

Ciclosporin population pharmacokinetics and Bayesian estimation in thoracic transplant recipients

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ABSTRACT

Background and objectives: Therapeutic drug monitoring of ciclosporin has been recognized as an essential tool in the management of allograft transplant recipients, as it could help improve their outcome. However, there is still no consensus about the optimal method for monitoring ciclosporin after thoracic transplantation. Better knowledge of the pharmacokinetics of ciclosporin in thoracic transplant patients and design of tools dedicated to ciclosporin monitoring could help its practice and its outcome in this population of patients. The aims of this study were: (i) to investigate the population pharmacokinetics of ciclosporin in thoracic (heart or lung) transplant patients, and study the influence of a range of potential covariates, including demographic, clinical and genetic factors, on pharmacokinetic parameters; and (ii) to develop a Bayesian estimator able to predict the individual pharmacokinetic parameters and exposures indices in this population of patients.

Methods: The analysis was performed with 187 full pharmacokinetic profiles obtained in 57 lung and 19 heart transplant patients within the first year post-transplantation. A population pharmacokinetic model was developed by nonlinear mixed effect modeling using NONMEM (version 7.1) from an index dataset (118 profiles). On the basis of this population model and a limited number of blood samples, a Bayesian estimator able to determine ciclosporin area under the blood concentration-time curve during a dosage interval was built and evaluated in the validation dataset (69 profiles).

Results: Ciclosporin pharmacokinetics was described using a two-compartment model with time-lagged first order absorption and first order elimination. The final population model included sex as a covariate: ciclosporin apparent oral clearance was on average 37% faster in male than in female patients (34.8 vs 25.4 L/h, $p < 0.001$). Good predictive performance of the Bayesian estimator was obtained using three blood concentrations measured at 40

minutes, 2 and 4 hours post-dose, with a non-significant bias of -5% between the estimated and the reference trapezoidal area under the curve and a good precision (relative mean square error=13%).

Conclusion: Ciclosporin population pharmacokinetic analysis in thoracic transplant patients (including patients with cystic fibrosis) showed a significant influence of sex on apparent clearance. The Bayesian estimator developed in this study yielded accurate prediction of ciclosporin exposure in this population throughout the first year post-transplantation. This tool may allow routine ciclosporin dose individualization.

INTRODUCTION

Ciclosporin, a member of the calcineurin inhibitors, is a potent immunosuppressant, which has long been used following solid organ transplantation and has become an essential component of standard treatment after heart or lung transplantation.

To optimize the pharmacological response of ciclosporin (i.e. minimizing side effects without increasing the risk of rejection) therapeutic drug monitoring (TDM) is mandatory. Indeed, Ciclosporin microemulsion (NEORAL®, Novartis, Basel, Switzerland) is characterized by a narrow therapeutic index, a large inter-individual variability of its pharmacokinetics and a poor correlation between blood ciclosporin concentrations and the given dose ^[1]. However, there is still no consensus about the optimal method for ciclosporin monitoring, and very few data are available on the practices and outcomes of ciclosporin monitoring after thoracic transplantation ^[2].

Currently, the most common practice consists in adjusting the administered dose using as exposure indices the trough concentration (C_{trough}) or the concentration measured 2 hours after dosing (C_2). However, the inter-dose area under the blood concentration-time curve

(AUC_τ), representing the global exposure to the drug, is probably the index most closely linked to the therapeutic as well as toxic effect of ciclosporin. At least in renal transplantation, AUC_τ has been advocated as the most informative and most relevant index of drug exposure ^[2-4]. Ciclosporin AUC_τ can be evaluated using different approaches. For ciclosporin monitoring in thoracic transplant recipients, few studies reported sparse sampling strategies in which algorithms provided AUC_τ estimation using a limited number of blood samples collected at precisely defined times ^[5-8]. In a critical analysis of these sparse sampling strategies, Monchaud et al. ^[2] reported that only one seemed to be clinically applicable in heart transplantation ^[5], whereas none were applicable to lung transplant patients. In parallel, maximum *a posteriori* probability Bayesian estimators, characterized by their flexibility with respect to sampling times and their ability to estimate simultaneously ciclosporin pharmacokinetic parameters and exposure indices, have been proposed in heart ^[9-11] and lung ^[12] transplantation. However, none were based on a population pharmacokinetic analysis performed in thoracic transplant patients. One of these Bayesian estimators was originally designed for renal transplant recipients and further used to calculate ciclosporin AUC_τ in heart transplant recipients, but it could not be validated in this second population ^[10]. Another one was developed based on parameters selected from previously published pharmacokinetic study in stable heart transplant recipients ^[13]. The others were developed based on population pharmacokinetic parameters estimated using the iterative two-stage method ^[9;12], and are limited to one type of graft and certain conditions.

