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Tacrolimus population pharmacokinetics in adult heart transplant patients

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What is already known about this subject:

- Tacrolimus is an immunosuppressant largely used in heart transplantation.
- Therapeutic drug monitoring is required to optimize the dose.
- Only a few population pharmacokinetic models for tacrolimus have been developed in heart transplant patients¹⁻³, while the others included mixed populations (HTX and other organs).

What this study adds:

- A 2 compartment with transit absorption population pharmacokinetic model was developed from full pharmacokinetics profiles in adult heart transplant patients (n=18, pk profiles= 47 and 546 concentrations).
- A bayesian estimator based on a limited sampling strategy (0h-1h-2h post administration of tacrolimus) was derived, yielding good predictive performances (AUC bias±SD = 2.72± 10.17% and imprecision = 9.9%).
- The probability of target attainment was performed for the AUC and the C0 to propose different starting doses depending on the CYP3A5 status, non expressors (0.11 mg.kg⁻¹.12h⁻¹ based on the AUC or the C0) or expressors (0.22 mg.kg⁻¹.12h⁻¹ based on AUC and 0.29 mg.kg⁻¹.12h⁻¹ based on C0).

Abstract

Introduction: Tacrolimus is an immunosuppressant largely used in heart transplantation. However, the calculation of its exposure based on the area under the curve (AUC) requires the use of a population pharmacokinetics model. The aims of this work were (i) to develop a population pharmacokinetic model for tacrolimus in heart transplant patients, (ii) to derive a maximum a posteriori bayesian estimator (MAP-BE) based on a limited sampling strategy (LSS) and (iii) to estimate probabilities of target attainment (PTA) for AUC and trough concentration (C₀).

Material and methods: Forty-seven PK profiles (546 concentrations) of eighteen heart transplant patients of the PIGREC study receiving tacrolimus (Prograf®) were included. The database was split into a development (80%) and a validation (20%) set. PK parameters were estimated in MONOLIX® and based on this model, a bayesian estimator using a LSS was built. Simulations were performed to calculate the PTA for AUC and C₀.

Results: The best model to describe the tacrolimus pharmacokinetics was a two-compartment model with a transit absorption and a linear elimination. Only the CYP3A5 covariate was kept in the final model. The derived MAP-BE based on the LSS (0-1-2h post-dose) yielded an AUC bias±SD=2.72±10.17%; and an imprecision of 9.9% in comparison to the reference AUC calculated using the trapezoidal rule. PTA based on AUC or C₀ allowed to propose new recommendations for starting doses (0.11mg.kg⁻¹.12h⁻¹ for the CYP3A5 non-expressor and 0.22mg.kg⁻¹.12h⁻¹ for the CYP3A5 expressor).

Conclusion: The MAP-BE developed should facilitate estimation of tacrolimus AUC in heart transplant patients.

Introduction

Tacrolimus is a narrow therapeutic index immunosuppressant, largely used in combination with mycophenolate mofetil in heart transplant patients⁴. Tacrolimus is characterized by a large inter-individual variability⁵ which renders mandatory therapeutic drug monitoring (TDM). The most frequently used marker for tacrolimus TDM is the trough concentration (C₀) even if, its correlation with rejection is still a matter of debate^{5,6}. The interdose area under the curve (AUC) of tacrolimus is theoretically the best exposure marker and has been proposed for TDM⁵. No prospective randomized study has investigated the association between AUC or C₀ and acute rejection in heart transplant recipients. A single study of 25 heart transplant patients treated with oral tacrolimus, showed that the AUC was significantly lower in rejection cases vs cases without rejection⁷. Nowadays, the C₀ target in heart transplant patients used in the two first months post transplantation is from 15 to 20 µg.L⁻¹⁵. Nevertheless, a study using a sample of 110 heart transplant patients has shown an increased risk of acute kidney injury within the first 2 weeks after transplantation for C₀>15 µg.L⁻¹⁸. This led the second conference of consensus on Tacrolimus to suggest revising the C₀ target. However, this has not yet been done. Currently, no AUC target has been defined in heart transplant patients.

