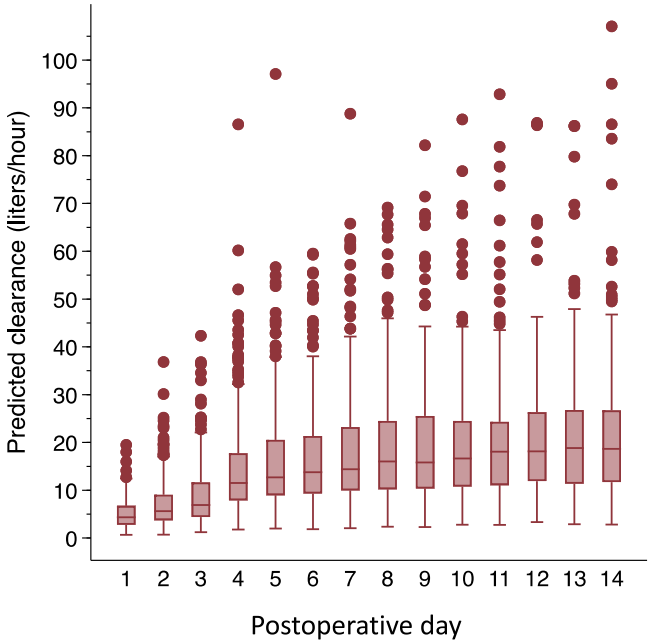

Figure 3 Box plot of population-predicted clearance stratified by postoperative day. The figure depicts the distribution of clearance on each postoperative day. The box and whiskers are interpreted as follows: the box represents the interquartile range (25th-75th percentiles); the line intersecting the box is the median; and dots represent outlier values; clearance values for each postoperative day were determined by the covariate profiles for each patient on that particular day, resulting in population-level marginal effect estimates.[28]

dysfunction. Although not definitive, our study was larger, focused specifically on the early postoperative period, and included multiple clinical covariates including CYP genotype and daily hematocrit, which explained a meaningful amount of tacrolimus level variability. Our findings provide preliminary evidence suggesting that population PK models that include lung-transplant–specific covariates may enable improved dosing compared to the current "trial and error" approach to tacrolimus dosing used in most transplant centers.[2]

A key observation from this study is the dynamic nature of tacrolimus CL during the early period after lung transplantation. Tacrolimus CL increased more than 3-fold over the study period, which may be due to changing critical illness physiology over time as patients recover from the surgical insult.[20] We hypothesized that transplant type and PGD would explain some of this underlying physiology. Transplant type is associated with the severity of the surgical insult, with bilateral transplants requiring longer duration of cardiopulmonary bypass, a median sternotomy, and potentially increased risk for ischemia-reperfusion injury.[29] PGD, a severe lung injury syndrome that occurs within 3 days of lung transplantation, is characterized by circulating markers of physiologic dysregulation and is a strong risk factor for early mortality.[30] Notably, these covariates had associations with tacrolimus CL that were different on postoperative days 0 to 3 vs later days. The time-varying association between transplant type and CL is consistent with an early impact of the surgical insult that wanes over time as patients recover. This highlights the importance of exploring time-varying coefficients for such variables in this population, as the relative lack of association with CL in the later time period may diminish these variables' predictive utility. Future analyses that include additional covariates related to critical illness (e.g., mechanical ventilation parameters, blood pressure, fluid resuscitation, vasopressor support) are warranted.

In agreement with previous studies,[10] we identified *CYP3A5* genotype as a strong predictor of tacrolimus CL. As CYP3A5 expression is more common in African Americans,[10] the impact of this covariate could be larger in other study cohorts. CYP3A4 genotype did not demonstrate a significant impact in multivariable analysis, which may be due in part to the low number of patients with this genotype in our cohort. We additionally identified voriconazole and amiodarone as predictors of reduced tacrolimus CL. These drugs are strong inhibitors of CYP3A4, the predominant tacrolimus metabolizing enzyme in those not expressing CYP3A5. Both drugs are key therapies in the lung transplant population. Voriconazole is commonly used for prophylaxis of invasive aspergillosis,[31] and amiodarone is used for the prevention and treatment of postoperative atrial fibrillation.[32]

Our study has limitations. The primary limitation is that tacrolimus concentrations consisted mainly of trough sampling. This limited our ability to explore 2-compartment models and to model the absorption process. This is particularly important, as tacrolimus absorption is highly variable in the setting of critical illness[3] and may vary across