Ciclosporin is mainly metabolized by the cytochrome P450 (CYP) isoenzymes CYP3A4 and CYP3A5 (expressed in the liver and the intestine mucosa) and is also a substrate of the efflux transporter P-glycoprotein (encoded by the *ABCB1* gene). Polymorphisms in the genes of

these proteins could have an impact on the pharmacokinetics of ciclosporin. Several investigations about the association between these SNPs and the pharmacokinetics of ciclosporin have been performed, yielding conflicting results ^[14].

Population pharmacokinetic studies can be used to identify and quantify the influence of demographic, clinical and genetic factors on drug pharmacokinetics and population models can be used further as *a priori* information for Bayesian forecasting. Several ciclosporin population pharmacokinetic studies have been reported in patients with solid-organ transplantation ^[15-21], but to our knowledge few have been published in heart ^[22;23], lung or heart-lung transplant recipients ^[24]. Given the potential pharmacokinetic differences between populations, the pharmacokinetics of immunosuppressants may be different in thoracic and liver or kidney transplant recipients, and thus deserve to be specifically studied.

The aims of the present study were: (i) to develop a population pharmacokinetic model for ciclosporin in adult heart and lung transplant recipients; and (ii) to develop a Bayesian estimator able to estimate ciclosporin 12-hour area under the blood concentration-time curve (AUC_{12}) exposure during a dosage interval using a limited sampling strategy.

METHODS

Patients and data collection

This study was part of the main goal of two multicenter pharmacokinetic trials intended to develop population pharmacokinetic models and Bayesian estimators for optimized dose adjustment of immunosuppressive drugs in thoracic transplant patients. Both these trials complied with legal requirements and the declaration of Helsinki, amended in Tokyo. They were approved by the Limousin regional ethic committee and authorized by the French Drug Agency (PIGREC, EudraCT number N° 2006-006832-23; STIMMUGREP not subject to registration in the EudraCT database). PIGREC was also registered within ClinicalTrials.gov (Identifier NCT00812786). All the patients included gave their written informed consent. The first study (PIGREC) enrolled heart transplant patients while the second one (STIMMUGREP) enrolled lung transplant patients with or without cystic fibrosis (CF). In these two observational pharmacokinetic studies, the choice of the immunosuppressive strategy was at the discretion of the investigators. The maintenance immunosuppressive regimen typically consisted of the association of several immunosuppressants (calcineurin inhibitor, i.e. ciclosporin or tacrolimus, and/or antimetabolite, i.e. azathioprine or mycophenolate mofetil, and/or mTOR inhibitor, i.e. everolimus) and oral corticosteroids. The patients were followed-up until the end of the first year post-transplantation. Full pharmacokinetic profiles were collected from each patient at one or more of the following post-transplantation periods: between day 7 and day 14, month 1, 3 and 12. Blood samples were collected at the following time-points: predose, 0.33, 0.67, 1, 1.5, 2, 3, 4, 6, 8, 10, and 12 hours (except for months 3 and 12 in cardiac transplant recipients), after the morning dose of the immunosuppressive drugs.

In the present pharmacokinetic study, only the profiles collected from patients receiving ciclosporin were analyzed.

The lung transplant recipients had orally received a combination of ciclosporin (Neoral®, soft gelatine capsules) twice daily, an antimetabolite (either azathioprine or mycophenolate mofetil) and a corticosteroid. The heart transplant recipients had orally received a combination of ciclosporin (Neoral®, soft gelatine capsules) corticosteroid and either mycophenolate mofetil or everolimus. No standardized target was defined for the purpose of these observational studies. For all the patients, ciclosporin dose adjustment was performed in each center in accordance with local practice, classically on the basis of morning trough blood concentrations (C_{trough}). For example, in heart transplant patients, ciclosporin doses were typically adjusted on the basis of C_{trough} to reach 170 to 230 ng/mL for the first month and around 150 ng/mL thereafter for the first year. Corticosteroids were administered according to the standard administration regimen of each center. Classically high doses were administered at the time of transplantation, close to 2mg/kg/day at day one and progressively reduced over the first six months to achieve a maintenance dose (of approximately 5 mg) which is administered thereafter. Concurrent medications known to interfere with ciclosporin pharmacokinetics could potentially be administered.

Ciclosporin assay

Ciclosporin determination was performed in whole blood using a turbulent-flow chromatography – tandem mass spectrometry technique whose system configuration, parameters, and analytical process were previously described in detail ^[25;26]. Briefly, online extraction was performed at 1.25 mL/min on a Cyclone P[®], 50- μ m particle size (50 x 0.5 mm, i.d.) column (Thermo Fisher, Les Ulis, France) in alkaline conditions. Chromatographic separation was performed in acidic conditions (phase A: 0.1 % formic acid in water and

phase B: 0.1 % formic acid in methanol) using a Propel MS C₁₈, 5-μm (50 x 3.0 mm, i.d.) column (Thermo Fisher) kept at 60°C with a constant flow-rate of 300 μL/min.