The large variability in tacrolimus exposure is caused by several factors including the cytochrome P450 family 3 subfamily A member 5 ([CYP3A5](#)) status (with a two times lower dose requirement in *CYP3A5**3 homozygous carriers⁹ in comparison to carriers of at least one *1 allele (*CYP3A5**1/*1 or *CYP3A5**1/*3), food intake, the type of organ transplanted, the time-period post-transplantation, age and hematocrit¹⁰.

Currently, only a few population pharmacokinetic models (POPPK) dedicated to heart transplant patients have been developed¹⁻³. Other tacrolimus developed models also include lung¹¹ or liver and kidney¹² transplant patients. In a recent work, Kirubakaran et al.¹ built a POPPK based on sparse data (mostly trough concentrations) on the basis of a previously published POPPK model¹³ to inform the effect of anti-fungal interaction¹ on tacrolimus apparent clearance. None of them investigated the predicted probability of AUC target attainment using simulations to improve the first dose of tacrolimus. An accurate estimation of the interdose AUC can be obtained by combining a Maximum a posteriori Bayesian estimator (MAP-BE) derived from a POPPK model and a limited sampling strategy (LSS).

The aims of this study were: (i) to develop a POPPK model of tacrolimus in a population of heart transplant patients; (ii) to develop a MAP-BE to estimate individual PK parameters and

tacrolimus exposure indices based on a limited sampling strategy (LSS); (iii) to estimate the probabilities of target attainment (PTA) for AUC and trough concentration (C₀) using Monte-Carlo simulations and to propose recommendations for tacrolimus starting doses.

Material and methods

Patients and samples

Heart transplant patients of the PIGREC study receiving tacrolimus (Prograf® *bid*) were selected to build the model. All the patients underwent heart transplantation between 2007 and 2009 and they all received basiliximab for 3 days post transplantation. On the 4th day, the association tacrolimus/mycophenolate mofetil was introduced. This multicentre trial, in which 7 French transplantation centres participated, was approved by the Limousin regional ethic committee and authorized by the French Drug Agency (PIGREC [Pharmacocinétique des Immunosuppresseurs chez les patients GREffés Cardiaques], EudraCT number N°2006-006832-23; ClinicalTrials.gov identifier NCT00812786). All the patients included gave their written informed consent. The initial dose of tacrolimus was 0.075 mg.kg⁻¹.day⁻¹. Forty-seven full-PK profiles (concentration data collected at T0, T20', T40', T60', T90', T2h, T3h, T4h, T6h, T8h and T9h + T12h) were collected from 18 adult heart recipients. Each patient underwent up to 4 post-transplant visits (P1=7 to 14 days: 128 concentrations, P2=1month: 126 concentrations, P3=3 month: 125 concentrations and P4=1 year after transplantation:167 concentrations). One additional blood sample was taken at D7-14 for pharmacogenetic analyses. The transplantation of any other organ before or during the PIGREC study was an exclusion criterion.

Assay

All the blood samples were analyzed in the Pharmacology, Toxicology and Pharmacovigilance Department of the Limoges University Hospital, using a validated turbulent flow chromatography-tandem mass spectrometry (TFC-MS/MS) method. Briefly, online extraction was performed at a high flow rate (1.25 ml min⁻¹) on a Cyclone P®, 50-mm particle size (50 µm I.D.) column (Cohesive technologies, Milton Keynes, UK) in alkaline conditions using ammonium acetate and acetic acid as mobile phase. Chromatographic separation was performed in acidic conditions using a Propel C18 MS, 5 mm (50 µm I.D.) column (Cohesive technologies, Milton Keynes, UK) heated to 60°C, with a constant flow rate of 300 ml min⁻¹. Detection was performed using a TSQ Quantum Discovery MS/MS system (Thermo-Fisher, Les Ulis, France) equipped with an orthogonal electrospray ionization source and controlled by the Xcalibur computer program. MS/MS detection was performed in the positive