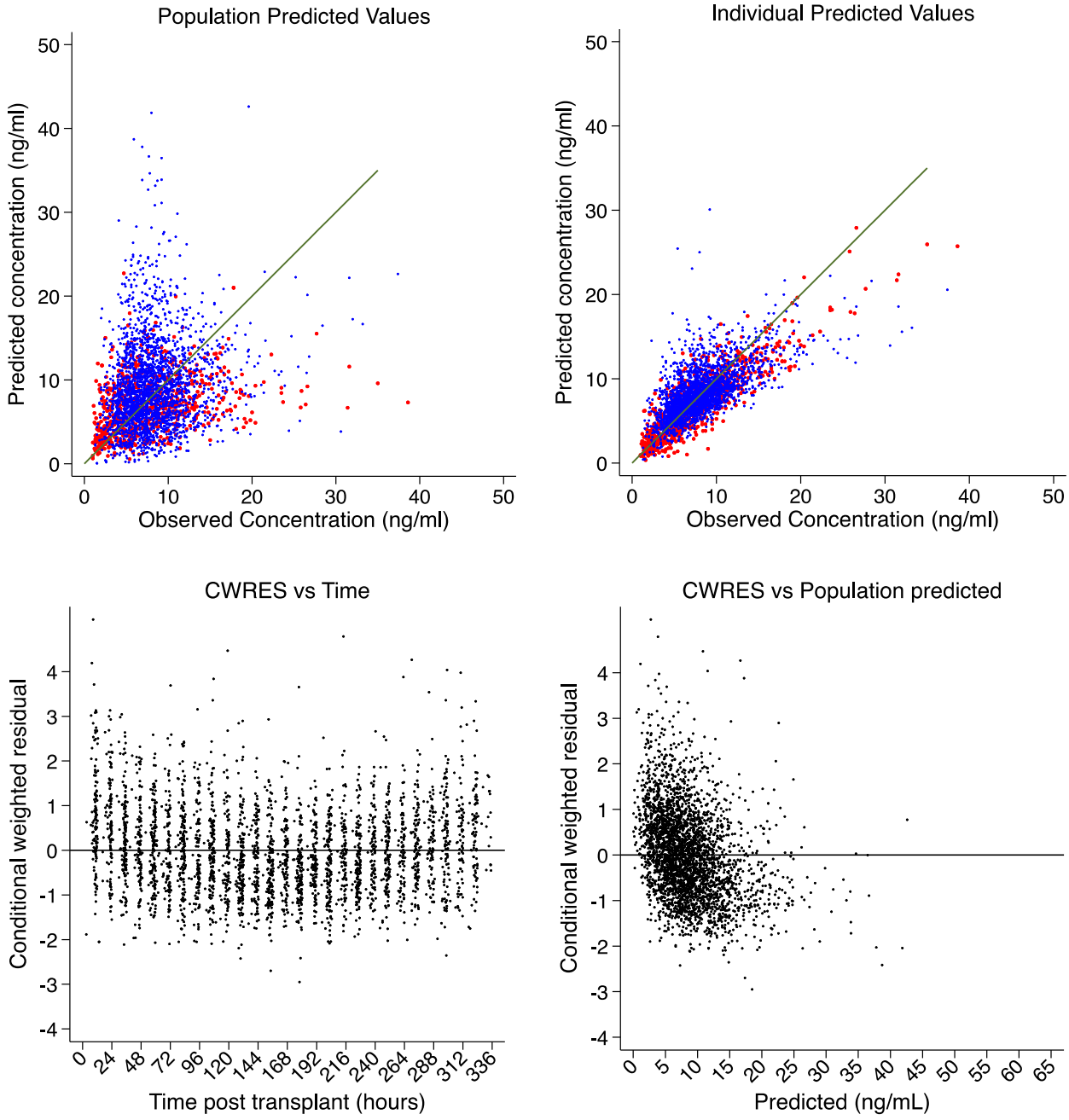

Figure 4 Diagnostic plots of the final model in the derivation cohort. Observed vs predicted plots stratified by postoperative day (red days 0-3, blue days 4-14). CWRES, conditional weighted residual error.

routes of administration (oral vs sublingual[3]). The insensitivity of our model to changes in absorption is reflected in the results of sensitivity analysis, wherein changing the fixed ka from 4.5 to 0.58 liter/hour had negligible effects on model parameters. Future studies employing rich concentration sampling are needed to explore factors affecting tacrolimus absorption variability. In addition, although our sample size was considerable, there was limited power to