Detection was performed using a TSQ Quantum Discovery tandem mass spectrometric system equipped with an orthogonal electrospray ionization source and controlled by the XCalibur software (Thermo Fisher). Tandem mass spectrometry detection was performed in the positive ion, multiple reaction monitoring mode following three transitions for ciclosporin (m/z 1220.0→1203.0 for quantification and m/z 1220.0→1185.0 and m/z 1220.0→425.0 for confirmation) and two transitions (m/z 1234.0→1217.0 for quantification and m/z 1234.0→119.0 for confirmation) for its analogue ciclosporin D, used as internal standard. To 100 μL of whole blood were added 200 μL of a methanol/aqueous zinc sulfate (70:30 v/v) containing the internal standard at 25 μg/L. The mixture was vortex-mixed for 30 s, centrifuged at 13000 rpm and the supernatant was introduced into a 200 μL-vial for injection. Calibration standards at 0, 10, 20, 50, 100, 200, 500, 1000, 2000 μg/L were prepared by spiking blank whole blood with ciclosporin. The lower limits of detection and quantification (LLQ) were 10 μg/L and 20 μg/L and calibration curves obtained using quadratic regression from the LLQ to 2000 μg/L yielded $r^2 > 0.998$. Inter-assay precision and accuracy were assessed by analyzing the MassCheck[®] Immunosuppressants Whole Blood Controls (Chromsystems Instruments & Chemicals GmbH, München, Germany) at 4 levels on 5 independent days, intra-assay precision and accuracy by analyzing 5 replicates of the 4 levels on the same day. The method showed good inter-assay precision and accuracy with relative standard deviation values (RSD) from -3.1 to 11.8% and mean relative error (MRE) from 4.0 to 11.7 %, as well as good intra-assay precision and accuracy with RSD from -1.1 to 9.1% and MRE from 1.8 to 6.4%.

Genotyping

Patients' genotypes were characterized for *CYP3A5* rs776746A>G (*CYP3A5**3 allele), *ABCB1* c.1236 C>T (rs1128503), c.2677 G>T (rs2032582) c.3435C>T (rs1045642) and *CYP3A4* intron rs 35599367C>T (*CYP3A4**22 allele) single-nucleotide polymorphisms (SNP), using validated TaqMan allelic discrimination assays on an ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Courtaboeuf, France). Linkage disequilibrium between *ABCB1* SNPs and patients most probable haplotype for this gene were determined using the PHASE V2.0 program ^[27]. Eight *ABCB1* haplotypes were identified. Patients were classified on the basis of the presence of *ABCB1* variant (TTT) haplotype (non-carrier, heterozygous and homozygous carrier).

Pharmacokinetic Analysis

The distribution of population parameters was studied using the nonlinear mixed effects model approach as implemented in NONMEM version 7.1 (ICON Development Solutions, Hanover, MD, USA) executed using Wings for NONMEM version 703 (developed by N. Holford, Auckland, New Zealand, available from <http://wfn.sourceforge.net>). All population pharmacokinetic analyses were done using the first-order conditional estimation (FOCE) method.

The whole dataset was randomly divided into two groups: an index group made up of 49 patients (118 pharmacokinetic profiles) was used to develop the population pharmacokinetic model and a validation group made up of 27 other patients (69 pharmacokinetic profiles) was used to evaluate the predictive performance of this population model.

Covariate-free model

A 2-compartment open model fitted the elimination phase, while two different approaches were tested to describe the absorption phase: (i) a first-order input with or

without a time-lag parameter; and (ii) a transit compartment model based on an Erlang distribution, which is a particular case of the gamma distribution used previously for ciclosporin modeling in kidney transplantation ^[28]. In order to discriminate between these nested absorption models, the coding used for the comparison was based on ADVAN5.

Both interindividual variability (IIV) and inter-occasion variability (IOV) were described by exponential error models (see equation below) and tested for each parameter:

$$P_i = \theta_i \times \exp(\eta_i + \eta_{ik})$$

where P_i is the individual value of the parameter, θ_i is the typical parameter value in the population, η_i is the IIV and η_{ik} is the IOV.

Diagonal matrix and full variance/covariance matrix was successively tested to estimate inter-patients random variabilities. Additive, proportional and combined (i.e. additive and proportional) error models were tested for the residual variance.