ion, multiple reaction monitoring mode following two transitions for tacrolimus (m/z 821.5→768.6; m/z 821.5→786.4) and two for the internal standard ascomycin (m/z 809.3→756.4; m/z 809.3→564.4). The method was developed using ascomycin as the internal standard due to the similar molecular weight with the tacrolimus and the absence of deuterated or labeled tacrolimus at that time. This method was fully validated for tacrolimus determination in whole blood. The calibration curve used is a 1/x weighted quadratic regression to obtain the best fit across the calibration range based on the standard error of the fit and minimization of calibrator's bias. The lower limit of quantitation (LLQ) was 1 µg.L⁻¹ and the calibration curves obtained from the LLQ up to 100 µg.L⁻¹ yielded r² > 0.998. The method was found to be accurate and precise with a bias of -4.4% to 0.6% and a low coefficient of variation (CV) of -3.8% to 6.4% (CV intra-day = 14.23% and CV inter-day = 11.7 % at the LLQ). It is worth noting that all the concentrations were above the LLQ.

Genotyping

Patients' genotypes were characterized for cytochrome 3A5 (CYP3A5) rs776746 A/G (CYP3A5*3 allele) using validated TaqMan allelic discrimination assays on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France).

Missing data:

Missing data were imputed using the K-nearest neighbour approach using the "VIM" R package¹⁴. The data imputations were only performed when missing data represented less than 20% of the data set. A Wilcoxon test was performed to determine if the distribution of the variables is affected by this method. Furthermore, some patients did not have a sample drawn at t12h. To circumvent this issue, the linearity of the elimination between 9-12 hours was checked after log transformation and in order to estimate the reference AUC using the trapezoidal rule, the T=12h was then extrapolated using a mono-exponential decay from the last point available:

$$C(t) = C_0 \times e^{-K_e \times t}$$

Pharmacokinetic modelling

The database was randomly divided, based on the PK profiles, into a development dataset (38 PK profiles and 433 concentrations) and a validation dataset (9 PK profiles and 113 concentrations). The two populations were compared using a Wilcoxon test. The POPPK analysis was performed using a nonlinear mixed effect approach using the Stochastic Approximation Expectation-Maximization (SAEM) algorithm in MONOLIX¹⁵.

Structural model development

Several structural models were investigated to fit the data: one or two-compartment models with first-order elimination and with a lag time or transit compartments to describe the absorption phase. Models were parametrized in terms of apparent clearance (CL/F), apparent volume of distribution (Vd), mean transit time, transit rate constant and absorption rate. Inter-individual variability was described using an exponential model. The covariance of the parameters was investigated during the modelling process. The best structural model and residual error model (comparison of additive, proportional or both) were selected based on the Bayesian information criterion (BIC) and the visual inspection of goodness-of-fit plots.

Covariate investigation

Covariates investigated on PK parameters were weight, age, hematocrit, CYP3A5, sex and the categorized time post-transplantation (7-14 days, 1-month, 3-month, 1 year).

Scatter plot and Pearson correlation coefficient were drawn between the Empirical Bayes Estimates and the continuous covariates and boxplots and ANOVA for the categorical covariable (CYP3A5, sex and time post-transplantation). The plots of covariates vs ETA CL from the base model were drawn.

Covariates were introduced individually into the structural model following a forward inclusion and backward elimination procedure. The covariate that decreases the BIC was kept in the structural model. The clinical relevance of the covariates was also evaluated based on changes in the interpatient variability.

Categorical covariates were introduced as follows:

$$\theta_j = \theta_{jTPV} \times e^{\theta_{COVi} \times COVi}$$

Continuous covariates were tested as follows:

$$\theta_j = \theta_{jTPV} \times \left(\frac{COVi}{COVi_{median}} \right)^{\theta_{COVi}}$$

- θ_j represent the value of the PK parameters j^{th} , $\theta_{j\text{TPV}}$ is the mean value of j^{th}
- θ_{COVi} a parameter estimated representing the effect of the i^{th} covariate (COVi)

Internal validation

Goodness-of-fit plots were drawn for the final model. The final model was internally evaluated using the prediction-corrected visual predictive check (pcVPC) ¹⁶ in the development and in the validation dataset.