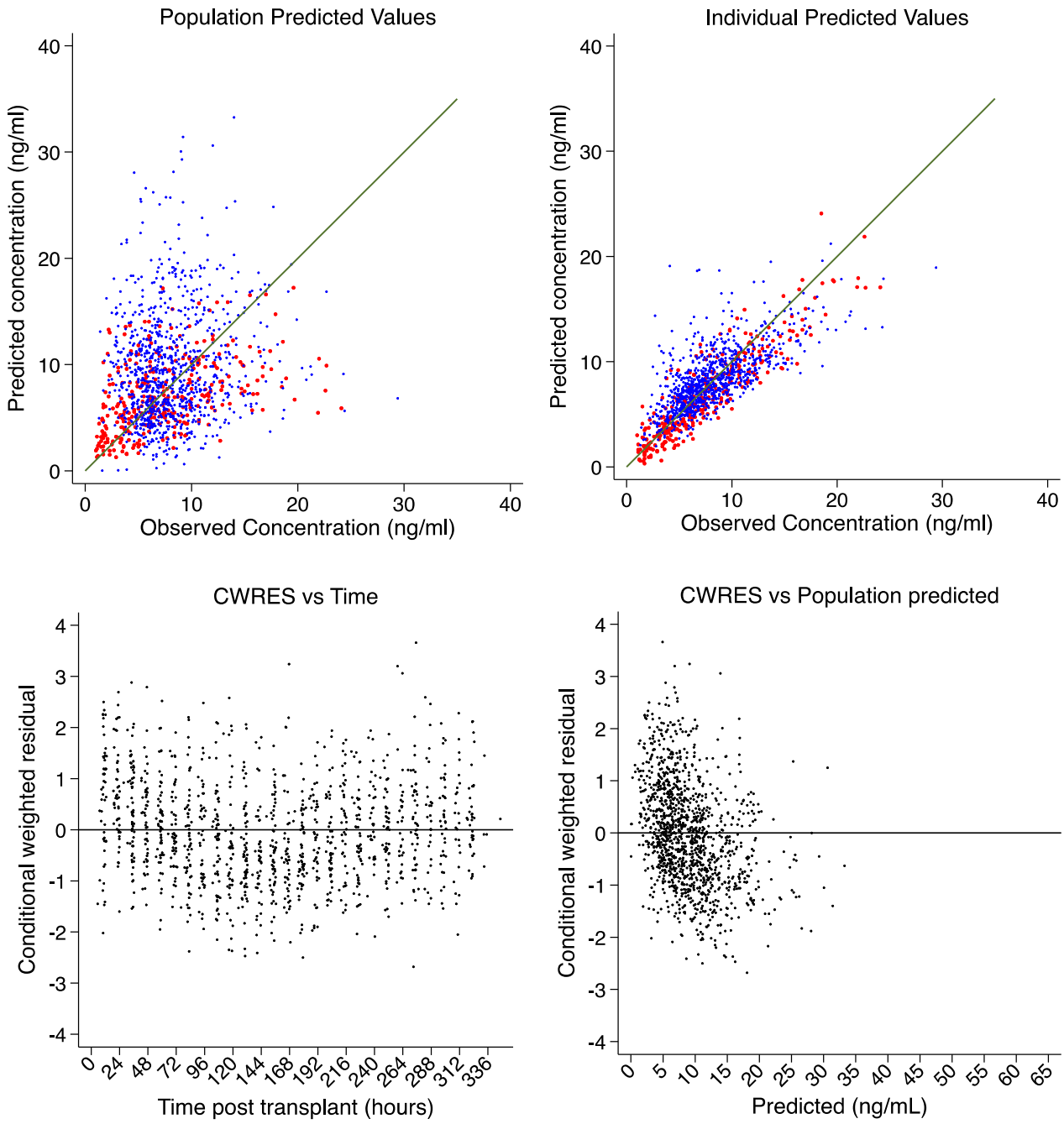

Figure 5 Diagnostic plots of the final model in the validation cohort. Observed vs predicted plots stratified by postoperative day (red days 0-3, blue days 4-14). CWRES, conditional weighted residual error.

estimate gene-drug interactions. Previous literature suggests the inhibitory effect of azole antifungals on tacrolimus metabolism varies by CYP3A5 genotype.[33] Additionally, our model did not account for assay variability, although the coefficient of variation (CV) of the tacrolimus assay in our clinical laboratory is < 10%. And, to the extent that the times of drug administration and concentration sampling were recorded with error (which is plausible in a clinical environment), this could also serve as a source of residual variability. Lastly, although we examined our final model in