The population model was built stepwise. The objective function value (OFV) provided by NONMEM[®], was used to compare nested models. Two nested models were considered significantly different from each other when the difference in OFV was larger than the critical value from a chi-squared distribution with degrees of freedom equal to the difference in the number of estimated parameters. A decrease of the OFV > 6.64 units shows a significant improvement of $p < 0.01$ for a nested model with one more degree of freedom. Model adequacy was evaluated using diagnostic plots.

Screening of covariates

The screening and selection of covariates were performed following a classic stepwise approach ^[29]. In the first step, a covariate-free population pharmacokinetic model was computed. Then, we graphically investigated the influence of covariates on the individual values of apparent clearance (CL/F), apparent volume of the central compartment after oral

administration (V_1/F) and absorption parameter. The continuous covariates evaluated in this study were age (years), bodyweight (kg), serum creatinine ($\mu\text{mol/L}$), hemoglobin (g/dL) and hematocrit (%). The categorical covariates were the type of graft, cystic fibrosis status (with or without CF), post-transplantation period, sex, co-administered immunosuppressant and *CYP3A5**3, *CYP3A4**22, *ABCB1* (c.1236C>T, c.2677G>T and c.3435C>T) genetic polymorphisms. The *ABCB1* haplotype was also considered as a potential covariate.

Each of the covariates of interest was introduced individually into the structural model to evaluate its relative impact on the individual estimates of CL/F, V_1/F and the absorption parameter.

The final model was developed following a forward inclusion and backward elimination procedure. A covariate was kept in the final population pharmacokinetic model when its removal resulted in an increase of at least 10.83 in the objective function ($p < 0.001$, 1 degree of freedom). The clinical relevance of the covariates was also appraised, taking into account the improvement of parameter estimation precision, the reduction in IIV, IOV, and residual variability. Furthermore, the difference between two models was evaluated using a visual predictive check.

Model Evaluation

The accuracy and robustness of the final population model were assessed by a bootstrap method. Briefly, 1000 bootstrap sets were obtained by resampling from the original dataset, each providing population pharmacokinetic parameter estimates. The median and 95% confidence interval values of each pharmacokinetic parameter estimated from the 1000 bootstrap sets were compared to the corresponding mean population values obtained with the original dataset. This procedure was performed using Wings for NONMEM.

The adequacy of the final population model was evaluated using a Visual Predictive check (VPC) performed using Rfn^[30] (link on <http://sourceforge.net>) via the R program^[31]. A total of 1000 datasets were simulated from the final model using the original dataset. Plots of the median and 90% confidence interval of the simulated concentration *versus* time profiles were generated, potentially stratified by relevant covariates, to check whether the distribution of the observed concentration-time profiles was reasonably contained within the confidence interval of the simulated profiles. As the ciclosporin dose was different in each patient, and its pharmacokinetics is linear, the prediction and the observation presented on VPC were dose-normalized on the basis of the mean dose administered in the index group.

Building of a Bayesian Estimator

The population parameters obtained from the index group were used as priors to compute the individual pharmacokinetic parameters in the patients of the validation group using Bayesian forecasting.

The best limited sampling strategy was selected using the D-optimality criterion implemented in the ADAPT-II® program^[32], on the basis of a combination of a maximum of two or three sampling times. The performance of the Bayesian estimator was evaluated by computation of the mean prediction error (as measure of bias) and root mean squared prediction error (RMSE, as measure of precision) of AUC₁₂ Bayesian estimates with respect to the reference values obtained with the linear trapezoidal method applied to the full profiles^[33].

All statistical analyses were performed in R^[31]. Comparisons of continuous variables were performed using the Mann-Whitney test while comparisons of categorical variables were

performed using the χ^2 -test, or the exact Fisher test when the number of patients per category was too small. Two-sided tests were used.

RESULTS

187 full pharmacokinetic profiles were obtained from 76 patients (19 heart transplant patients and 57 lung transplant patients, including 16 patients with cystic fibrosis). The main demographic, biological and pharmacogenetic characteristics of the patients enrolled are reported in Table I. Information on serum creatinine, hematocrit and hemoglobin was not available for 6 observed profiles (obtained from 5 different patients). These missing covariates were replaced by the values of the covariate observed at the closest sampling period for the same patient, or if any, by their median in the corresponding dataset.

Genotyping results were, for each SNP, consistent with the Hardy-Weinberg equilibrium.

Population pharmacokinetic modeling

A two-compartment model with time-lagged first-order absorption best described the concentration data. The pharmacokinetic model was characterized by six parameters: k_a (absorption rate constant, h^{-1}), t_{lag} (lag time, h), V_1/F (apparent volume of the central compartment, L), Q/F (intercompartmental clearance after oral administration, L/h), V_2/F (apparent volume of the peripheral compartment, L) and CL/F (apparent oral clearance, L/h). The typical value for bioavailability (F) of ciclosporin was fixed at 100%. Introduction of IIV on F , k_a , Q/F , V_1/F , CL/F and t_{lag} and of IOV on k_a , V_1/F and CL/F significantly improved the fit of the model. The residual error was described using a model combining additive and proportional parts.