Bayesian estimator

The final model developed was used as prior to develop a MAP-BE that could estimate tacrolimus AUC based on a 3-samples strategy. Several LSS using samples within the first 4 hours post-dose were investigated in the development set and compared based on the relative mean prediction error (rMPE = biais) and the root mean square error (RMSE = imprecision).

$$rMPE (\%): \frac{\sum_{i=1}^n \left(\frac{pei}{AUC_{ref}} \right)}{n} \times 100$$

$$RMSE (\%) = \sqrt{\frac{\sum_{i=1}^n \left(\frac{pei}{AUC_{ref}} \right)^2}{n}} \times 100$$

- n represents the number of pairs of estimated and measured AUC, AUCref is the calculated AUC using the trapezoidal rule and pei is the difference between the estimated and the reference AUC

As the sample at t=0h is routinely drawn for TDM, only LSS including this sample were selected.

Finally, the predictive performance of the MAP-BE based on the best LSS was tested in the validation dataset based on the RMSE and the rMPE in comparison to the reference AUC and the number of patients with a rMPE > 20%.

Simulation of dose regimens

The probability of target attainment (PTA) was determined from simulations performed in SIMULX-2021R1 ¹⁷: for both CYP3A5 genotype status (Expressor and Non expressor), 1000 simulations were drawn for different tacrolimus *bid* dose (2.5 mg, 5 mg, 7.5 mg, 10 mg, 15mg

and 20mg) and the PTA was determined for different C₀ targets (between 2.5 and 20 µg.L⁻¹) and for different AUC targets (between 100 and 350 µg. h. L⁻¹). To determine the AUC target in the early phase period, we used the regression equations between the C₀ and the AUC developed by Saint Marcoux et al.¹⁸ even if we acknowledge that the correlation is not perfect (supplemental figure 1).

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY¹⁹ and are permanently archived in the Concise Guide to PHARMACOLOGY 2019/20.^{20,21}

Results:

Patient's characteristics:

Baseline characteristics of the patients, overall and in the development and validation sets are reported in the Table 1. There were no significant differences between the 2 datasets.

Pharmacokinetics model:

Structural model:

A structural pharmacokinetics model with 2 compartments, transit absorption and a linear elimination best described the tacrolimus pharmacokinetics (supplemental Table 1). Based on one of our previous articles in renal transplant patients²², the inter transit-compartment absorption rate (K_{tr}) was fixed at 5.74 h⁻¹ to help the model to converge. The proportional error model was selected as it yielded the lowest BIC (with additive error model BIC: -3842; with proportional error model BIC : -3969).

Missing data:

There was no covariate with more than 20% of missing data (Sex= 4.2%, Age= 6.4%, CYP3A5= 0%, body weight = 8.8% and Hematocrit= 8.5%). The data imputation did not change the variable distributions as presented in supplemental Table 2 and supplemental Figure 2.

Covariate analysis:

The covariate significantly associated with the CL/F was the CYP3A5 status (supplemental Table 3) with a theta of 0.62. The addition on this covariate led to a decrease in the BIC by 19 (from -3969 to -3986).

$$Cl/F = TVCL * e^{\theta_{CYP3A5} \times CYP3A5}$$

θ_{CYP3A5} (= 0.62) is the change in the apparent clearance in CYP3A5 expressors (CYP3A5 = 1 for expressors leading to EXP(0.62) and 0 for CYP3A5 non expressors leading to EXP(0)) ; TVCL - population mean value of apparent clearance; Cl/F - apparent clearance

The scatter plots and boxplots of the association between the covariates and the random effect of CL are presented in supplemental figure 3. Some models had similar BICs but the Relative Standard Error (%) of the kinetics parameters and the imprecision were too high.