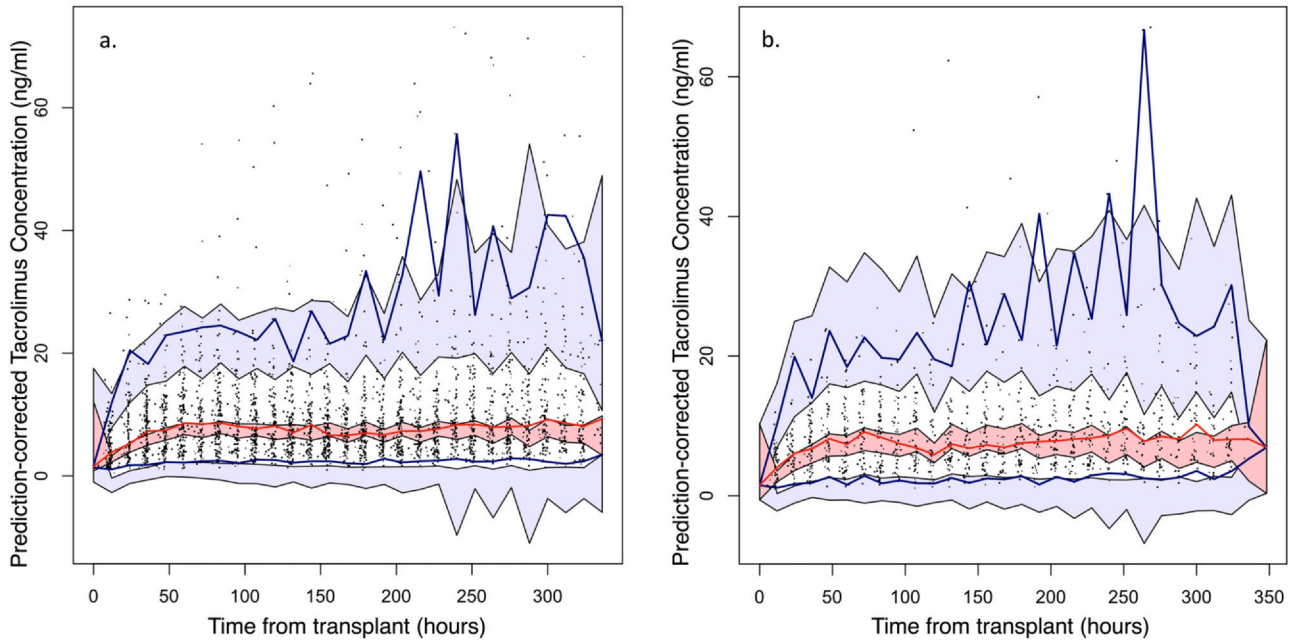

Figure 6 Prediction-corrected visual predictive checks. Panel (a) depicts the prediction-corrected visual predictive checks (pc-VPC) for the derivation dataset and (b) depicts the pc-VPC for the validation dataset. The black dots are the observed prediction-corrected concentrations. The red line represents the median and the blue solid lines are the 2.5th and 97.5th percentiles of observed prediction-corrected concentrations. The shaded areas represent 95% confidence intervals of median, 2.5th, and 97.5th percentiles of the simulated values. Adequate fit of the model would be demonstrated by the lines falling completely within the corresponding confidence bands. These figures suggest that the model has adequate fit to the data during the first week of the study period but has suboptimal fit in the latter half of the study period.

a validation cohort, this cohort consisted of other patients transplanted at the same center, which could limit generalizability.

Conclusion

Tacrolimus CL is highly dynamic during the early postlung transplant period. Population PK models that include lung-transplant–specific covariates may enable precision dosing algorithms that account for this highly dynamic CL. Future multicenter studies including a broader set of covariates are warranted.

Author contributions

Todd A. Miano: Conceptualization, Methodology, Formal analysis, Writing - Orginal Draft; **Athena F. Zuppa**: Methodology, Writing - Review and Editing; **Rui Feng**: Writing - Review and Editing; **Stephen Griffiths**: Data curation, Writing - Review and Editing; **Laurel Kalman**: Writing - Review and Editing; **Michelle Oyster**: Writing - Review and Editing; Project administration; **Edward Cantu**: Writing - Review and Editing; **Wei Yang**: Writing - Review and Editing; **Joshua M. Diamond**: Writing - Review and Editing; **Jason D. Christie**: Writing - Review and Editing, Supervision; **Marc H. Scheetz**: Methodology, Writing - Review and Editing; **Michael G. S. Shashaty**: Conceptualization, Writing - Review and Editing, Supervision.

Disclosure statement

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jhlto.2024.100134.

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