Univariate analysis showed that the following eight covariates led to a significant decrease of the objective function value: CF status on F and CL/F, type of transplantation on CL/F, sex on V₁/F and CL/F, bodyweight on CL/F, *CYP3A5* genotype on CL/F and *ABCB1* haplotype on CL/F. However, the type of transplantation was not considered as relevant because it systematically led to a significant increase of the IIV on CL/F and did not decrease the residual error. This covariate was not retained for the multivariate analysis.

As a result of the forward inclusion and backward elimination procedure, only one covariate resulted in a significant association: sex with CL/F. Inclusion of this covariate resulted in a decrease in the IIV and the IOV on CL/F (from 18.7 to 13.8% and from 31.5 to 27.2%, respectively), as compared to the covariate-free model. The final model was characterized by a residual error of 25.8% for the proportional part and 12.4 ng/mL for the additive part (i.e. lower than the limit of quantification of the analytical method, LLQ=20 ng/mL). The population pharmacokinetic parameters obtained with this final model are reported in table II.

The goodness-of-fit plots in the final model are shown in figure 1. The plots of predicted and individually predicted versus observed concentrations showed no structural bias. Conditional weighted residuals (CWRES) were equally distributed regardless of the predicted concentrations and over time suggesting no bias in the model predictions.

The median parameter estimates obtained from the bootstrap process (1000 runs) were similar to the estimates obtained with the original dataset and the confidence intervals were reasonably narrow and did not include zero (table II).

The results of the VPCs stratified on sex and based on dose-normalized concentrations are presented in figure 2. They show good agreement between the prediction obtained from

1000 simulations and the observations in male and in female patients. Although, observations were within the 90% confidence interval, they were not distributed symmetrically relative to the median prediction in males ($p < 0.05$), showing the tendency of the model to underestimate the concentrations in these patients.

Design of a Bayesian estimator

The optimal limited-sampling schedule based on three time-points was 40 min, 2h and 4h. This sampling schedule was tested in the validation dataset, with patient characteristics similar to the index group (Table I). In this validation dataset, two pharmacokinetic profiles did not contain enough information on the selected times to reliably estimate AUC_{12} using the trapezoidal and Bayesian methods. Thus, the performance of the Bayesian estimator was evaluated in 67 PK profiles. The comparison between Bayesian AUC estimates and reference AUC values led to a non-significant mean relative bias of -5.0% (from -29.7 to 42.6%; $p = 0.282$), an acceptable precision (RMSE=13%) and a determination coefficient value (r^2) of 0.905. The bias on AUC_{12} was larger than $\pm 25\%$ in 2 profiles (i.e. 2.9% of the profiles). No difference in bias was observed when comparing male versus female patients or when comparing patients with versus patients without CF.

DISCUSSION

In this study, a population pharmacokinetic model has been developed for ciclosporin in heart and lung (with or without CF) transplant recipients, including sex as the only factor influencing the drug pharmacokinetics. Based on this population model, a Bayesian estimator for predicting ciclosporin exposure in heart and lung transplant patients using only 3 blood samples collected 40min, 2h and 4 h after dosing was built and validated.

Concentration profiles were described using a classical two-compartment model with time-lagged first-order absorption. The Erlang absorption model, previously used for ciclosporin

[16;28;34] being more appropriate for a drug with such a highly variable absorption time, did not improve the fit of the present data significantly. Several significant covariates were identified by univariate analysis, but multivariate analysis led us to retain only the influence of sex on ciclosporin oral CL/F, as it significantly decreased its IIV and IOV and provided the lowest OFV. In the final population model including this covariate, the apparent clearance of ciclosporin was 37% higher in male patients indicates that men could require larger ciclosporin doses than do women. No clear relationship between sex and CL/F had been previously established for ciclosporin. A sex-dependent racial difference in the disposition of ciclosporin was reported in a small number of healthy patients [35] and *in vitro* studies have suggested that women clear CYP3A4 substrates more rapidly than men do, which may result in a higher apparent volume of distribution and CL/F [36], in apparent contradiction with the present results. The influence of this covariate needs to be confirmed as the observed effect could be due to confounding factors. Indeed, when tested individually, several covariates including bodyweight influenced significantly the CL/F of ciclosporin. Sex was finally retained in the final model because it appeared to be one of the most influential covariate by the multivariate analysis and because its introduction into the model led to the best predictive performance of the Bayesian estimation.