The pharmacokinetics parameters of the final model with covariates are presented in Table 2. The diagnostic plots for the final model are presented in Figure 1. There was an excellent correlation ($r=0.98$) between individual predictions and observations. The residuals of individual prediction and time were homogenously distributed around 0.

Model Evaluation

The pcVPC of the final model overlaid with the observations of the development and validation datasets are presented in Figure 2A and 2B. The observed percentiles are in line with the predicted percentiles of the development dataset. In the validation set, even if they did not overlap perfectly, the observations percentiles were within the 95% predicted confident interval.

Bayesian estimation:

In the development dataset, the 0-1-2 hours post dose LSS was associated with the best performances and was selected for the evaluation in the validation dataset (Table 3). This LSS led to good performances: rMPE mean \pm SD = $2.72 \pm 10.17\%$; RMSE=9.9% (best and worst fit are presented in supplemental Figure 4). Bland Altman plot between the reference AUC and

the MAP-BE LSS AUC is presented in Figure 3. No patient had a relative bias out of a +/-20% range.

Probability of target attainment as function of CYP3A5 status:

The results of the PTA for C0 and AUC are presented in Figures 4 and 5, respectively. The target currently used for C0 in the early phase post heart transplantation is between 15-20 $\mu\text{g}\cdot\text{L}^{-1}$ and for the AUC between 270-350 $\mu\text{g}\cdot\text{h}\cdot\text{L}^{-1}$. As these dose proposals are only valid for the starting dose of tacrolimus (afterwards TDM is used), only early targets post transplantation were investigated. Based on the PTA with C0 targets (considering a mean weight in France of 69 kg)²³, a dose of 7.5 mg (0.11 $\text{mg}\cdot\text{kg}^{-1}$) for a patient CYP3A5 non expressor and 20 mg (0.29 $\text{mg}\cdot\text{kg}^{-1}$) for a patient expressing CYP3A5 has to be administered for 85 % of the simulated patients to reach the early phase targets (Figure 4). Similarly, for the AUC targets, a dose of 7.5 mg (0.11 $\text{mg}\cdot\text{kg}^{-1}$) of tacrolimus twice a day in the non expressor group and at least 15 mg (0.22 $\text{mg}\cdot\text{kg}^{-1}$) in the expressor group has to be administered for 85 % of the simulation to reach the early phase targets (Figure 5).

Discussion:

In this work, we developed a parametric POPPK model to describe the pharmacokinetics of tacrolimus after heart transplantation. The final model had two-compartments with transit absorption phase and first order elimination. Transit compartment models have already been used to describe the tacrolimus absorption^{22,24} while other models used a lag time^{12,25,26}. The best LSS found in this work used 3 samples at 0, 1 hour and 2 hours post-dose. We acknowledge that the 0, 1 hour and 3 hours post dosing strategy also yields acceptable and similar results, but we chose to keep the best strategy. Several previous studies which developed LSS for tacrolimus were based on samples drawn at 0, 1 hour and 3 hours (in kidney or liver transplantation)^{22,24,27}. For example, in the Marquet et al. study²⁸ in kidney transplant patients, the authors selected a LSS based on samples drawn at 0, 1 hour and 3 hours post dose but showed that the result with samples at 0, 1 hour and 2 hours post dose were similar. Interestingly, in lung transplant patients a LSS based on 20 min, 2 hours and 4 hours has been previously selected showing that samples at 2 and 4 hours could be of interest in heart and lung transplant patients²⁴.

The performance of the LSS was then evaluated in an external dataset obtained by data splitting and showed accurate performances in line with similar studies in kidney or lung transplantations

(the bias and imprecision of our study are respectively of 2.7% and 9.9% vs. values between 0.1% and 2.4% for the bias or 7.7% and 9.8% for the imprecision in the other studies)^{22,24,29}.

To help the model convergence we fixed the K_{tr} , based on a previous study carried out on renal transplant patients²². Without fixing this parameter, the BIC was significantly increased (BIC = -3974).

A comparison to the PK parameters of Woillard et al. 2011 in renal transplant patients showed that in heart transplant patients, the apparent clearance was slightly decreased (from 0.37 to 0.20 L.h⁻¹.Kg⁻¹) and the central compartment volume was strongly decreased (from 3.66 to 0.22 L.Kg⁻¹)²². Similarly, our apparent clearance is in line with the apparent clearance of other heart transplant patient models (0.27 L.h⁻¹.Kg⁻¹ for Kirubakaran et al.¹, 0.22 L.h⁻¹.Kg⁻¹ Gong et al.² and 0.21 L.h⁻¹.Kg⁻¹ for Han et al.³). The same was observed for our apparent central volume of distribution which was also strongly decreased compared to the heart transplant model (2.55 L.Kg⁻¹ for Kirubakaran et al.¹, 11.52 L.Kg⁻¹ for Gong et al.² and 12.17 L.Kg⁻¹ for Han et al.³). In lung and heart transplant patients, Sikma et al.¹¹ observed an apparent central volume of distribution around 2.99 L.Kg⁻¹ which is also far from our value. However, even if most of the reported values for the tacrolimus apparent central volume of distribution are between 2.55 L.Kg⁻¹ & 12.30 L.Kg⁻¹^{1-3,11,30} some studies in other organ transplant populations also reported small central compartment volume values³¹⁻³³. It should be noted that the shrinkage value of the mean transit time is high (140%) but this was not a problem as we are only interested by the apparent clearance value.

In our final model, the CYP3A5 polymorphism was selected based on the tacrolimus apparent clearance. The CYP3A5 polymorphism effect is largely known and has been previously described⁵. This polymorphism leads to an alternative splice site in the pre-mRNA and results in a truncated enzyme³⁴. Individuals that carry at least one *CYP3A5*1* allele are considered to be CYP3A5 expressors (including *CYP3A5*1/*3* and *CYP3A5*1/*1*). These patients require a higher starting dose compared with *CYP3A5*3/*3* carriers to reach the predefined target exposure early after transplantation³⁵. The hematocrit has been shown to influence the apparent clearance in renal transplant patients²² and in heart transplant patients¹, but its effect was not found to be significant in the present study, even if the range of the hematocrit values was quite large (between 26 and 47 %).

The inter-occasion variability was investigated as a categorical variable because the model was struggling to converge with the standard way to include it (estimation of IOV in addition to inter-patient variability). We had thus to hypothesize that all the PK profiles were independent, which is acceptable given the fact that the lag between the two occasions was at least two weeks.

Finally, we did not observe an effect of the categorised time post transplantation on the PK parameters.

For the PTA of C₀, as expected, the expression of the CYP3A5 increased the apparent clearance of tacrolimus and higher doses were required to attain the targets compared to the non expressor group. To illustrate this effect, for a C₀ target =15 µg.L⁻¹, a dose of 7.5 mg would lead to 80% of patients reaching the target in the non expressor group whilst only 40% would reach the target in the expressor group. In the latter group, a dose of 20 mg would be required to reach the same target. Similarly for AUC, for an AUC target = 270 µg.h.L⁻¹, a dose of 7.5 mg would lead in the non expressor group to a probability of target attainment of 85%. In the expressor group, this would lead only to 60%; the optimal dose being 15mg. Since the AUC is theoretically the best marker of the tacrolimus exposure⁵, the observed differences between the doses proposal based on the C₀ or the AUC were expected. The comparison of the dose proposals between our study and the study of Woillard et al. ⁹ performed on renal transplant patients showed some differences as their PTA showed that a dose of 7.5 mg in the non expressor group and 15 mg in the expressor group was required to attain a target of C₀= 10µg.L⁻¹. The differences with the present study are most likely due to the different type of transplantation. However, the magnitude of the differences between expressors and non expressors remains similar. We chose to compare our dose proposal based on our AUC PTA to the summary of product characteristics of the tacrolimus and some differences were also observed. Indeed, the dose recommendation is 0.075 mg.kg⁻¹.day⁻¹ ³⁶ to attain a AUC target of 270 µg.h.L⁻¹ and we proposed in this study a dose of 0.11mg.kg⁻¹.12h⁻¹ for a non expressor patient and a dose of 0.22 mg.kg⁻¹.12h⁻¹ for an expressor patient which represents a large increase. Obviously, these proposals need to be considered only for the starting dose as after that, TDM must be used to provide dose individualisation.