In our univariate analysis, the bioavailability of ciclosporin was on average 30% lower in patients with CF. This difference has been previously reported [37] and could be attributed to gastrointestinal disorders and malabsorption of lipids due to pancreatic insufficiency. Although the incorporation of this covariate into the sex-dependent model was statistically significant and markedly reduced IIV on bioavailability, CF status was not retained at the end of the multivariate analysis. Indeed, the performances of the two population pharmacokinetic models (including CF status and sex or sex only) were quite equivalent.

Moreover, Bayesian estimation, when tested in the validation dataset on the basis of the population pharmacokinetic model including the two covariates, did not provide accurate prediction of ciclosporin exposure, in particular in patients with CF for whom very large biases were obtained.

Associations observed between *CYP3A5* 6986A>G and CL/F and between *ABCB1* haplotype and CL/F were statistically significant in univariate analysis only, but not in the multivariate analysis. An effect of *ABCB1* (so-called *MDR-1*) haplotype on ciclosporin pharmacokinetics was described in Asian heart ^[38] and Chinese kidney ^[18] transplant recipients, whereas other studies found no such correlation ^[19;39;40]. The present study confirms that the contribution of *ABCB1* genetic variability to interindividual differences in ciclosporin pharmacokinetics is either null or weak.

In the present population model, the confidence intervals evaluated by bootstrap analysis were reasonably narrow. The CL/F estimates was similar to values reported in lung and heart-lung transplant recipients (22.1 [range 19.5-24.7] L/h) ^[24], as well as in a population including kidney and heart transplant recipient (30.5 L/h on average) ^[19]. Moreover, the non-explained interindividual and inter-occasion variabilities on this parameter were relatively low (13.8% and 27.2%, respectively). The apparent clearance constitutes the most relevant pharmacokinetic parameter as it is crucial for the estimation of the individual exposure (i.e. AUC).

On the basis of this final population model and consistent with our experience with other immunosuppressive drugs, a Bayesian estimator was developed for the prediction of ciclosporin AUC₁₂ using three concentration-time points compatible with clinical practice for therapeutic drug monitoring: 40 min, 2 hours and 4 hours post-dose. This Bayesian estimator provided good predictions of AUC₁₂ in heart and lung (with and without CF) transplant

recipients. The predictive performance of the Bayesian estimator was validated in patients of the validation group with an acceptable bias between estimated and observed AUC_{12} (mean -5.0%, range -29.7% to +42.6%; $p=0.282$) and a very satisfactory estimation precision (RMSE 13%). The two extreme values of bias were observed for two profiles obtained in early post-transplant period (between day 7 and day 14, i.e. period during which the profiles can be erratic) from lung transplant patients with hardly any common characteristic (-29.7% and +42.6% for a woman with CF and a man without CF, respectively).

Whereas many studies in thoracic transplantation having put in evidence a poor correlation between the C_{trough} and ciclosporin exposure ^[2], C_{trough} and C_2 were moderately correlated with AUC_{12} ($r^2 = 0.5785$ and 0.6356 for C_{trough} and C_2 , respectively) in this study. It suggests that approximately 60% of the variability in AUC_{12} is explained by either the C_{trough} or C_2 level in this patient group, while the other 40% are unexplained. This is a classic situation for immunosuppressive drugs, which results in the fact that a large range of AUC values can still be observed among patients with the same C_2 or C_{trough} level. Herein, for similar C_{trough} or C_2 values, AUC_{12} values varying in a 1 to 2 ratio were observed. The figure 3 illustrates this variability. For example, in two lung transplant patients (without CF), the two C_{trough} were very close (187.38 $\mu\text{g/L}$ and 187.25 $\mu\text{g/L}$) while a variability of AUC_{12} was observed (i.e. 3.47 $\mu\text{g.h/L}$ and 5.29 $\mu\text{g.h/L}$, respectively). Whereas monitoring on the basis of the single concentration would induce a same recommendation of dose for the two profiles, AUC_{12} monitoring would inevitably lead to different dose recommendations. Variability in C_{trough}/AUC_{12} and C_2/AUC_{12} relationships observed in the first year after a lung or heart transplantation raised a doubt about the accuracy of monitoring based on the single concentration values in certain patients. For instance, flat profiles and delayed absorption were reported in transplant patients with CF, for whom AUC monitoring could probably be

more safety. Only a prospective trial compared ciclosporin dose adjustment based on the full AUC and the C_{trough} or C_2 could help to propose optimal method for monitoring ciclosporin in thoracic transplant recipients.

To the best of our knowledge, this is the first Bayesian estimator able to predict ciclosporin exposure as well in heart as in lung transplant patients with cystic fibrosis or not.