However, this study has some limitations. First, it was performed with the bid formulation of tacrolimus as the once daily formulation was not available at that time. However, nowadays, the once a day formulation is also used in this population. The model developed in this article is limited to the bid formulation. Moreover, despite the fact that this study is multicentric, (seven French hospitals participated) and the data were collected under the same treatment protocol allowing generalisability of the results on a French and by extension European scale, the model is unlikely to be generalizable to other heart transplant patient populations (Chinese, American etc...). Furthermore, in our study, some PK profiles had required extrapolation of the AUC using a monoexponential decay. Before doing this extrapolation, we determined that the elimination was linear between 9-12 hours post dose. However, this extrapolation could slightly

impact the trapezoidal rule AUC. Also, to determine the AUC target, we used the relation between the C₀ and the AUC calculated on a kidney transplant population and their correlation coefficient was only about 0.7. Finally, to complete all kinetic profiles, some missing covariates were imputed using the K-nearest neighbours' method which imputes data based on the most similar patients. This approach is quite innovative and avoids imputing the missing data based on the population median as usually done. It is of note that a model for tacrolimus in heart transplant recipients has already been implemented in the ISBA website (<https://pharmacochu-limoges.fr>). This model was developed using an Iterative Two Stage Bayesian approach in which the absorption phase was described using a double gamma distribution. Our article presents a Nonlinear Mixed Effects Models approach allowing to better evaluate the inter-individual variability and additionally provides recommendations for the starting dose.

Conclusions:

In this work, we developed a POPPK model and derived a MAP-BE based on a LSS for tacrolimus in heart transplant patients that can be used for TDM based on the AUC in routine practice. We also proposed optimal first doses recommendations to reach predefined C₀ or AUC depending on the CYP3A5 status.

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Conflict's interest

The authors have no competing interests to declare in relation to this work.

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Data availability

The data that support the findings of this study are available, on reasonable request, from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author contribution

AP, AD, JBW performed the analysis and wrote the paper, PM, CM and ML conceived the analysis, organized and collected data

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Table 1: The characteristics of heart transplant patients receiving tacrolimus (P1= 7-14 days, P2 = 1 month, P3 = 3 month, P4= 1 year).

Variable	Overall Patient: 18 Pk profiles: 47	Development Patient: 11 Pk profiles: 38	Validation Patient:7 Pk profiles : 9	p-value
Age (years)	44 [22 – 63]	45 [22 - 63]	37 [22-57]	0.177
Sex (male/female)	39 (83%) / 8 (17%)	31 (82%) / 7 (18%)	8 (89%) / 1 (11%)	0.542
Weight	66 [34 - 93]	67 [36 - 93]	63 [34 - 83]	0.444
Hematocrit (%)	36.5 [25.8 – 47.0]	35.5 [25.8 – 47.0]	37.8 [32.0-45.0]	0.341
Tacrolimus dose (mg twice a day)	3.0 [0.5 – 8.5]	3.0 [0.5 – 8.5]	3.5 [1.0-8.0]	0.683
CYP3A5 status* (expressor/ non expressor)	10 (21%)/ 37 (79%)	7 (18%) / 31 (82%)	3 (33%)/ 6 (73%)	0.437
Post transplantation delay (P1/P2/P3/P4)	11 (23%)/ 11(23%)/ 11(23%)/ 14 (30%)	8(21%)/ 10(26%)/ 8(21%)/ 12(36%)	3(30%)/1(10%)/ 3(30%)/ 2(30%)	0.948