The previous Bayesian estimators available in thoracic transplantation were designed for patients with characteristics different from ours. The Bayesian estimators reported by Rousseau et al. ^[12] were developed and validated in stable lung transplant patients (i.e. patients with no evidence of acute rejection episode within the previous 3 months) whereas ours is dedicated to the lung transplant patients over the first year post-transplantation and it is known that the PK of CsA vary over the first months following transplantation. Ray et al. ^[11] proposed a Bayesian estimator for stable (>12 months post-transplantation) heart transplant recipients only. Solari et al. ^[10] reported the estimation of ciclosporin AUC_{12} in heart transplant patients using a Bayesian estimator originally designed for renal transplant recipients but no validation of this estimator was reported. The Bayesian estimators reported by Monchaud et al. ^[9] for heart transplant patients over the first year transplantation were developed using the standard two-stage method. Unlike the population approach, where the population included in the study should represent the general population, this method requires homogeneous populations. Multiple estimators were thus developed for each post-transplantation period (week 1, month 3, month 12 and month 3 and 12 combined) and their predictive performances were assessed separately. The values of biases and precisions reported in this study were similar to those obtained in our study.

Other sparse sampling strategies were proposed in the literature for estimation of AUC using Bayesian method in heart (0-1h-3h^[9;10] and 0-1h-2h^[11]) and in lung (0-1h-3h^[12]) transplant recipients. Bayesian estimation based on the present population model using these strategies led to less good predictive performances than 40min, 2h and 4h, with regard to ciclosporin AUC₁₂ estimation (Table III).

Cinical implications

Ciclosporin monitoring on the basis of the C_{trough} or C₂ is widely used in most clinical settings as it is the easiest means for individual dose adjustment. Indeed, only one blood sample is required and the clinician can easily calculate the dose needed to reach a target. C_{trough} or C₂ targets were determined empirically on the basis of clinicians' experiences and published data, as no consensus has been published after thoracic transplantation, unlike kidney transplantation. Ciclosporin monitoring on the basis of AUC Bayesian estimate may seem less easy to implement because it requires a specific computer program and a trained pharmacologist authorized to validate the results. However user-friendly solutions can be proposed through, for example, an expert system, such as the ISBA system - Immunosuppressants Bayesian dose Adjustment- which is accessible to the transplantation centers via a website (at:<https://pharmaco.chu-limoges.fr>). Using validated pharmacokinetic population models and Bayesian estimators, this expert system may provide to clinicians, AUC estimates, fitted concentration-time curve and recommended dose-adjustment to reach therapeutic range. The current limit for individual dose-adjustment of CsA based on AUC is that, there is no ciclosporin AUC₁₂ target consensually recommended after heart and lung transplantation (as well as after kidney transplantation). However, on the basis of the mean C_{trough}/AUC₁₂ or C₂/AUC₁₂ relationships, ciclosporin AUC ranges expected corresponding to C_{trough} or C₂ target values could be determined. Thus, if a patient exhibits

either side effects or acute rejection despite C_{trough} and/or C_2 values close to the targets, the determination of AUC could provide relevant information on under or over drug-exposure. Although this study does not allow recommendations for optimal ciclosporin monitoring in thoracic transplantation, the developed Bayesian estimator could be very useful to conduct a prospective study designed to evaluate the relationship between ciclosporin global exposure and surrogate markers of efficacy or toxicity and to define optimized target AUC values.

Conclusion

In conclusion, sex was found to influence significantly the oral apparent clearance of ciclosporin, while none of the other biometric or pharmacogenetic covariates tested did. The Bayesian estimator based on the population pharmacokinetic model developed here is suitable for clinical practice and could be helpful for the adjustment of the immunosuppressive therapy. Moreover, this tool could be useful to determine the most relevant ciclosporin exposure indices or to define the AUC_{12} target(s) in this specific population of lung and heart transplant recipients.

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Table I. Patient characteristics.

	Index group (n = 49)	Validation group (n = 27)	p-value ^c
Number of profiles	118	69	
Type of graft (heart/lung)	11/38	8/19	0.489
Pathology ^a (CF/non-CF)	12/26	4/15	0.404
Sex (F/M)	15/34	6/21	0.403
Age ^b (years)	50 (19-66)	52 (18-65)	0.655
Weight ^b (kg)	63 (30-113)	63 (43-105)	0.698
Ciclosporin dose (mg)	150 (40-400)	150 (60-750)	0.632
Serum creatinine ^a (μmol/L)	80 (35-318)	89.5 (18-274)	0.144
Haematocrit ^b (%)	32 (23-44)	33 (25-65)	0.321
Hemoglobin ^b (g/dL)	10.7 (7.8-15.6)	11.1 (7.9-14)	0.465
<i>CYP3A5</i> *3 allele (n)			0.195
Non carriers (AA)	0	2	
Heterozygous (AG)	9	4	
Homozygous carriers (GG)	40	21	
<i>CYP3A4</i> *22 allele (n)			
Non carriers (CC)	42	NA	
Heterozygous (CT)	6	NA	
Homozygous carriers (TT)	1	NA	
<i>ABCB1</i> c.1236C>T genotype (n)			0.136
CC	18	11	
CT	21	15	
TT	10	1	
<i>ABCB1</i> c.2677G>T genotype (n)			0.129
GG	17	13	
GT	22	13	
TT	10	1	
<i>ABCB1</i> c.3435C>T genotype (n)			0.089
CC	13	10	
CT	22	15	
TT	14	2	
<i>ABCB1</i> variant (TTT) haplotype (n)			0.081
Non carriers	20	13	
Heterozygous	21	14	
Homozygous carriers	8	0	

^aLung transplant patients' characteristic; ^bData are presented as median (range); ^cA two-sided p-value less than 0.05 was considered statistically significant. ^{NA}Genotyping not performed; M, male; F, female; CF, cystic fibrosis.

Table II. Final ciclosporin population model, and results of the bootstrap internal validation procedure.

Model parameters		Final model original dataset estimate (SE) ^(a)	Bootstrap ^(a)	
			Median	2.5th-97.5th percentiles
Fixed effect parameters				
ka (h ⁻¹)		1.04 (0.23)	1.07	0.75 - 1.61
Q/F (L/h)		26 (3.7)	27.4	20.1 - 36.1
V ₁ /F (L)		86.3 (9.02)	87.8	69.5 – 104.0
V ₂ /F (L)		1350 (302)	906	511.9 – 1490
CL/F = $\theta_4 * \theta_{10}^{SEX}$				
θ_4 (L/h)		25.4 (3.5)	24.3	18.5 – 32.6
θ_{10}		1.37 (0.24)	1.39	0.94 – 1.90
tlag (h)		0.302 (0.04)	0.310	0.29 – 0.44
Inter-individual variability				
F (%)		14.5 (13.5)	17.0	0.5 – 54.6
ka (%)		43.1 (15.3)	51.0	11.9 – 76.7
Q/F (%)		39.6 (9.1)	43.7	24.9 – 58.6
V ₁ /F (%)		36.2 (13.9)	24.0	8.3 – 54.8
CL/F (%)		13.8 (13.0)	16.9	5.9 – 54.8
tlag (%)		55.4 (9.7)	44.9	26.9 – 62.9
Inter-occasion variability				
ka (%)		67.6 (10.1)	66.2	46.2 – 84.3
V ₁ /F (%)		31.0 (11.0)	36.2	15.4 – 54.8
CL/F (%)		27.2 (8.0)	22.5	17.4 – 54.7
Residual variability				
Proportional (%)		25.8 (3.0)	23.8	18.3 -30.0
Additive (ng/mL)		12.4 (7.4)	6.72	5.1 -34.1

^(a)Statistics from 1000 bootstrap runs.

SE, standard error; ka, absorption rate constant; Q/F apparent inter-compartment clearance; V₁/F, apparent central volume of distribution; V₂/F, apparent peripheral volume of distribution; CL/F, apparent oral clearance; tlag, lag time; F, bioavailability; SEX=0 if female and 1 if male.

Table III. Comparison of trapezoidal AUC₁₂ with AUC₁₂ Bayesian estimates (n=67) using different previously reported limited sampling strategies

Sampling times	r ²	Relative Bias (%) (RMSE ^a %)	Extreme values of relative bias (%)	Number of profiles with relative bias <-25% or >+25%
C _{trough} -C ₁ -C ₃ ^[9-10;12]	0.8703	-0.8 (13)	-29.7 ; +45.5	5
C _{trough} -C ₁ -C ₂ ^[11]	0.8299	+0.8 (15)	-33.0 ; +49.5	6
C _{0.67} -C ₂ -C ₄	0.9013	-5.0 (13)	-29.7 ; +42.6	2

^aRMSE= root mean squared prediction error

Legend of figures

Figure 1. Goodness-of-fit plots of the final model: (a) model-predicted *versus* observed blood ciclosporin concentrations; (b) individual-predicted *versus* observed blood ciclosporin concentrations; (c) conditional weighted residuals *versus* model-predicted ciclosporin concentrations (CWRES); and (d) conditional weighted residuals *versus* time.

Figure 2. Visual predictive check. Comparison of observed ciclosporin blood concentrations with the median (solid line) and 90% tolerance interval (dashed line) obtained from 1000 simulated datasets. The VPC are separately presented for (a) female patients and (b) male patients. Concentrations were standardized to a 171 mg ciclosporin dose.

Figure 3. Four examples of whole blood concentration-time profiles obtained at the same post-transplantation period in four patients who received ciclosporin, with (a) close residual concentrations and (b) close concentrations measured two hours after dosing, showing marked variability in the area under the concentration-time curve from 0 to 12 hours (AUC_{12}).