Continue covariate: mean[range]; Categorical covariate: n (%). P value was obtained using the Wilcoxon test. Descriptions for each PK profile were considered as independent *Number of patients in the overall population: expressor = 5 / Non expressor = 13

Table 2: Tacrolimus pharmacokinetics parameters in heart transplant patients of the structural and the final models. The final model in the development set was the one used for the development of the BE

Parameters	Base model in development set		Final model in development set (Model used for the BE)		Final model in all patients		BE model in validation set
	Estimate	RSE (%)	estimate	RSE (%)	estimate	RSE (%)	Shrinkage (%)
Ktr (h-1)	5.74 (fixed)	-	5.74		5.74		
Mtt (h)	0.50	11.39	0.48	11.46	0.57	13.16	-
Ka	1.04	8.41	0.84	14.39	0.99	20.27	-
Cl/F (L. h-1)	15.48	10.94	13.87	10.89	13.51	9.57	-
V1/F (L)	21.17	34.43	19.35	34.02	19.02	56.99	-
Q/F (L.h-1)	78.30	11.22	65.30	11.90	69.59	13.94	-
V2/F (L)	498.80	16.68	540.65	14.49	453.25	13.02	-
IIV Mtt %CV	59	16.02	57	16.21	84	12.22	- 140
IIV Ka %CV	16	49.67	53	21.71	53	35.80	11.6
IIV Cl %CV	73	12.74	67	13.29	67	13.46	-2.25
IIV V1 %CV	251	17.59	156	22.64	243	27.86	-13.6
IIV Q %CV	59	16.44	70	15.37	77	17.99	6.82
IIV V2 %CV	96	16.19	58	19.76	58	23.04	- 26.9
Beta Cl CYP3A5	-	-	0.62	27.21	0.74	19.35	-
Proportional error %CV	12	4.30	12	4.26	12	3.96	-

: BE- Bayesian estimator; MTT - mean transit time; Ka- absorption rate constant; Ktr – transit absorption rate constant; Cl - apparent clearance; Q - inter-compartmental apparent clearance; V1 – apparent central volume of distribution; F - oral bioavailability; V2- apparent peripheral volume of distribution; IIV - inter individual variability. CV =

$$\sqrt{(e^{sd^2} - 1)} \quad ^{37}(\text{CV} - \text{Coefficient of variation; SD} - \text{Standard deviation})$$

Table 3: LSS comparison in the development and in the validation set

	LSS	RMSE (%)	Relative Mean bias (%)	Patient with bias >+/- 20%
Development set	C0.C1.C2	13.3	5.7	5
	C0.C1.C3	14.1	8.0	6
	C0.C1.C4	14.4	8.8	5
	C0.C2.C4	17.5	9.3	10
Validation Set	C0.C1.C2	9.9	2.7	0

: LSS - Limited sampling strategy; RMSE- Root mean square error

Figure legends

Figure 1: Diagnostic plots for the final model of individual (A) or population predictions (B) versus observed tacrolimus concentrations ($\mu\text{g.L}^{-1}$) and weighted residuals as function of time (h) (C) or individual prediction (D) in the development population

Figure 2: pcVPC of the final model overlaid with the observation ($\mu\text{g.L}^{-1}$) of the development (A) and validation (B) dataset versus time (h). (area = prediction interval; dotted = predicted percentiles; line = empirical percentiles, red line = median concentration, blue line = lower and upper concentration; black dots = observations)

Figure 3: Bland-Altman plot between the reference and the Maximum a posteriori Bayesian estimation Limited sampling strategy area under the curve in the validation dataset

Figure 4: Proportions of simulated patients achieving different tacrolimus bid C₀ (Trough concentration) targets for different dosage regimens and depending on the CYP3A5 status.

Figure 5: Proportions of simulated patients achieving different tacrolimus bid AUC (Area under the curve) targets for different dosage regimens and depending on the CYP3A5 status.